THE UNIVERSITY OF MANITOBA

THE EFFECT OF FERTILIZER NITROGEN ON YIELD, PROTEIN CONTENT AND SYMBIOTIC NITROGEN FIXATION IN SOYBEANS (*Glycine max* L. var. *Maple Presto*)

by

Peter Jonathan Regitnig

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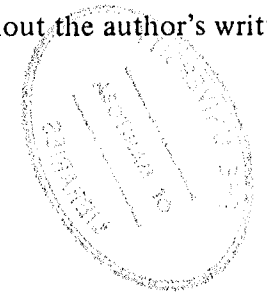

Abstract

Maple Presto soybeans are an early maturing variety of soybeans which were developed specifically for cool climate conditions. Such as those found in Manitoba. Field and growth chamber experiments were conducted to evaluate the effect of fertilizer nitrogen on the yield, protein and symbiotic nitrogen fixation potential of Maple Presto soybeans. The fertilizer treatments consisted of 0-100 kgN/ha and 0-360 ppm N in field and growth chamber studies respectively. Fertilizer treatments were labelled with ^{15}N to facilitate the evaluation of symbiotic nitrogen fixation. Dry matter and seed yield, protein content and total N and total fertilizer N uptake and soil N uptake were measured. The ^{15}N "A" value method, ^{15}N assisted difference method, classical difference method, acetylene-ethylene assay and nodule rating technique were used to evaluate symbiotic nitrogen fixation in these experiments.

Maple Presto soybeans, when inoculated with an effective Rhizobium strain obtain their nitrogen from soil and by Dinitrogen fixation. Results from the experiments showed that on low to medium NO_3-N soils these two sources were not able to fully satisfy the soybeans demand for nitrogen. Fertilizer nitrogen applications of 30 and 100 kgN/ha resulted in highly significant seed yield increases while significant protein increases occurred with applications of 100 kgN/ha when soil N was present in moderate amounts along with the added fertilizer. When fertilizer nitrogen was not applied seed protein levels fell below the standards required for processing in Manitoba. Maple Presto soybeans were shown to fix up to 51 kgN/ha or 66% of their total N content, the

percent of the total and the total amount fixed decreasing with fertilizer addition. Substantial symbiotic nitrogen fixation occurred subsequent to flowering in these soybeans, maximum fixation occurring at the mid pod fill stage of development.

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1. INTRODUCTION

Soybeans (Glycine max) were introduced to Canada as a forage crop but are now grown primarily as an oil-protein seed crop. In the 1970's nearly all Canada's hectarage was in southern Ontario, however, with the advent of early maturing varieties the potential area of production has been expanded. Manitoba is one area where the potential now exists to produce soybeans and there is interest in obtaining information on production of early maturing varieties such as Maple Presto under Manitoba conditions, particularly with respect to maintaining high seed yield and protein content. Research was therefore initiated by the Department of Soil Science at the University of Manitoba to determine the soil fertility and nutritional requirements of Maple Presto soybeans. There was also interest in providing information on the amount of nitrogen symbiotically fixed by early maturing soybean varities such as Maple Presto.

Specific research in the area of the nitrogen requirements of Maple Presto soybeans was initiated by Regitnig (1979) who worked on the effects of nitrogen fertilization on symbiotic nitrogen fixation, yield and protein content. Work in Alberta by Dubetz (1979) also examined the nitrogen requirements of Maple Presto soybeans using applied nitrogen fertilizer. Both these workers found significant protein responses to added fertilizer nitrogen. Dubetz also observed a significant yield response when nitrogen was added. Each of these experiments was carried out on soil with relatively high available NO_3-N.

The work resulting in this dissertation was undertaken to continue to examine the effect of added nitrogen on Maple Presto soybeans with specific emphasis on: 1) Yield response of soybeans to nitrogen application, 2) Protein response of soybeans to nitrogen application, 3) The amount of symbiotic nitrogen fixed by soybeans and the effect of different levels of added nitrogen on fixation and nitrogen uptake, 4) Methods of measuring symbiotic nitrogen fixation in soybeans.

2. LITERATURE REVIEW

2.1 Yield, Symbiotic Nitrogen Fixation and Chemical Composition of Soybeans: Effect of N

Soybeans are a member of the Leguminosae family and can obtain nitrogen by fixation of atmospheric nitrogen when in symbiotic association with Rhizobium Japonicum. This has led to the expectation that soybeans will obtain a large quantity of their nitrogen supply from this source (Sorensen and Penas, 1978). Ohlrogge (1963) reported that soybeans yielding 3362 kg/ha required 314 kgN/ha of which 30 to 50% was symbiotically fixed. Weber (1966b) indicated that symbiotic nitrogen fixation could supply 78 kg N/ha or 40% of the total requirements of a 2690 kg/ha crop.

Soybeans can obtain nitrogen from the soil or from fertilizer sources as well as by fixation of atmospheric N. DeMooy et al. (1973) were of the general opinion that soil and symbiotic sources of nitrogen are perhaps not adequate for maximum yields. The reason for applying fertilizer nitrogen would therefore be to supply the plant with nitrogen when soil and symbiotic sources could not meet total plant requirements. Fertilizer addition would not, however, directly supplement the other two sources of nitrogen because of the inverse relationship between supplemental nitrogen and symbiotic nitrogen. A reduction in the total N supplied by symbiotic fixation has generally been observed as a result of the addition of mineral nitrogen (Norman and Krampitz, 1946; Thornton, 1947 and Weber 1966b). These workers all reported that the percentage of the total nitrogen resulting from fixation was reduced

with added nitrogen, however, the total amount of nitrogen fixed has, in some cases, increased as a result of increased dry matter accumulation (Allos and Bartholonew, 1955, 1959).

The literature is not in agreement on the question of whether nitrogen fertilizer application is advantageous to soybean production. Sorensen and Penas (1978) indicated that some reports have shown yield increases with added nitrogen although these increases were often small, while a number of workers also reported no response to nitrogen fertilizer. DeMooy et al. (1973) stated that although fertilizer additions may contribute to supplying nitrogen for maximum yields it is generally felt that it would not be profitable even if the other two sources were not adequate. Jones et al. (1982) while reviewing the literature, were quite adamant that, in light of the ability of legumes to utilize N directly from the soil air "growers should not waste scarce and expensive nitrogen fertilizers on legumes when there is a global need to conserve petroleum-based N fertilizers".

Examination of specific investigations on nitrogen addition to soybeans further reveal the differences in response which have been observed and their relationship to symbiotic nitrogen fixation in the plant. As early as 1946, Norman and Krampitz established that maximum yields were not obtained when symbiotic nitrogen was the only source of nitrogen for the soybean plant. They indicated that yield increases could be realized if fertilizer nitrogen was added.

Ham et al. (1975) suggested that soybeans do not supply enough symbiotic nitrogen to maximize yield. He stated this based on observations that nitrogen application increased seed yield and protein content.

This conclusion was contradicted by results from Welch et al. (1973) who did experiments at ten different field locations in Illinois. They worked with residual nitrogen, organic sources of nitrogen and inorganic fertilizer nitrogen and found that soybeans did not respond to nitrogen additions in any of the experiments. In only 3 out of 133 instances were significant yield responses shown to occur and this occured at uneconomical fertilizer rates. The soils used in these experiments were lower in available nitrogen than most in the surrounding area although no nitrogen levels were given.

Results from Beard and Hoover (1971) show that the addition of nitrogen caused leaves of soybeans to be less chlorotic, however, this did not result in an increase in seed yield of soybeans in the field. Shibles et al. (1975) reported that responses of soybeans to nitrogen application have run the range of substantial (rare) to nil (frequent). Cartter and Hartwig (1963) also reported that a lack of response of soybeans to nitrogen application was typical of many trials on well nodulated soybeans.

Other workers have examined the application of nitrogen to soybeans and its effect on growth and development of the plant, as well as parameters such as yield and protein. Soybeans were found to have the greatest requirement for nitrogen at the stage between full bloom and mid pod fill (Harper, 1971). The total accumulation of N has been found to follow patterns similar to that of dry matter accumulation (Hanway and Weber, 1971), N accumulation preceeding the production of dry matter (Neunylov and Slabko, 1968a). Estimates made by Weber et al. (1971) indicate that more than 80% of the nitrogen fixed by soybeans is fixed

between the flowering and green bean stages of development corresponding to the period of greatest nitrogen demand. The occurence of fixation was observed from 3 to 4 weeks after seeding to near maturity 12 to 13 weeks later (Hardy et al., 1968; Weber, et al. 1971). Mengel and Kirkby (1982) were not in agreement with this stating that nitrogen supplied by the nodules was terminated abruptly at the completion of flowering. Withholding N at the period 2 to 3 weeks before bloom was shown to be the most detrimental in terms of yield (Iwata and Utada, 1967). The greatest dry matter production has been shown to occur when nitrogen was applied at planting (Neunylov and Slabko, 1968a). This indicates that although critical periods exist for nitrogen accumulation an adequate supply over the entire growing season is important.

Hardy (1959) reported no response of soybeans to nitrogen fertilizer added at different growth stages. Pal and Saxena (1976) found nitrogen fertilizer had little effect on nitrogen accumulation in different plant parts or whole plants, on nitrogen accumulation rate, nitrogen concentration or seed yield per plant in nodulating soybean isolines. These parameters were also measured in non-nodulating isolines to which nitrogen had been applied. Non-nodulating plants responded positively to added nitrogen until they reached a similar nitrogen status as the nodulating plants. This occured at application rates of 200 kg N/ha. It was reported that the soils in these experiments were high in total N but no indication was given whether this had any effect on the lack of response in the nodulating soybeans.

Many workers reporting in the literature have not indicated the nitrogen status of the soil and therefore have not indicated whether significant amounts were supplied from this source. In light of the soybeans need for an adequate seasonal N supply, the lack of response to added nitrogen reported in some experiments may be in part a reflection of failure to consider the soil nitrogen supply when results are examined. It seems desirable, therefore, to report either a soil analysis or a plant standard to indicate soil nitrogen present.

Sorensen and Penas (1978) observed yield increases with added nitrogen at 9 of 13 field sites over a 3 year period. Seed production varied from 1810 Kg/ha to 3640 Kg/ha. They suggested that yield responses could occur if soil nitrate levels are low and or soil PH is low. Visual differences in plant color only appeared on some sites which had yield responses. Yield responses were said to result from increased seed size when nitrogen was added. Other workers have also reported yield increases as a result of seed size as well as seed number (Brevedan et al., 1978). Sorensen and Penas also showed that nitrogen application resulted in a significant increase in nitrogen concentration in plants sampled at the end of flowering, however, the increases were not directly related to the yield increases that were observed. They concluded that the nitrogen content of the plant at this stage may not be a good indicator of yield response to addition of nitrogen.

The use of non-nodulating standard crops in several experiments allowed for an estimation of the contribution of symbiotic fixation in supplying nitrogen to the legume. Hanway and Weber (1971) stated that nitrogen fertilizer applications increased the nitrogen concentration in

all nodulating and non-nodulating soybean plant parts. They also reported that nutrient translocation to seeds in the process of filling had pronounced effects on decreasing the nutrient concentration of other parts of the plant irrespective of fertilizer application. This occurence was, therefore, not likely a result of an inadequate nitrogen supply. When nitrogen was not applied, it was shown that nitrogen concentration in non-nodulating soybeans was consistently lower than in nodulating soybeans. Application of 672 kg N/ha to non-nodulating soybeans resulted in slightly higher total plant N than in nodulating soybeans which had no nitrogen applied. Based on estimates made using the difference in nitrogen concentration between nodulating and non-nodulating plants it appeared that fixation was assuming a major role in supplying the plants' nitrogen requirements.

Similar results were observed by Weber (1966) who studied the response of nodulating and non-nodulating soybeans to nitrogen applications in the field. He observed an increase in seed yield, seed size, seed protein, plant height and dry matter yield of the non-nodulating isoline with added nitrogen. Under the same conditions the nodulating isoline showed very small responses of these parameters to added nitrogen. Yields were similar for both isolines if enough nitrogen was added to supply the requirements of the non-nodulating variety. Bhangoo and Albritton (1976) found at low nitrogen applications a nodulating isoline had better grain yields than a non-nodulating isoline. At high nitrogen applications the yields between the two isolines were similar. They also found that some soil nitrogen or fertilizer nitrogen was essential in obtaining maximum yields for the nodulating isoline.

Diebert et al (1979) used ^{15}N labelled nitrogen fertilizer while carrying out field trials with nodulating and non-nodulating soybeans. Three rates of fertilizer, 45, 89 and 134 kg N/ha applied at planting and full bloom were used along with a zero treatment. The soil was described as having modest nitrogen fertility based on soil analysis. Plant samples were collected at full bloom, early pod fill and maturity. It was shown that nitrogen application did not significantly affect yield or nitrogen concentration of nodulating soybeans at final harvest. These parameters were consistently increased in the non nodulating isoline and this increase was consistently higher with delayed application of fertilizer. It was concluded from this observation that soybeans may be more efficient in utilizing soil nitrogen and applied nitrogen at later stages of growth when vegetative growth gives way to pod filling.

Brevedan et al. (1978) also reported that soybean yields increased over the control in field and greenhouse studies when nitrogen was added during the period of initial flowering to the end of flowering but that additions during pod filling had no effect. These results coupled with earlier observations that an adequate supply of nitrogen is required over the entire growing season in spite of critical periods which exist (Neunylov and Slabko, 1968a; Iwata and Utada, 1967), show that soybeans will respond to proper nitrogen management.

Diebert et al. (1979) also reported that once nodules were established fixation could adequately supply plant nitrogen requirements. Plants were observed to obtain approximately 66% of their nitrogen requirements through symbiotic fixation. Measurements of the percentage

of seed nitrogen that was symbiotically fixed indicated that fertilizer nitrogen applied at planting at rates greater than 45 kg N/ha caused a reduction in nitrogen fixation while later applications after flowering had little effect on changing the amount of nitrogen fixed at any of the rates used. Allos and Bartholomew (1955) observed similar effects of nitrogen application on symbiotic fixation when the nitrogen was applied at seeding in the greenhouse. Small amounts of applied nitrogen did not have much of an adverse effect on fixation while larger amounts caused notable reductions in the amount of nitrogen fixed. Experiments seem to indicate that although nitrogen addition inhibits symbiotic nitrogen fixation it is never totally inhibited (Allos and Batholomew, 1955; Norman and Krampitz, 1946; Thornton, 1947). It also appears from these experiments that fertilizer nitrogen need not be inhibitory to symbiotic fixation if properly managed.

A number of workers have examined specific effects of added nitrogen on nodulation of soybeans. Good viable nodulation is generally an indication that the soybean is actively fixing nitrogen. In demonstrating the inverse relationship between nitrogen application and symbiotic nitrogen fixation in the plant, Thornton (1947) showed that added nitrogen was depressing nodulation. Nodules were observed to be smaller and distributed more on lateral roots in treatments where nitrogen had been applied.

Weber (1966b) demonstrated the effects of added fertilizer nitrogen on nodulation of field grown soybeans in soil to which ground corn cobs were added to partly immmobilize soil nitrogen present. Nodules were found to be smaller with increasing addition of supplemental nitrogen.

The total weight and number of nodules were also observed to decrease with increasing amounts of fertilizer N.

Harper and Cooper (1971) worked on nodulation response in soybeans to different rates and placement of nitrogen. They observed that nodule fresh weight and haemoglobin content were generally decreased when fertilizer was applied whereas the number of nodules which were formed were not significantly affected. It appeared that the application of nitrogen had a greater effect on nodular development than on nodular infection and therefore the number of nodules formed. Nutman (1965) stated that fixation was directly related to nodular tissue produced as well as haemoglobin content.

Harper and Cooper found that nodulation varied with placement of nitrogen when 150 ppm N was used in soil columns. Nodulation was inhibited to a greater extent when the nitrogen was applied throughtout the 30.5 cm. column than when it was applied to the lower 10 cm. of the column. At 40 ppmm N there were no differences in nodulation due to placement.

The nitrogen carrier used is another variable which appears to have an effect on the reponse of soybeans to applied nitrogen. Uziakowa (1959) examined the effect of different sources of nitrogen on soybean growth and nodule formation. When NH_4NO_3, HNO_3, urea and NH_4OH were applied frequently in small amounts the dry matter yield increased 30 to 80% at flowering with urea showing the best response. Urea was also the best carrier at maturity increasing dry matter yields 18% over the control. NH_4OH had a depressive effect on final yield. Total nitrogen accumulation was increased 42% at flowering and 19% at maturity when

urea was applied while other carriers showed smaller effects. Nodule formation was decreased to different degrees depending on the nitrogen carrier applied, NH_4OH having the greatest effect with progressively less depression by HNO_3, NH_4NO_3 and urea respectively. Diatloff (1968) reported that nitrate forms of nitrogen had a greater effect on inhibiting nodulation than ammonium. He showed that 160 ppm of NO_3-N inhibited nodulation of excised soybean roots, while only partial inhibition was observed with 224 ppm NH_4-N.

A number of environmental factors could also potentially affect nodule formation or functioning and, therefore, affect the responses observed to applied nitrogen. Low soil pH may have adverse effects on the nitrogen fixation process primarily through the limiting effects it has on rhizobium survival and/or proliferation in the rhizosphere (Vincent, 1965). Infection would therefore be limited due to lower numbers of bacteria present. Vincent stated direct effects of low pH on nodule formation and functioning were harder to observe.

Soil temperature has also been found to have an influence on symbiotic nitrogen fixation. Kuo and Boersma (1971) reported that nitrogen fixation and dry matter yield of three week old soybeans increased when temperatures were raised from 15.6°C to 27°C and then declined as temperatures were raised above 27°C. Vincent (1965) stated that temperature has an influence on the survival and proliferation of rhizobia, nodule formation and nodule functioning.

Soil moisture has been observed to effect symbiotic fixation and response to nitrogen addition. Sprent (1976) reported that moisture stress and waterlogging reduce symbiotic fixation by inhibiting the

functioning of existing nodules as well as reducing nodulation. Rhizobia survival is probably the dominant factor in reducing fixation under water stress early in the season as the bacteria are likely to die rapidly as the soil dries (Vincent, 1958). A release of soil nitrogen as a result of mineralization after summer rainfall may also affect symbiotic fixation and response to added nitrogen particularly if it occurs at periods of maximum nitrogen requirement (Runge and Odell, 1960). Seed yield response to nitrogen has been observed in hot, dry summers whereas no increases were observed in years of normal precipitation (Lyons and Early, 1952). This level of moisture stress therefore appeared to be more limiting to symbiotic nitrogen fixation than to potential plant growth. DeMooy et al. (1973) reported that many U.S. workers observed responses to added nitrogen fertilizer in the dry summer of 1967.

Soil salinity has also been observed to decrease nodulation in soybeans. Bernstein and Ogata (1966) observed that salinity decreased nodule weight by 23% of the control. Decreased dry matter yields as a result of salinity were relatively less with the addition of nitrogen fertilizer.

2.2 METHODS OF MEASURING SYMBIOTIC NITROGEN FIXATION

A number of methods for determining the amount of symbiotic nitrogen fixation in nodulating plants have been summarized in the literature. Hardy and Holsten (1977) outline three different approaches available for assessing symbiotic nitrogen fixation. The methods are based on 1) growth, unique morphology and haemoglobin, 2) nitrogen increment, N_2 uptake, or nitrogen isotope enrichment, 3) reduction products or alternate substrates. They indicate the two most significant methods are ^{15}N enrichment and $C_2H_2 \longrightarrow C_2H_4$ assay. Three methods will be outlined in this review. These methods were used in determining results found in this manuscript.

The first method outlined is referred to as the difference method for measuring symbiotic nitrogen fixation in legumes as used by Weber (1966). This method has also been referred to as special controls for legumes (Hardy and Holsten, 1977). This concept can be broken up into three approaches. One approach is concerned with the difference in total nitrogen between inoculated and uninoculated plants of the same variety of legume. Another determines the difference in total nitrogen between a nodulating and non-nodulating isoline of the same legume. A third approach measures the difference in total nitrogen between a cereal plant and a nodulating legume. These three approaches determine the amount of nitrogen fixed by a crop by subtracting the total nitrogen in the above ground portions of a nodulating crop from that in the above ground portions of a non nodulating crop. The non-nodulating crop is represented by the uninoculated legume, the non-nodulating isoline or the cereal plant. It is assumed that each of these non-nodulating crops

will take up a similar amount of soil nitrogen. This amount of soil nitrogen is assumed to be similar to the soil nitrogen taken up by the nodulating legume grown in the same environment. Any additional nitrogen in the nodulating legume is therefore said to be nitrogen which resulted from symbiotic fixation.

Weber would appear to justify the use of the difference method based on the concept that combined nitrogen is used preferentially over symbiotically fixed nitrogen and that nitrogen is fixed only when inadequate amounts of combined nitrogen are present (Allos and Bartholemew, 1959). Weber's results along with those of Thorton (1947) would seem to lend support to this conclusion. Results from Allos and Bartholemew (1959) do, however, show that although this conclusion was true of a number of legumes it must be qualified. The addition of nitrogen to soybeans increased the growth of the legumes and therefore the need for absorption of nitrogen. This in effect stimulated the fixation process. Therefore, total fixation was related to the amount of growth that took place in the plant. Hardy and Holsten (1977) reviewed the difference method and indicated they do not feel it provides a good absolute quantitative value for symbiotic nitrogen fixation. They do not recommend its use in light of other methods that are now available.

The difference method was slightly modified by adding fertilizer to both the fixing and non-fixing plants (Richards and Soper, 1979). This method required the use of ^{15}N labelled nitrogen fertilizer. When plants were harvested $^{15}N/^{14}N$ ratio analysis was carried out to determine the amount of labelled nitrogen present. By calculating the dilution of fertilizier ^{15}N in the plant the amount of nitrogen derived

from fertilizer was known. This value was subtracted from the non-nodulating plants to determine the contribution of soil nitrogen to the plant. The calculation can be represented by the following equation:

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

where S = quantity of nitrogen symbiotically fixed,

P = total nitrogen in 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).

A second method which has been used to measure symbiotic nitrogen fixation in legumes involves the use of an "A_n" value, which is based on a concept proposed by Fried and Dean (1952). They proposed that the availability of a nutrient in the soil could be determined in terms of a standard amount of nutrient, which is usually a fertilizer. They made a major assumption in their concept which stated that if two sources of nutrient are available to the plant then the plant will take up from each of these sources in proportion to their respective availabilities. The following equation was proposed for determining the amount of available nutrient in the soil.

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

where A = available nutrient in the soil

B = standard (fertilizer) applied

Y = the proportion of nutrient in the plant derived from the standard

If Y could be determined then "A" could be calculated. It was suggested that radioactive or stable isotopes would be suitable to measure the value of Y. This method assumed that all the standard fertilizer was available to the plant. The "A" value determined was in fertilizer equivalent units. This value, according to Fried and Dean, should not be affected by the rate of standard applied. This conclusion was supported by work from Legg and Stanford (1967) and Hunter and Carter (1965). Fried and Dean concluded that the standards which are used will determine to a large degree what can be said about the "A" values obtained. This implies that a fertilizer standard may interact differently in different systems being measured (Fried, 1967). Fried and Dean determined that factors such as character of the standard, fixation of the standard, soil acidity and placement of the standard may put some confinements on interpretations made about the "A" value but do not invalidate it. Broadbent (1970) examined effects of fertilizer rate, placement and type on calculated "A" values. He concluded that the "A" values usefulness will depend on how carefully the experimental conditions where it is measured are defined.

Fried and Broeshart (1975) determined that the concept of the "A" value could be used to measure the amount of symbiotic nitrogen fixation taking place in a legume crop. The "A" value for a non-nodulating crop was subtracted from the "A" value for a nodulating crop to obtain a value for fixed nitrogen in terms of the fertilizer standard. This can be explained by realizing that the "A" value for the nodulating crop actually represents both available soil nitrogen and symbiotically fixed nitrogen whereas the "A" value for the non-nodulating crop represents

available soil nitrogen only. Subtracting the values was said to give the amount of nitrogen fixed in terms of the fertilizer standard. Since this value was in fertilizer equivalent units it was multiplied by the percent utilization of fertilizer by the legume to obtain the actual amount of nitrogen fixed. The percent utilization of fertilizer was calculated using ^{15}N labelled nitrogen fertilizer to determine the amount of fertilizer taken up by the plant and dividing by the rate of fertilizer applied.

The "A" value or any other method utilizing ^{15}N enriched nitrogen assumes that there is no descrimination between ^{15}N and ^{14}N isotopes or that living systems can distinguish between isotopes only with difficulty (Hauck and Brenner, 1976). Hardy and Holsten (1977) state that this is generally valid for N_2 fixing systems. Their review of the literature indicated that fractionation does occur in complex systems like the soil, perhaps at the stage of denitrification. Marriote et al (1980) reported on fractionation of nitrogen isotopes by 42 different plant species. They stated that fractionation during nitrate absorption by higher plants after a long growth period was very low or even nonexistent. A few plants exhibited an isotope descrimination of one percent or more. Only one plant, pearl millet, was observed to have significant descrimination between isotopes. Soybeans were not examined in these tests.

Hauck and Bremner (1976) reported that methods utilizing ^{15}N also assume that elements containing two or more isotopes in the natural state have a constant isotope composition. One final assumption is that chemical identity of isotopes is maintained in biological systems.

These assumptions are central to the use of ^{15}N labelled fertilizer in a biological system and although none is totally valid for all experimental conditions, they are said to be generally true.

The final method for measuring symbiotic nitrogen fixation is the acetylene reduction assay. This assay is an indirect method for measuring fixation in which an alternate substrate is used to measure the nodule activity. Specifically for this determination, soybean nodules reduce nitrogen in the atmosphere to ammonia but can also reduce acetylene to ethylene. By using a conversion from the substrate to nitrogen, symbiotic nitrogen fixation can be calculated. An equation for quantitative calculation of symbiotic nitrogen fixation is given by Hardy and Holsten (1977) as follows:

$$gN_2(C_2H_2) \text{ fixed/hr. sample} = \frac{e-b-i}{s}.c.r.v.\frac{l}{t}.\frac{l}{f} \times 28$$

where e, b, i and s are peak height, or area, for C_2H_4 in analyzed sample of 50 ml from, respectively, (i) experimental sample incubated with C_2H_2, (ii) experimental sample pre-incubated 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/liter at STP.

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

v = volume of incubation chamber in liters at STP.

t = time of incubation in hours.

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

28 = molecular weight of N_2.

To determine the conversion factor, the ratio of moles of C_2H_2 reduced to moles of N_2 fixed must be examined. The theoretical value for this ratio is three. This value is thought to be valid in most natural samples, while a value of 4 seems to be more appropriate for nitrogenase in vitro (Hardy and Holsten, 1977). Hardy et al (1968) reported on advantages of this method. They stated that as little as 1 μμ mole could be detected. This procedure has three times the sensitivity of ^{15}N analysis. Equipment and substrates are easy to use and because of their nature and the nature of the analysis a mobile unit is possible. Gas samples can also be stored readily in gastight containers. The product, ethylene, is easily separated and quantified on a gas chromotograph and the procedure is very rapid.

Rennie et al (1978) suggested that this assay was not without its limitations. C_2H_2 reduction is a short term measurement. Diurnal and seasonal variation in nitrogen fixation causes doubt to arise when short term values are extrapolated to total nitrogen fixed by the plant over an entire season. It was observed that N_2/C_2H_2 ratios for different systems vary widely and that it was recommended that each be determined experimentally. Conclusions from Hardy and Holsten (1977) concurred with this suggestion.

The effects of fertilizer nitrogen addition on symbiotic nitrogen fixation in legumes has been extensively examined using the acetylene reduction assay. Hardy and Holsten (1977) indicate it has surpassed the

use of ^{15}N in nitrogen fixation studies. The acetylene reduction assay indicates conclusively that decreases in symbiotic fixation occur with added fertilizer. This assay has been used successfully for measuring profiles of symbiotic nitrogen fixation over time.

When examining different methods available for measuring symbiotic nitrogen fixation in legumes advantages and limitations are evident in each method. It would appear that care must be taken in how results are interpreted but that meaningful information can be extracted using these methods.

3. METHODS AND MATERIALS

3.1 Field Experiments (1979). Nitrogen as a limiting factor in the production of Maple Presto soybeans was examined during the summer of 1979 under field conditions. All experiments were located in the Morden - Winkler area of southern Manitoba. Three sites were planted and were designated Enns, Toews and Nikkel for the purpose of distinguishing them.

3.1.1. Soils. Three soil types were used in this study. The three sites, Enns, Toews and Nikkel, were located on a Reinland series (imperfectly drained gleyed carbonated rego black fine sandy loam), Hockfeld series (well drained orthic black fine sandy loam) and Neuenberg series (imperfectly to moderately well drained gleyed carbonated rego black very fine sandy loam) respectively (Smith and Michalyna, 1973).

Soil samples were taken at the four corners of each site at seeding time at depths of 0 - 15 cm, 15 - 30 cm, 30 - 60 cm, 60 - 90 cm and 90 - 120 cm. The soil was placed in plastic bags and 2 - 3 drops of toluene were added to inhibit mineralization. The samples were frozen as soon as possible and thawed at a later date for chemical analysis. Subsamples were taken and oven dried at 105°C for 24 hours for nitrate nitrogen determination. Other chemical analysis was carried out on air dry samples. All samples were ground to pass through a 2 mm. sieve prior to analysis.

3.1.2. Experimental Design and Procedure. A randomized complete block design consisting of four replicates and nine treatments was used for each experimental site. Soybeans (Glycine max (L) var. Maple Presto) were used as the test crop and fababeans (Vicia Faba (L.) var.

Diana) were planted as a comparison crop with respect to symbiotic nitrogen fixation. Previous studies have shown that fababeans are capable of fixing substantial amounts of nitrogen (Richards, 1977). Non-nodulating soybeans were planted as a standard for measuring symbiotic nitrogen fixation in the Maple Presto soybeans. The non-nodulating soybean variety was not an isoline of Maple Presto but was the earliest maturing variety which could be obtained. These soybeans were obtained from the University of Minnesota through the efforts of Dr. G. Ham. The name of the variety was not obtained.

Treatment plots were 7.6 meters long, 2.7 meters wide and contained three seed rows with 61 cm. spacing. This row spacing was dictated by the seeding equipment available. The blocks of treatments were separated by 1.5 metre pathways and the entire experiment was surrounded on three sides by 3.7 meter guard rows and on the fourth side by another experiment. Three extra rows were also added to each of the nodulating soybean treatments for the purpose of conducting an acetylene reduction assay.

The nodulating soybeans were inoculated with Nitragin Corporation(1) S culture inoculum applied in a slurry at twice the recommended rate of 418 g/100kg of seed. Soybeans were also pretreated with Dupont Arasan 50 - Red fungicide to inhibit seed decay and seedling blights. This was applied at a rate of 174g/100 kg of seed for both soybean varieties.

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(1) Supplier: The Nitragin Company, Milwaukee, Wisconsin 53219.

Fababeans were treated with Nitragin Corporation (1) Q culture inoculum applied in a slurry at twice the recommended rate of 418 g/100 kg of seed.

Seeding was done with a two row V-belt seeder which was equipped to sideband fertilizer 5 cm. below and 5 cm. to either side of the seed row. A seeding rate of 120 kg/ha was used for both varities of soybeans and a rate of 180 kg/ha was used for fababeans. Seeding depth was 2.5 cm. for all crops. Trifluralin was applied at a rate of 0.84 kg/ha at each site and was incorporated with a tandem disc before seeding. This herbicide was used primarily to control green foxtail while other weed control was carried out by mechanical means or by hand. The 61 cm. row spacing facilitated easy mechanical weed control. Seeding was completed on June 7th, 8th and 9th, 1979 for Enns, Toews and Nikkel respectively.

The treatments used in this experiment are listed in Table 1. Nitrogen was applied at 30 and 100 kg N/ha to the nodulating and non-nodulating soybeans. A 0 N treatment was also used for both these varities. Fababeans and uninoculated Maple Presto soybeans to which no inoculum was applied received an application of 30 kg N/ha. The nitrogen carriers used were urea (46-0-0) and ammonium nitrate (34-0-0), the former being applied at Enns and the latter at Toews and Nikkel. The use of different nitrogen carriers was determined by the quantity of urea and ammonium nitrate ^{15}N carriers that were available.

Reagent grade ^{15}N labelled nitrogen was applied in a one and a half meter band 5 cm. below and 5 cm. to either side of the seed row. Application was carried out at the same time as the unlabelled fertilizer using the V-belt seeder. The ^{15}N labelled fertilizer was placed in

TABLE 1

Treatments used in 1979 soybean field experiment

Treatment number	Nitrogen added (Kg N/ha)	Type of plant
1	0	Non-nodulating soybeans
*2	30	Non-nodulating soybeans
*3	100	Non-nodulating soybeans
4!	0	Nodulating soybeans
*5!	30	Nodulating soybeans
*6!	100	Nodulating soybeans
*7	30	Uninoculated soybeans
*8	30	Fababeans

* Indicates ^{15}N labelled nitrogen was applied.

! Indicates treatments with extra rows for the purpose of conducting an acetylene reduction assay.

the middle of the center row of each subplot which had nitrogen applied. The urea was labelled 5.28 atom % ^{15}N and 3.21 atom % N abundance for the 30 and 100 kg N/ha rates of application respectively. The ammonium nitrate was labelled 4.36 atom % ^{15} N and 3.21 atom % ^{15}N abundance for the 30 and 100 kgN/ha rates of application respectively.

Two methods utilizing ^{15}N were used to determine symbiotic nitrogen fixation. These methods were the ^{15}N assisted difference method and the "A" value method. A description of the methods is found in the literature review.

Nutrients other than nitrogen were applied to all sulplots in order that deficiences of these nutrients would not occur. Phosphorus at a rate of 30 kg P_2O_5/ha was sidebanded as monocalcium phosphate (0-46-0). Potassium at a rate of 70 kg K_2O/ha was broadcast as potassium chloride (0-0-62). Sulfur at a rate of 30 kg S/ha was broadcast as agrosul (90%S). Zinc at 3 kgZn/ha was broadcast in solution as sequestrene zinc chelate (14.2%Zn). Copper at 10 kg Cu/ha was also broadcast in solution as copper sulfate ($CuSO_4$ $5H_2O$).

Six harvests of aerial dry matter from the nodulating soybeans were taken throughout the growing season with intervals of seven to twelve days between them. Specific harvest times and developmental stages are listed in Table 2. Two meters of plant material was sampled from each treatment at each harvest and yield and protein content of the dry matter were determined on an oven dry basis.

Acetylene reduction determinations were carried out at each mid-season harvest. Four plants were selected in each two meter row from which dry matter yields were to be obtained. Plants were selected in

TABLE 2

Dates and Stages of Midseason Harvests

Days after Seeding	Date	Development Stage
26	July 3rd	4-5th trifoliate leaf stage
36	July 13th	Early flowering
43	July 20th	Flowering
50	July 27th	Early pod formation
61	August 7th	Pod Filling
70	August 16th	Early leaf senescence

0.5 meter intervals along the row so that the choice of plants was not biased by any observed differences in growth. A volume of soil with a surface radius of approximately 7 cm. around the stem down to a depth of approximately 15 cm. was removed with a spade. The soil was shaken from the roots and each root sample placed in a nine hundred and nine milliliter mason jar with a serum stopper in the lid. The lid was put on the jar and twenty milliliters of acetylene was added through the serum stopper with a graduated thirty milliliter syringe. The nodules on the roots were left for one hour to reduce acetylene to ethylene. This was done in a shaded area so nodule functioning would not be quickly reduced due to rapid dehydration. A twenty milliliter sample of gas was taken with the syringe and after dispelling a few milliliters, 10 milliliters was stored in a 10 milliliter vacutainer (2) until it was analyzed on a gas chromatograph. This procedure was obtained from the plant science department of the University of Manitoba and was similar to that described by Hardy and Holsten (1977).

Nodule counts were done at the same time as the acetylene reduction assay. This involved rating the roots which were removed from the soil on a scale between zero and five. Zero represented no evidence of nodulation and five represented seventeen or more nodules, which was considered abundant nodulation. Other numbers on the scale were as follows:

1 = 1 - 4 nodules, 2 = 5 - 8 nodules, 3 = 9 - 12 nodules and

4 = 13 - 16 nodules.

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(2) supplier: Becton Dickinson and Co. Canada Ltd., 2464 South Sheridan Way, Mississauga, Ontario, L5J 2M8

Plants which were to be analyzed for $^{14}N/^{15}N$ ratios were harvested at a stage just before leaf senescence was initated. This was done so that the loss of leaf material and therefore nitrogen would be minimized. The harvest was taken on August 17th which was 71, 70 and 69 days after seeding for Enns, Toews and Nikkel respectively. A total of two meters of plant material was sampled from the center row of each subplot. Samples from rows where ^{15}N had been applied were divided into one meter of sample which was obtained from the area of ^{15}N application and one meter from the rest of the harvest row. These samples were kept separate from one another so that isotope analysis could be carried out on the ^{15}N enriched sample. All samples were air dried to a uniform moisture content and yield weights were obtained. Samples which were to be analyzed for isotope ratios were ground in a Wiley mill in their entirety, to pass through a two millimeter sieve. A subsample was taken and ground for samples which only required protein analysis of the dry matter.

A final harvest was taken for all soybean treatments at maturity for the purpose of determining seed yield and seed protein content. Fababeans, although not mature, were harvested to determine the yield of dry matter. They were in the later stages of pod filling when this harvest was taken. All harvests were carried out by hand. The final harvest dates were September 15th for nodulating soybeans and fababeans at Enns and Toews and September 17th at Nikkel. This was 98 days after seeding at Enns and Nikkel and 97 days at Toews. The non-nodulating soybeans matured later than the nodulating soybeans and were harvested on September 21st at all sites. This was 104, 103 and 102 days after

seeding for Enns, Toews and Nikkel respectively. Final harvest samples were taken from a three meter length along the center row of each treatment. Samples were air dried, thrashed and cleaned so seed yield could be obtained. Subsamples were taken and oven dried to determine the moisture content of the air dry material. The seeds were ground in a Wiley mill to pass through a two millimeter sieve. All values calculated for yield and protein were determined on an oven dry basis.

3.2 GROWTH CHAMBER EXPERIMENT A

3.2.1. Soils A Hockfeld soil (Smith and Michalyna, 1973) located near Morden, Manitoba, was chosen for use in this growth chamber study. Soil was taken in the summer of 1979 from the 0 - 15 cm. depth and was immediately dried at 30°C in order that mineralization of nitrogen would be minimized. The soil was mixed thoroughly while in the process of drying. The soil was sieved to pass through a two millimeter mesh sieve and a representative sample was taken for chemical analysis.

3.2.2. Experimental Design and Procedure A completely randomized experiment containing three replicates and thirteen treatments was undertaken using inoculated soybeans (Glycine Max (L.) var. Maple Presto) as the test crop and barley (Hordeum Vulgare (L.) var Conquest) and unincoculated Maple Presto soybeans as reference crops. The treatments are outlined in Table 3.

Nitrogen was applied to the test crop at rates of 0, 20, 40, 60, 100 and 200 ppm at seeding and at 60 ppm at three time periods, 51, 64 and 81 days, after seeding. A single rate of 60 ppm N at seeding along with a zero treatment were used with each of the two reference crops. All treatments received 185 ppm K, 50 ppm P, 50 ppmS, 8 ppmZn and 4 ppmCu. These rates were calculated on an air dry soil basis.

Pots used in the experiment were washed with tap water and rinsed with distilled water. Nutrients were pipetted in solution form to five kilograms of air dry soil and the soil was mixed thoroughly. Nitrogen applications after seeding were pipetted in solution form on the soil surface for each pot. The $^{15}NH_4{}^{15}NO_3$ applied was labelled with 5.37 atom % ^{15}N excess for all treatments where nitrogen was applied except for the 100 and 200 ppm applications which were labelled with 2.28 atom

TABLE 3

Treatments used in Soybean Growth Chamber Experiment A

Treatment number	Nitrogen added (ppm)	Type of plant	Time of Nitrogen Application
1	0	Inoculated Soybeans	-
2	20	Inoculated Soybeans	Seeding
3	40	Inoculated Soybeans	Seeding
4	60	Inoculated Soybeans	Seeding
5	100	Inoculated Soybeans	Seeding
6	200	Inoculated Soybeans	Seeding
7	60	Inoculated Soybeans	7 weeks
8	60	Inoculated Soybeans	Early flower
9	60	Inoculated Soybeans	Early pod fill
10	0	Uninoculated Soybeans	-
11	60	Uninoculated Soybeans	Seeding
12	0	Barley	-
13	60	Barley	Seeding

% ^{15}N excess. The $^{15}NH_4{}^{15}NO_3$ labelled at 5.37 atom %^{15}N excess was mixed in solution to a concentration of 10 mgN/ml and $^{15}NH_4{}^{15}NO_3$ labelled at 2.28 atoms %^{15}N excess was mixed in solution to a concentration of 8 mgN/ml.

Conditions in the growth chamber were as follows: Daylength = 15 hours, 8 A.M. to 11 P.M., Humidity = 80% (night), 50% (day). Temperature was initially 18°C (day), 15°C (night) and was adjusted to 25.5°C (day), 18°C (night) 28 days after seeding.

Soybean seeds were pregerminated for four days before seeding by placing the seeds between wet paper towels. The seeds were inoculated with Nitragin Corporation S culture inoculum applied in a slurry at many times the recommended rate of 418 g/100 kg of seed. The experiment was seeded and pots were immediately watered to field capacity (22%). After emergence soybeans were thinned to two plants per pot and barley to four plants per pot.

Barley plants were grown for 79 days after seeding to a stage when heads were filling. Soybeans were grown for 88 days after seeding to the stage when pod filling was occuring. Above ground plant material was harvested for each crop and oven dried for forty-eight hours at 60°C. Dry weights were measured and plants were ground in a Wiley mill to pass through a two millimeter sieve. Protein and ^{15}N ratio analysis were carried out on the ground materials. Tap roots were removed from the pots using a garden trowel after harvest of the above ground material. A conical volume of soil approximately eight centimeters in diameter at the soil surface and ten centimeters deep was removed from the area of emergence of each plant. The soil was washed off the roots with water and nodules were counted.

3.3 GROWTH CHAMBER EXPERIMENT B

3.3.1 Soils. A Reinland soil (Smith and Michalyna, 1973) located near Winker, Manitoba was chosen for use in this growth chamber study. Soil was taken in the summer of 1980 from the 0-15 cm depth and immediately dried at 30°C in order that mineralization of nitrogen would be minimized. The soil was mixed thoroughly while in the process of drying. The soil was sieved to pass through a three millimeter mesh sieve and a representative sample was taken for chemical analysis.

3.3.2 Experimental Design and Procedure. A completely randomized experiment containing three replicates and thirteen treatments was undertaken using soybeans (Glycine max (L.) var Maple Presto) as the test crop and barley (Hardeum Vulgare (L.) var Conquest) as the reference crop. The treatments are outlined in Table 4.

Nitrogen was applied to the test crop at rates of 0, 20, 40, 60, 120, 240 and 360 ppm at seeding and at 100 ppm at two time periods, 37 and 52 days, after seeding. Nitrogen was applied to the barley at rates of 0, 60 and 120 ppm at seeding. All treatments received 80 ppm P and 100 ppm K as KH_2PO_4, 100 ppm K as K_2SO_4, 8 ppmZn as $ZnSO_4.7H_2O$, 6 ppm Cu as $CuSO_4.5H_2O$ and 48 ppm S from the last three carriers. These rates were calculated on an air dry soil basis. The K, Zn and Cu were dissolved together in solution and 100 ml was alloted per pot in order to give the correct application of nutrients. P was also applied in solution using 50 ml aliquots.

The pots used in the experiment were washed with tap water and then rinsed three times with distilled water. Five kilograms of air dry soil for each pot was spread on a sheet of paper and nutrients were pipetted

TABLE 4

Treatments used in Soybean Growth Chamber Experiment B

Treatment number	Nitrogen added (ppm)	Type of plant	Time of Nitrogen Application
1	0	Soybeans	-
2	20	Soybeans	Seeding
3	40	Soybeans	Seeding
4	60	Soybeans	Seeding
5	120	Soybeans	Seeding
6	240	Soybeans	Seeding
7	360	Soybeans	Seeding
8	100	Soybeans	Bloom (37 days)
9	100	Soybeans	Early pod fill (52 days)
10	0	Soybeans	-
11	0	Barley	-
12	60	Barley	Seeding
13	120	Barley	Seeding

evenly over the surface area. The nutrients were mixed in the soil by rolling the soil using the paper and then thoroughly mixing by hand. The soil volume for each pot was weighed and divided in half on a weight basis. The volume of soil which was to be put in the bottom half of the pot was mixed with $^{15}NH_4{}^{15}NO_3$ in solution using the same application and mixing procedure as was used for the other nutrients. The concentrations of the solutions were 4 mg N/ml for the treatments from 20 to 120 ppm N including the late applications of 100 ppm N and 24 mg N/ml for the 240 and 360 ppm N treatments. The placement of nitrogen in the bottom half of the pot was carried out in order to reduce nitrogen loss through volatilization and curtail seedling damage. This was of particular concern for the treatments with high applications of nitrogen. Nitrogen applications after seeding were pipetted in solution on the soil surface for each pot. $^{15}NH_4{}^{15}NO_3$ was labelled with 1.86 atom % ^{15}N excess for the 20, 40, 60, 100 and 120 ppm applications and with 0.99 atom $\%^{15}N$ excess for the 240 and 360 ppm applications.

Conditions in the growth chamber were as follows: Temperature = 15°C (night), 20°C (day), Daylength = 15 hours, 8 A.M. to 11 P.M., Humidity = 80% (night), 50% (day).

Soybean and barley seeds were placed in water for approximately twenty minutes before planting to promote imbibition. Soybean seeds were inoculated with Nitragin Corporation S culture inoculum applied in a slurry at many times the recommended rate of 418 g/100 kg of seed. Six seeds were planted per pot at two centimeters below the soil surface. After seeding the pots were watered to fifty percent field capacity. They were kept at fifty percent field capacity from planting

to emergence, eighty percent field capacity from emergence to the primary leaf stage and full field capacity (27.3%) from the primary leaf stage to harvest. Plants were watered on a daily basis after seeding to keep the soil at the desired moisture level. Soybeans were thinned to two plants per pot at the primary leaf stage and barley to two plants per pot at the two leaf stage.

The plants were grown for fifty-nine days after seeding to the stage when pod filling was occuring. Above ground plant material was harvested and oven dried for forty eight hours at 60°C. Oven dry weight was determined and plants were ground in a Wiley mill to pass through a two millimeter sieve. Protein and ^{15}N ratio analysis were carried out on the ground plant material. Tap roots were removed from the soil using a garden trowel after harvest of the above ground material. A conical volume of soil approximately eight centimeters in diameter at the soil surface and ten centimeters deep was removed from the area of emergence of each plant. The soil was washed off the roots with water and nodules were counted.

The soil from each pot was spread out on brown paper and mixed by hand after which it was air dried at 30°C for forty eight hours. Mixing was continued during the drying period to promote uniform drying. A representative sample was taken from each soil and ground to pass through a two millimeter sieve. This sample was used for nitrate analysis.

3.4 ANALYTICAL PROCEDURES

3.4.1 Soil Analysis

Soil NO_3-N was determined for all samples except growth chamber experiment B residual samples using Harper's modified phenoldisulphonic acid method (Harper, 1924). Ten grams of air dried soil were extracted with 50.0 ml of a solution containing 0.02 M $CuSO_4.5H_2O$ and 0.06% Ag_2SO_4. Nitrate was measured colorimetrically as the nitrate form of phenoldisulphonic acid in an alkaline solution using a Cecil Instruments 202 Ultraviolet Spectrophotometer at 415 nm. Soil NO_3-N from growth chamber experiment B were determined by hydrazine reduction using a modification of the automated colorimetric procedure of Kamphake et al. (1967), the method currently used by the Manitoba Provincial Soil Testing Laboratory. Bulk density data were used to convert ppm NO_3-N into kg NO_3-N/ha for field samples.

$NaHCO_3$ extractable phosphorus was determined by a modified Olson et al. (1954) method using 5 grams of soil extracted with 100 ml $NaHCO_3$ and one gram of pretreated charcoal. The samples were shaken for thirty minutes, filtered and phosphorus level in the extractant determined using Murphy and Riley's acid molybdate method (1962).

NH_4OAc extractable K was determined using five grams of soil which was shaken for one hour in 100 ml of solution containing 1.0 M NH_4OAc and 250 ppm $LiNO_3$. The solution was filtered and potassium concentration in the filtrate determined using a Perkin-Elmer model 303 atomic absorption spectrophotometer.

SO_4-S was determined by the method described by Lazrus et al. (1966). A 1:20 soil to 0.001 M $CaCl_2$ mixture was shaken for 30 minutes and filtered. An aliquot from the filtrate was diluted 1 to 41 with distilled water and reacted with $BaCl_2$ at pH 2.5 - 3.0. Exactly enough methylthymol blue to complex the original amount Ba present was added and the pH adjusted to 12.5 to 13. The amount of uncomplexed methylthymol blue as measured at 460 nm on a Technicon Auto Analyzer II reflected the amount of sulfate in the sample.

DTPA (diethylenetriaminepentacetic acid) extractable Cu,Zn and Mn were determined by the method described by Lindsay and Norvell (1969). Twenty five grams of soil were shaken with a DTPA extracting solution (0.005M DTPA, 0.01 M $CaCl_2$ and 0.1 M triethanolamine) for two hours. The solution was filtered and determinations were made using a Perkin-Elmer model 303 atomic absorption spectrophotometer.

Organic matter was determined by the Walkley-Black method as described by Allison (1965). An automatic titrator was used to back titrate excess $K_2Cr_2O_7$ with $FeSO_4$.

The inorganic carbonate content was determined using a one gram soil sample which was digested in 40 mls of 0.1M HCl for ten minutes. The CO_2 evolved was collected using a Nesbitt tube containing ascarite and the change in weight of the ascarite was taken to be the weight of CO_2 evolved.

Soil pH was determined on a water-saturated soil paste using a standard glass-calomel combination electrode. Conductivity was determined on the some soil paste using a Radiometer conductivity meter with a standard conductivity cell.

Field capacity was determined using a plastic cylinder into which air dry soil was placed. The cylinder had a cotton cloth at the bottom to hold the soil in. Water was added to the soil until the wetting front had moved half way down the cylinder and parafilm was placed over the open end of the cylinder. After 48 hours, a sample was taken from the center of the wetted soil, weighed and oven dried at 105°C for 48 hours. The oven dry soil was weighed and the field capacity calculated on an oven dry basis.

3.4.2 PLANT ANALYSIS

a) Total Nitrogen Content. Total plant nitrogen was determined using the modified Kjeldhal-Gunning method described by Jackson (1958). The digestion accelerator used was a Kelpak (1) No. 2, which contained .3 g $CuSO_4$ and 10.0 g K_2SO_4. This method was used for analyzing both dry matter and seed samples.

b) Excess Atom % ^{15}N. Abundance of tracer ^{15}N of enriched plant material was determined by mass spectrometric analysis using a modification of the method described by Bremner (1965). 50 mls of 0.1 N H_2SO_4 was used as a trap to collect NH_3 released from plant material and NH_3 content was determined by back titration with standard 0.1N NaOH. The titrated distillate was acidified with a drop of concentrated H_2SO_4 and evaporated on a hot plate to a volume of approximately ten milliliters. This solution was stored in a glass test tube covered with parafilm until further analysis was carried out. Samples of ^{15}N fertilizer standards also underwent mass spectrometric analysis using the same procedure as was used for ^{15}N enriched plant material. NH_4NO_3 fertilizer required the use of 1.0 to 1.5 grams of Devarda's alloy (45% A1, 50%, Cu, 5% Zn) during distillation, to reduce NO_3 to NH_4 prior to gas preparation (Bremner, 1965).

Nitrogen gas was manufactured by oxidation of the NH_4 solution with sodium hypobromite in the absence of air. This reaction was carried out

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(1) Supplier: Canadian Lab. Supplier, Ltd., 80 Jutland Road, Toronto, Ontario

in gas sample tubes (1) which were used in conjunction with a vacuum system similar to that used by Fehr (1969) and McGill (1971). The use of liquid nitrogen for removing water vapor was discarded and a method suggested by C.M. Cho (2) was used. This method consisted of adding a strong desiccant (KOH) to the gas collection tube to remove water vapor.

N_2 was analyzed for $^{14}N/^{15}N$ ratios using a Micromass 602 mass spectrometer. Atom percent ^{15}N calculations were made from measured ion current intensities of mass twenty eight and twenty nine using a single collector scanning method. The equation used for calculating atom percent ^{15}N from the ratios obtained from the mass spectrometer was given by Bremner (1965):

$$(1)\quad \text{Atom \% } {}^{15}N = \frac{100}{2R + 1} \qquad (2)\quad R = \frac{({}^{14}N{}^{14}N)}{({}^{14}N{}^{15}N)}$$

Where R is the measured ratio of ion currents corresponding to mass 28 ($^{14}N^{14}N$) and mass 29 ($^{11}N^{15}N$). Bremner suggests a definition for atom percent ^{15}N as follows:

$$(3)\quad \text{Atom \% } {}^{15}N = \frac{({}^{14}N{}^{15}N) + 2({}^{15}N{}^{15}N)}{2({}^{14}N{}^{14}N) + 2({}^{14}N{}^{15}N) + 2({}^{15}N{}^{15}N)} \times 100$$

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1) Supplier: Eck and Krebs Inc. 27-09 40th Avenue, Long Island City, New York

(2) C.M. Cho, Professor, Department of Soil Science, University of Manitoba, Winnipeg, Manitoba. R3T 2N2

Calculations can be made using only mass 28 and 29 ion currents because mass thirty ion currents can be eliminated from the equation based on the following equilibrium:

$$^{14}N^{14}N + {}^{15}N^{15}N \longrightarrow 2\,{}^{14}N^{15}N$$

(4) where $K_{eq} = \dfrac{(^{14}N^{15}N)^2}{(^{14}N^{14}N)(^{15}N^{15}N)} = 4.0$

The equilibrium constant of 4.0 has been justified by theory and experiment. The validity of equation 1 can be demonstrated by combining equations 2, 3 and 4.

3.4.3 ^{15}N Calculations

Atom % ^{15}N excess in the plant or fertilizer material was determined by subtracting the percent natural abundance from the percent abundance in the enriched material. Natural abundance was determined from samples which were not enriched with ^{15}N and was very close to the currently accepted value of 0.366 (Hauck and Brenner, 1976). The atom % ^{15}N excess in the plant and fertilizer material was used to determine the nitogen derived from the fertilizer (Ndff). This value was expressed as a percent of the nitrogen fertilizer which was originally added:

$$\%Ndff = \frac{\%\,^{15}N \text{ excess in the plant material}}{\%\,^{15}N \text{ excess in the original fertilizer}} \times 100$$

The %Ndff, dry matter yield and nitrogen content were used to calculate the fertilizer nitrogen taken up by the plant:

Fertilizer N taken up by the plant (KgN/ha) = Yield (Kg/ha) x total N (%) x Ndff

From this calculation the percent utilization of fertilizer was calculated by:

$$\% \text{ Utilization of Fertilizer} = \frac{\text{Fertilizer N in the plant}}{\text{Amount of fertilizer added}} \times 100$$

3.4.4 Ethylene Analysis

Ethylene in vacutainers was analyzed using a Varian aerograph model 1200 gas chromatograph and a Varian chart recorder. The column was packed with Poropak T. Calibrating gas[1], which contained 1000 ppm ethylene with a balance of helium, was used as a standard to obtain standard curves to which the field samples could be compared. One milliliter of sample was removed from the vacutainer and injected into the gas chromatograph using Gastight[2] syringes. Micromoles of ethylene were determined using the standard curves. The chromatograph settings were as follows: injector temperature = 120°C, detector temperature = 160°C, oven temperature = 90°C, hydrogen flow rate = 21 ml/minute, oxygen flow rate = 103 ml/minute, nitrogen flow rate = 21 ml/min.

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[1] Supplier: Applied Science Laboratories, P.O. Box 440, State College, Pennsylvania, 16801.

[2] Supplier: Chromatographic Specialties, 300 Laurier Blvd., Brockville, Ontario.

4. RESULTS AND DISCUSSION

4.1 Field Experiments (1979)

4.1.1 Soils. Field experiments were located in the Morden-Winkler area on the soil types indicated in the methods and materials section of this manuscript. Chemical and physical properties of the soils used in these experiments are outlined in Table 5. Soil NO_3-N (0-60 cm), according to provincial soil testing guidelines (Manitoba Soil Fertility Advisory Committee, 1977), was very low in the Hockfeld FSL and Reinland FSL and was high in the Neuenberg VFSL. NO_3-N levels from 60-120 cm in the Reinland FSL were substantially higher than levels closer to the soil surface and this could have influenced plant growth once roots were deep enough to utilize the nitrogen from this source. Initial soil nitrate levels are important in light of the role of nitrogen in these experiments.

4.1.2 The Effect of Nitrogen Addition on Yield and Protein Content of Maple Presto Soybeans. Climatic conditions in 1979 were generally favourable for growing soybeans. Yield and protein content of Maple Presto soybeans were evaluated during the 1979 growing season as well as at maturity. Supplemental nitrogen was found to be an important factor in attaining high dry matter yields for this crop (Table 6). Significant dry matter yield increases at 70 days after seeding occurred with additions of 100 kg N/ha to the nodulating soybeans. Nikkel was the only site where additions of 30 kg N/ha significantly increased the yield of dry matter although non-significant increases occurred at the other sites.

Non-nodulating soybeans also showed significant increases in the yield of dry matter with applications of 30 and 100 kg N/ha on soils

TABLE 5

CHARACTERISTICS OF SOILS IN THE 1979 FIELD EXPERIMENTS

Site Characteristics	Nikkel	Toews	Enns
Legal Description	NE 30-3-5W	NW 15-3-5W	NE 29-3-4W
Soil Series	Neuenberg	Hockfeld	Reinland
Subgroup	gleyed carbonated Rego Black	Orthic Black	gleyed Carbonated Rego Black
Textural Class	very fine sandy loam	fine sandy loam	fine sandy loam
pH (0-15 cm)	8.2	7.2	7.4
% organic matter (0-15 cm)	2.5	2.3	2.5
Conductivity (0-15 cm)	0.26	0.17	0.16
(ds/m) (15-30 cm)	0.29	0.23	0.17
% $CaCO_3$ (0-15 cm)	2.3	0.6	1.2
equivalent (15-30 cm)	7.3	3.7	3.7
NO_3-N (0-60 cm)	48.3	13.3	13.6
(kgN/ha) (60-120 cm)	20.1	11.0	36.0
$NaHCO_3$ extractable PO_4-P (kgP/ha) (0-15 cm)	17.2	27.7	29.7
NH_4OAC extractable K (kgK/ha) (0-15 cm)	174.7	262.0	250.3
$CaCl_2$ extractable SO_4-S (kgS/ha) (0-60 cm)	140.69	15.33	17.72
DTPA extractable Zn (ppm) (0-15 cm)	0.88	0.71	0.98
DTPA extractable Cu (ppm) (0-15 cm)	0.57	0.43	0.46
DTPA extractable Mn (ppm) (0-15 cm)	30.1	13.1	16.0

TABLE 6

EFFECT OF NITROGEN APPLICATION ON YIELD OF DRY MATTER FROM AERIAL PORTIONS OF SOYBEANS

	Nitrogen Applied (kgN/ha)	Yield of Dry Matter (kg/ha)(1)		
		Enns	Toews	Nikkel
Non Nodulating Soybeans	0	3165 a*	2336 a	3497 ab
	30	4297 cd	4149 b	3776 abc
	100	4482 cd	4824 b	4122 bc
Nodulating Soybeans	0	3391 ab	3987 b	3011 a
	30	4132 bcd	4821 b	4108 bc
	100	4747 d	6558 c	4603 c
Uninoculated Soybeans	30	4118 bcd	4609 b	3941 abc
Fababeans	30	3708 abc	4168 b	3631 abc

* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

(1) Dry matter was sampled when the Maple Presto soybeans were at 5% leaf senescence (70 days).

which were low in NO_3-N (Enns and Toews). Non nodulating soybeans at Nikkel were not observed to respond significantly to nitrogen addition. This observation clarifies the importance of reflecting back on soil nitrate nitrogen levels when response to nitrogen fertilizer application is being considered. Non-nodulating plants at the Nikkel site had more soil N to feed from than plants at the other two sites and therefore yield increases as a result of supplemental nitrogen addition were smaller at this site. Alternately, the non-nodulating soybeans at the Toews site exhibited a large increase in dry matter as a result of nitrogen fertilizer addition. The low dry matter yield observed when no nitrogen was applied indicated the severe soil nitrogen deficiency at this site.

Dry matter yields of the uninoculated soybeans with fertilizer additions of 30 kg N/ha were very similar to those observed for the nodulating soybeans with the same fertilizer application. This indicated that dry matter accumulation in the nodulating soybeans was not increased as a result of symbiotic fixation at this rate of fertilizer application.

Fababeans were at the early pod fill stage of development when aerial dry matter yields were taken. The dry matter yields obtained were similar to those found by Richards (1977) in field experiments with the same variety sampled at the same developmental stage. It was hoped that a comparison of dry matter yields among different years along with comparisions of symbiotic nitrogen fixation would indicate how 1979 compared with other years in terms of growth and fixation. These observations could then be loosely applied to growth and fixation of Maple Presto soybeans in 1979 as compared to what might be expected in other years.

Highly significant increases in nodulating soybean seed yields were observed with nitrogen additions of 30 kgN/ha (table 7). Further seed yield increases were observed where 100 kgN/ha was applied and these were highly significant in comparison to both the zero and 30 kgN/ha applications. This indicated that the level of supplemental nitrogen required by Maple Presto soybeans for maximum seed yield production on these soils clearly exceeded 30 kgN/ha and may have been in excess of 100 kgN/ha. Results from Hnatowich and Soper (1980) showed seed yields of Maple Presto soybeans with additons of 200 kgN/ha were very similar to yields observed with 100 kgN/ha suggesting that yields may be maximized with additions of 100 kgN/ha. Significant responses observed by Hnatowich and Soper were largely due to increases in seed size with added nitrogen.

The magnitude of seed yield response between additions of 0 and 100 kgN/ha was consistent for Enns, Toews and Nikkel, increases being in the order of 400 kg/ha. The maximum seed yield obtained was 1942 kg/ha at Toews.

Seed yield responses closely followed dry matter increases in the nodulating soybeans when nitrogen was added. The degree of significance of the response was higher for seed yield than for aerial dry matter indicating that fertilizer nitrogen addition had a greater effect on increasing seed yield than on increasing dry matter yield as a whole.

Seed and dry matter responses to added fertilizer appear to be a function of the soil NO_3-N present at seeding. Regitnig (1979) working on soils with a large supply of available nitrate nitrogen reported no significant seed yield or dry matter increase when 30 or 100 kgN/ha were added to Maple Presto soybeans. Dubetz (1979) reported on soils which

TABLE 7

EFFECT OF NITROGEN APPLICATION ON SEED YIELD OF SOYBEANS

	Nitrogen Applied (kgN/ha)	Seed Yield (kg/ha)		
		Enns	Toews	Nikkel
Non Nodulating Soybeans	0	1262 a*	604 a	1178 a
	30	1346 b	906 b	1433 c
	100	1776 e	1667 e	1698 d
Nodulating Soybeans	0	1500 c	1539 d	1299 b
	30	1592 d	1733 f	1411 c
	100	1874 f	1942 g	1753 d
Uninoculated Soybeans	30	1417 b	1332 c	1315 b

* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.01

had 29 ppm NO_3-N down to 30 cm which was higher than levels observed at the sites discussed in this manuscript. He found that seed yield and plant height were significantly increased with increasing increments of 40, 80 and 120 kgN/ha on irrigated land in southern Alberta. It can be concluded that at soil NO_3-N levels at or below those observed in this manuscript (48KgN/ha, to 60 cm) and row spacing less than or equal to 61 cm the addition of nitrogen fertilizer at rates equal to or exceeding 30 kgN/ha will result in highly significant seed yield responses in Maple Presto soybeans. Yield responses to fertilizer addition may occur at soil nitrate levels above those in this manuscript but will diminish when large amounts of soil nitrate nitrogen are present.

The non-nodulating soybeans also exhibited a highly significant seed yield response to nitrogen additions of 30 kgN/ha and 100 kgN/ha at all field locations. The 100 kgN/ha fertilizer addition resulted in seed yields which displayed highly significant increases over the 30 kgN/ha treatment as well as the zero treatment. Response to nitrogen addition was especially pronounced at Toews and is an indication of a severe nitrogen deficiency. Comparison of seed yields among sites indicated that 100 kgN/ha of supplemental nitrogen increased yields at Toews to the levels observed at the other sites with the same nitrogen additions. This suggests that non-nodulating soybeans at Toews utilized applied fertilizer N more efficiently to increase yields than the other sites or that nitrogen application stimulated soil nitrogen uptake to a proportionately greater degree at this site or finally that this was the maximum yield response which could be realized through nitrogen addition.

Uninoculated soybeans with 30 kg/ha of added nitrogen fertilizer were observed to have lower seed yields than inoculated soybeans with the same rate of fertilizer at all study sites. This uninoculated soybean treatment was also observed to have lower seed yields than inoculated soybeans which had no nitrogen addition at Enns and Toews. Uninoculated soybeans were assumed to be void of any nodulation and this was verified by periodic examination of randomly selected roots. This implies that a significant amount of nitrogen was supplied to the nodulating plants for seed yield as a result of symbiotic fixation. This was not true of dry matter yields sampled at early leaf senescence. As discussed previously, comparisons between uninoculated soybeans and nodulating soybeans with the same rate of fertilizer application displayed similar dry matter yields for both treatments. This would indicate that symbiotically fixed nitrogen is utilized more specifically in seed yield production than in the accumulation of dry matter as a whole. Similar comparisons made between nodulating and non-nodulating soybeans with 0 and 30 kgN/ha corroborated this conclusion with the exception of the zero treatment at Toews which was under large enough nitrogen stress that symbiotic fixation in the nodulating soybeans significantly increased both seed and dry matter yield over that in the non-nodulating soybeans. In light of these considerations it is apparent that symbiotically fixed nitrogen plays an important role in increasing seed yield production on soils with low amounts of available nitrogen even when small amounts (30 kgN/ha) of supplementary fertilizer nitrogen are added at seeding.

Dry matter measurements were conducted at six stages throughout the growing season for the nodulating soybeans (Table 8, Figures 1-3). The increases observed as a function of fertilizer application generally followed those observed for final seed yield and dry matter yield at seventy days after seeding. Dry matter was significantly increased with the addition of 100 kgN/ha at all experimental sites. The occurence of this response tended to take place earlier on sites which had lower amounts of soil NO_3-N. Soybeans at Toews and Enns therefore exhibited a significant dry matter response to additions of 100 kgN/ha at early flowering and flowering respectively whereas soybeans at Nikkel did not respond significantly until pod filling. Significant dry matter increases were also observed with the addition of 30 kgN/ha at Enns and Toews beginning at the early flowering stage of development. Significant increases in dry matter yield between the 30 and 100 kgN/ha treatment only occured at Toews and this was likely a function of the severe nitrogen stress at this site.

The maximum rate of dry matter accumulation occurred between 50 and 61 days after seeding for all nodulating soybean treatments at each experimental site. This coincided with the early pod fill stage of development. The maximum growth rates for the 0, 30 and 100 kgN/ha treatments were 145, 192 and 263 kg plant material/ha/day, respectively and occurred at the Toews site. Although significant yield differences occurred early in the growing season between treatments with applications of 0, 30 and 100 kgN/ha, the largest differences in yield were observed at early pod fill and were maintained to seventy days after seeding at all sites. The degree of response at this stage also appeared to be a function of the soil NO_3-N content. Soybeans at Toews showed

TABLE 8

EFFECT OF NITROGEN APPLICATON ON SOYBEAN DRY MATTER YIELD THROUGHOUT THE GROWING SEASON

Location and Nitrogen Rates (kgN/ha)		kg/ha of Dry Matter at Different Sampling Times (days after seeding)					
		26 (1)	36	43	50	61	70
Enns	0	158 a*	354 a	783 a	1264 a	2535 a	3391 a
	30	166 a	638 b	1051 b	2013 ab	3259 b	4132 ab
	100	190 a	441 a	983 b	2032 b	3562 b	4747 b
Toews	0	197 a	442 a	796 a	1516 a	3112 a	3987 a
	30	207 a	680 b	1022 a	2151 b	4264 b	4820 b
	100	231 a	1001 c	1432 b	2742 c	5636 c	6558 c
Nikkel	0	152 a	425 a	824 a	1443 a	2424 a	3011 a
	30	170 a	476 a	917 a	2009 a	3359 b	4108 ab
	100	206 a	561 a	901 a	1932 a	3290 b	4603 b

* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

(1) Developmental Stages: (Statistical determinations were done separately for each site and harvest)
26 = 4-5 trifoliate leaf stage, 36 = early flowering, 43 = flowering, 50 = early pod formation, 61 = pod filling, 70 = early leaf senescence.

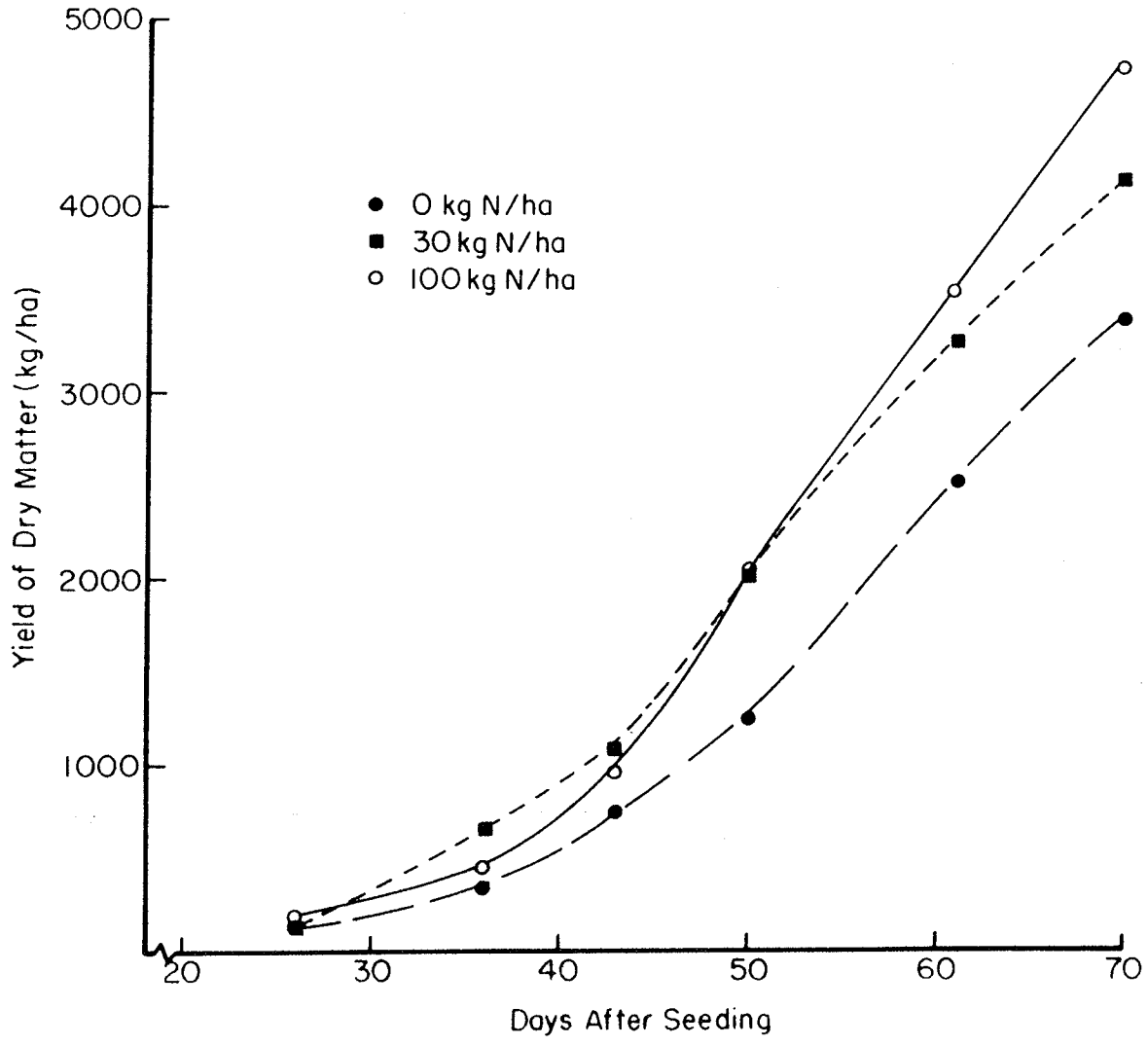

Figure 1. Effect of nitrogen application on soybean dry matter yield throughout the growing season (Enns).

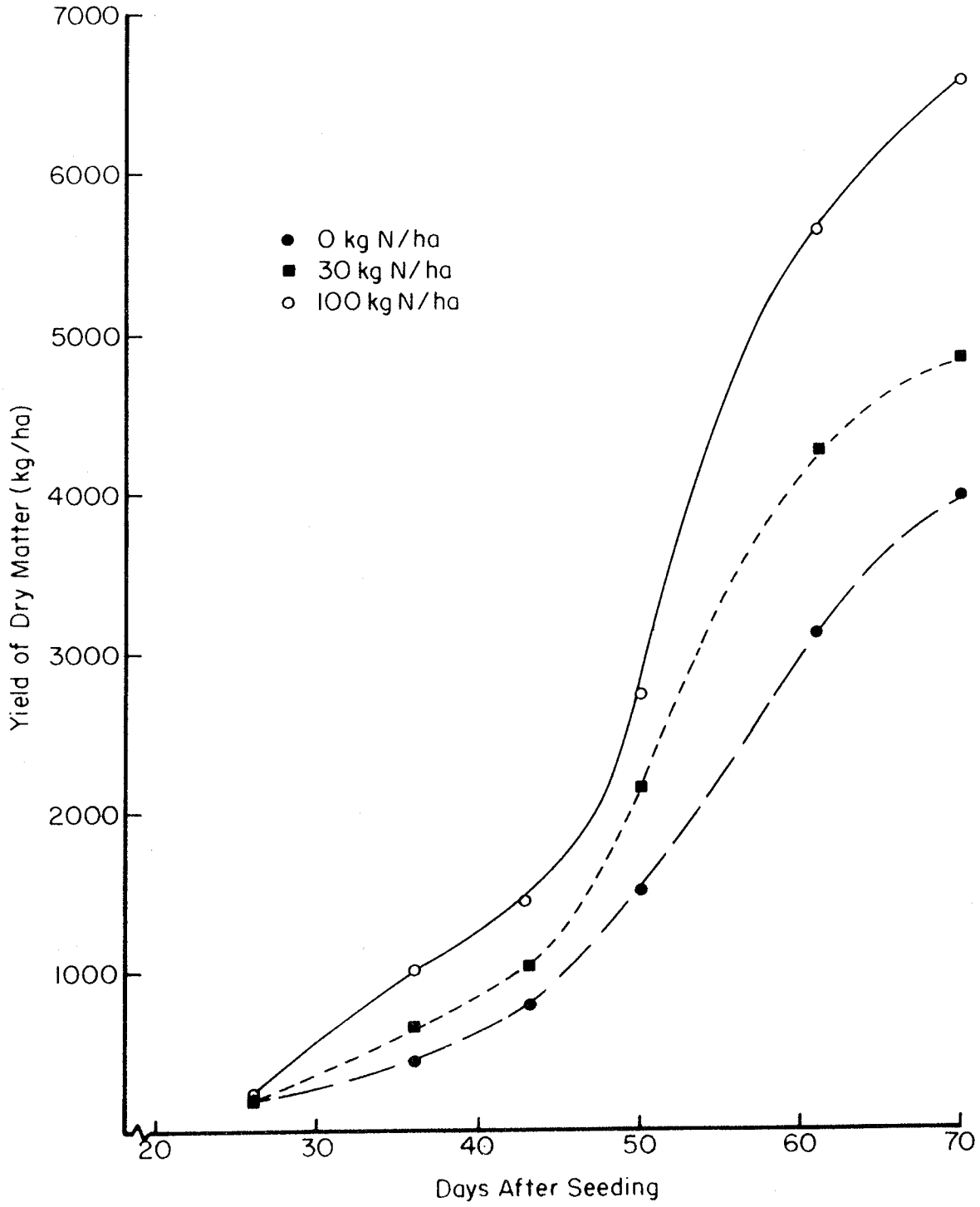

Figure 2. Effect of nitrogen application on soybean dry matter yield throughout the growing season (Toews).

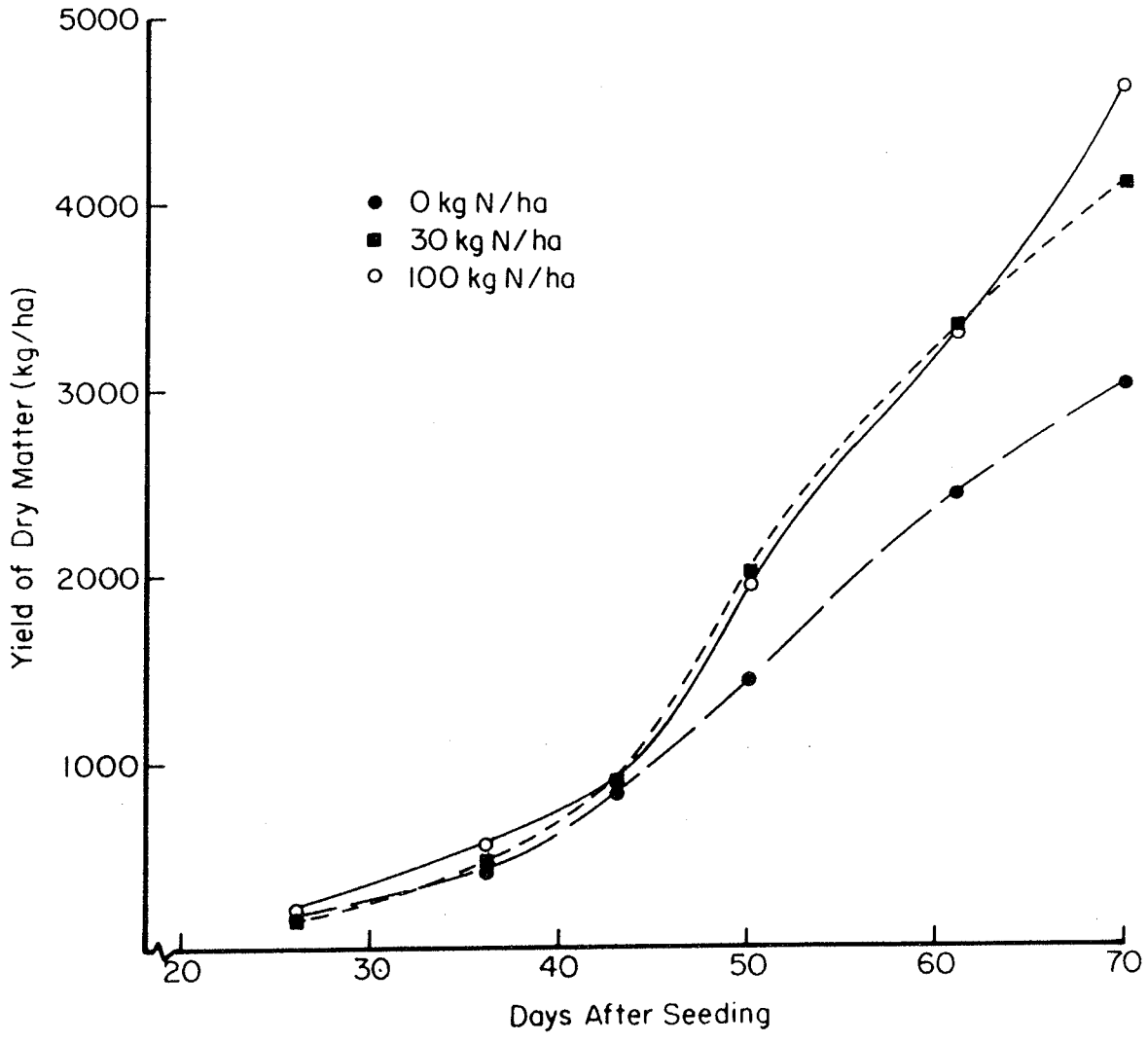

Figure 3. Effect of nitrogen application on soybean dry matter yield throughout the growing season (Nikkel).

greater differences in yield in response to applied nitrogen than plants at Enns or Nikkel.

Greater dry matter response to applied fertilizer at Toews relative to the other two sites did not appear to have a large influence on seed yield responses among the sites. Seed yield responses to applied fertilizer were highly significant at all sites. These results suggest that the time of initiation of a dry matter response or the degree of difference in dry matter yield are related to observed seed yield responses in Maple Presto soybeans but are not conclusive in determining the extent of seed yield response.

Seed protein responses were observed with added nitrogen but these responses were not as dramatic or as frequent as seed yield responses (Table 9). Nikkel was the only site which showed a significant seed protein increase with added nitrogen for the nodulating soybeans. This increase occurred with an application of 100 kgN/ha and was significantly greater than either the zero or 30 kgN/ha treatment. An application of 100 kgN/ha therefore supplied enough nitrogen for the nodulating soybeans to realize a significant seed yield and protein increase at this site. Nikkel was the only site with enough available nitrogen from soil and symbiotically fixed sources to support both a yield and protein increase for nodulating soybeans when supplemental nitrogen was added. Protein responses to added nitrogen in nodulating soybeans did not appear on sites where the soil was low in NO_3-N. Plants at these sites utilized all the added nitrogen for increasing seed yield.

These results are corroborated by those of Regitnig (1979) and Dubetz (1979). On a soil which was very high in NO_3-N Regitnig reported

TABLE 9

EFFECT OF NITROGEN APPLICATION ON PROTEIN CONTENT OF SOYBEAN SEED

	Nitrogen Applied (kgN/ha)	Percent Protein		
		Enns	Toews	Nikkel
Non-Nodulating Soybeans	0	30.8 a*	26.6 a	30.4 a
	30	29.8 a	27.1 ab	31.3 a
	100	35.4 b	31.4 c	36.0 c
Nodulating Soybeans	0	37.2 b	33.6 d	34.5 c
	30	35.3 b	32.8 cd	34.7 c
	100	37.0 b	34.3 d	40.4 d
Uninoculated Soybeans	30	32.7 a	28.6 b	33.0 b

* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

protein responses of Maple Presto soybeans to nitrogen additions of 100 kgN/ha. Likewise, Dubetz found significant protein responses to nitrogen applications from 40 to 120 kgN/ha on soils relatively high in NO_3-N. Hnatowich et al. (1981) working with the early maturing Maple Amber variety of soybeans found significant yield and protein increases with nitrogen additions of up to 200 kgN/ha on a soil very high in NO_3-N. It appears that there is a large potential for increasing yield and/or protein content in these early maturing varieties of soybeans based on the fertilizer rates which have effected responses in these parameters.

Nitrogen studies by Regitnig (1979), Dubetz (1979) and those reported in this manuscript indicate maximum protein levels of 36 to 40% for Maple Presto soybeans. These values would be at or just below acceptable levels required for soybean crushing plants in southern Manitoba. Acceptable levels have been indicated at 39 to 40 percent protein for whole beans at zero percent moisture (Gwyer, personal communication). Reports from Manitoba indicate that maintaining consistently high protein content in soybean seed has been a problem (Redekop, 1980; Gwyer, personal communication). Observations of low protein in soybeans with no added nitrogen and in soybeans with low rates of added nitrogen at all sites in this study are consistent with these reports.

The protein content of nodulating soybeans with no applied nitrogen or with nitrogen applications of 30 kgN/ha was observed to be significantly increased over that of an uninoculated treatment with 30 kgN/ha applied nitrogen. This indicated that both these nodulating treatments supplied enough nitrogen through symbiotic fixation to realize a significant protein response. In the case of the treatment to which no

fertilizer was applied, the amount of nitrogen supplied through symbiotic fixation for seed protein was larger in terms of its effect than 30 kgN/ha fertilizer nitrogen applied at seeding. These results show that symbiotic nitrogen fixation had an effect on the protein content of Maple Presto soybeans and could be said to be important in reaching the goal of obtaining soybeans with an acceptable level of seed protein for crushing.

Significant seed protein responses were observed for the non-nodulating soybeans with an application of 100 kgN/ha. This increase was greater than both the zero and the 30 kgN/ha treatment. As with the nodulating soybeans, although highly significant seed yield increases were observed with 30 kgN/ha applications, protein responses were not observed. Like yield responses, seed protein responses of the non-nodulating soybeans reflected the initial soil NO_3-N status. The protein content of non-nodulating soybeans at Toews was substantially reduced from that of the other two sites, particularily where no nitrogen was applied. The effect of this nitrogen stress was not eliminated even at nitrogen applications of 100 kgN/ha. This reduction of protein with low soil NO_3-N was also observed when comparing uninoculated soybean treatments between sites.

Protein measurements throughout the season showed an initial response to nitrogen addition at all sites (Table 10). This response occurred in the absence of significant increases in dry matter yield at the same growth stage. As the season progressed plants accumulated dry matter at an increased rate and as significant differences in dry matter appeared initial differences in plant protein diminished. Plant protein

TABLE 10

EFFECT OF NITROGEN APPLICATON ON SOYBEAN PROTEIN CONTENT THROUGHOUT THE GROWING SEASON

Location and Nitrogen Rates (kgN/ha)		Percent Protein at Different Sampling Times (days after seeding)					
		26 (1)	36	43	50	61	70
Enns	0	13.6 a*	17.8 a	17.3 a	18.5 a	17.8 ab	16.9 a
	30	18.2 b	21.6 b	19.8 ab	17.9 a	16.3 a	14.4 a
	100	22.0 c	25.7 c	22.6 b	21.1 b	19.2 b	15.1 a
Toews	0	13.8 a	15.3 a	17.0 a	14.3 a	13.9 a	12.0 a
	30	18.9 b	20.6 b	20.9 b	15.2 a	12.7 a	11.9 a
	100	22.7 c	23.3 c	24.1 c	20.1 b	13.6 a	11.2 a
Nikkel	0	16.3 a	15.8 a	17.2 a	14.7 a	14.7 a	14.4 a
	30	20.2 b	19.9 b	22.2 b	17.1 b	17.7 a	15.7 ab
	100	21.1 b	23.8 c	24.7 c	22.0 c	17.8 a	17.6 b

* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05 (Statistical determinations were done separately for each site and harvest).

(1) Developmental Stages: 26 = 4-5 trifoliate leaf stage, 36 = early flowering, 43 = flowering, 50 = early pod formation, 61 = pod filling, 70 = early leaf senescence.

decreased to the greatest degree as the plants entered the rapid growth phase between flowering and early leaf senescence. These results contrast observations for later maturing varieties by Hanway and Weber (1971) who reported a decrease in total plant protein content from early in the season to the early pod fill stage of development and an increase from that stage to maturity. This was the result of an increase in nitrogen concentration in the seeds since all other plant parts decreased in protein content to maturity. Soybeans at Nikkel were the only plants which maintained a significant protein increase with added nitrogen to the early leaf senescence stage of development. This was likely a result of the greater available soil nitrogen at this site which allowed for less protein dilution as dry matter increased.

Visual differences in color and size of the nodulating and non-nodulating soybeans were observed throughout the growing season. These differences corresponded to the yield and protein responses which were discussed. Increases in the plant size of Maple Presto soybeans was observed between treatments where either no nitrogen or inoculum had been applied and where nitrogen had been applied at 30 and 100 kgN/ha rates. Increases in plant height of the 100 kgN/ha treatment over the 30 kgN/ha treatment was only observed where soil nitrogen stress was more pronounced (Toews site). Chlorosis of the leaves of Maple Presto soybeans was observed in inoculated treatments with no nitrogen application or uninoculated treatments with additions of 30 kgN/ha. Nodulating soybeans with additions of 30 and 100 kgN/ha appeared to maintain healthy green leaves. Chlorosis of leaves was in evidence 26 days after seeding for plants at Enns and Toews and was especially pronounced at Toews. Differences in plant height began to appear soon after this.

Visual responses in plant height and leaf color were observed in plants at Nikkel 43 days after seeding but were not as great as those at Enns and Toews.

Differences in leaf senescence were also observed at all experimental sites. Uninoculated Maple Presto soybeans senesced earlier than the nodulated treatments. Nodulated treatments were observed to senesce earlier when no nitrogen was applied than when 30 or 100 kgN/ha were applied. This same response to applied fertilizer was even more pronounced in the non-nodulating soybeans. At all sites it appeared that maturity of the soybeans was extended with the addition of nitrogen and this was expecially true where there was greater soil NO_3-N deficiency.

4.1.3 THE EFFECT OF NITROGEN ADDITION ON NITROGEN UPTAKE INTO MAPLE PRESTO SOYBEANS

Nitrogen uptake into above ground portions of soybeans at early leaf senescence was significantly affected by nitrogen fertilizer addition (Table 11). Nitrogen uptake of nodulating soybeans was shown to be significantly increased with the addition of 100 kgN/ha. This was also observed when 100 kgN/ha was added to the non-nodulating soybeans. The non-nodulating soybeans were found to respond to additions of 30 kgN/ha at Enns and Toews. Uninoculated soybeans with 30 kg/ha of applied nitrogen had significantly lower nitrogen uptake than nodulated soybeans with the same fertilizer application at Toews. Symbiotic fixation, therefore, appears to be supplying nitrogen for plant growth in this treatment. Symbiotic fixation appears to be supplying less nitrogen to the nodulating soybeans in this treatment at the other sites as evidenced by the absence of any significant difference in nitrogen uptake between the nodulating and uninoculated soybeans. These results showed that both nodulating and non-nodulating soybeans required fertilizer nitrogen addition on these soils, if high nitrogen uptake is desired.

Comparisons between treatments of non-nodulating soybeans and uninoculated soybeans with 30 kgN/ha revealed no significant differences in nitrogen uptake. This indicates along with visual observations that the uninoculated soybeans were in fact not nodulated, as nodulation theoretically would result in differences in aerial nitrogen uptake between the treatments. Either treatment could, therefore, be used to measure soil nitrogen available to the nodulated soybeans.

The total nitrogen in the seed of nodulating and non-nodulating soybeans followed increases observed for aerial dry matter with added

TABLE 11

EFFECT OF NITROGEN APPLICATION ON TOTAL N UPTAKE OF AERIAL PORTIONS OF SOYBEANS

	Nitrogen Applied (kgN/ha)	Nitrogen Uptake (kg/ha)		
		Enns	Toews	Nikkel
Non Nodulating Soybeans	0	45 a	26 a	54 a
	30	73 b	50 b	66 a
	100	119 c	103 e	101 b
Nodulating Soybeans	0	92 b	77 cd	71 ab
	30	94 b	94 de	86 ab
	100	130 c	140 f	140 c
Uninoculated Soybeans	30	83 b	64 bc	78 ab

* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

nitrogen (Table 12). It was apparent that increases in nitrogen uptake of nodulating soybean seed resulted to a larger degree in increased seed yield rather than increased nitrogen concentration in the seed, as indicated by previously discussed yield and protein measurements.

Significant increases in seed nitrogen uptake of the nodulating soybeans did not occur with 30 kg/ha added nitrogen. Highly significant seed yield increases observed with these additions were, therefore, not a direct result of acquiring significantly more nitrogen in the seed portions of the plants. The corollary to this is that the protein content in the seed should have decreased at this rate of nitrogen application. Although, significant reductions do not occur in protein content at this rate (Table 9) trends indicate that a slight dilution took place in seeds from Enns and Toews. With applications of 100 kgN/ha at these two sites, protein content was maintained at the level of the zero treatment while seed yield increased, accounting for the increased nitrogen uptake observed. Only in plants at Nikkel was increased nitrogen uptake found to result in both yield and protein increases. Seed yield, therefore, approached optimum levels before protein was increased as a result of added nitrogen. This is supported by Regitnig (1979) who reported protein increases with added nitrogen on a high nitrogen soil where yields appeared to be maximized. Contrary to these results Dubetz (1979) reported a concurrent stepwise increase of both yield and protein as nitrogen was added.

Significant increases in total N uptake of aerial portions of the nodulating soybeans were observed throughout the growing season with applications of 100 kgN/ha (Table 13). In some cases applications of 30 kgN/ha significantly increased the total nitrogen in the plant but this

TABLE 12

EFFECT OF NITROGEN APPLICATION ON TOTAL N UPTAKE OF SOYBEAN SEED

	Nitrogen Applied (kgN/ha)	Nitrogen Uptake (kg/ha)		
		Enns	Toews	Nikkel
Non-Nodulating Soybeans	0	63 a*	26 a	58 a
	30	65 a	40 b	72 a
	100	101 cd	84 d	98 bc
Nodulating Soybeans	0	89 bc	83 d	72 a
	30	90 bc	91 d	78 ab
	100	111 d	107 e	114 c
Uninoculated Soybeans	30	74 ab	61 c	70 a

* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

TABLE 13

EFFECT OF NITROGEN APPLICATON ON TOTAL N UPTAKE OF AERIAL PORTIONS OF SOYBEANS THROUGHOUT THE GROWING SEASON

Location and Nitrogen Rates (kgN/ha)		Nitrogen Uptake at Different Sampling Times - kg/ha (days after seeding)					
		26 (1)	36	43	50	61	70
Enns	0	4 a*	10 a	22 a	38 a	72 a	92 a
	30	5 a	22 b	34 b	58 b	86 a	94 a
	100	7 b	18 b	36 b	69 c	113 b	130 b
Toews	0	4 a	11 a	22 a	34 a	69 a	77 a
	30	6 b	22 b	34 b	48 a	87 a	94 a
	100	9 c	38 c	55 c	88 b	123 b	140 b
Nikkel	0	4 a	11 a	23 a	34 a	55 a	71 a
	30	5 ab	15 ab	33 b	55 ab	90 a	86 a
	100	7 b	21 b	36 b	67 b	95 a	140 b

* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05 (Statistical determinations were done separately for each site and harvest).

(1) Developmental Stages: 26 = 4-5 trifoliate leaf stage, 36 = early flowering, 43 = flowering, 50 = early pod formation, 61 = pod filling, 70 = early leaf senescence.

was not as consistent a response and did not appear after the initiation of pod filling. There was a distinct increase in nitrogen uptake between the 43 and 61 day sampling periods which correspond to the stage between flowering and early pod fill (Figures 4-6). This agreed with results reported by Hanway and Weber (1971) who stated that nitrogen accumulation closely followed dry matter accumulation and that maximum uptake occurred between full bloom and late pod filling. Small and Ohlrogge (1973) suggested that high soybean grain yields are associated with the nitrogen nutrition of the plant at the pod filling stage of development.

The data shows that the total accumulation of nitrogen was below the maximum uptake capacity of Maple Presto soybeans at all periods during the growing season when no nitrogen fertilizer was added. It was also evident that by early leaf senescence plants at all sites were below their maximum uptake capacity with applications of 30 kgN/ha as indicated by the significant increases in uptake between the 30 and 100 kgN/ha treatments. This occurred earlier in the season for plants at sites with lower soil NO_3-N, significant increases being observed between the 30 and 100 kgN/ha treatments at early pod formation at Enns and over the entire growing season at Toews. At early leaf senescence plants at Enns and Toews may have been below their maximum uptake capacity even with 100 kg/ha of applied nitrogen as evidenced by the lack of protein response at these sites with this rate of application (Table 10). It appears that sufficient nitrogen at all stages of growth was not supplied to Maple presto soybeans at Enns and Toews but may have been supplied to soybeans to which 100 kgN/ha was applied at Nikkel.

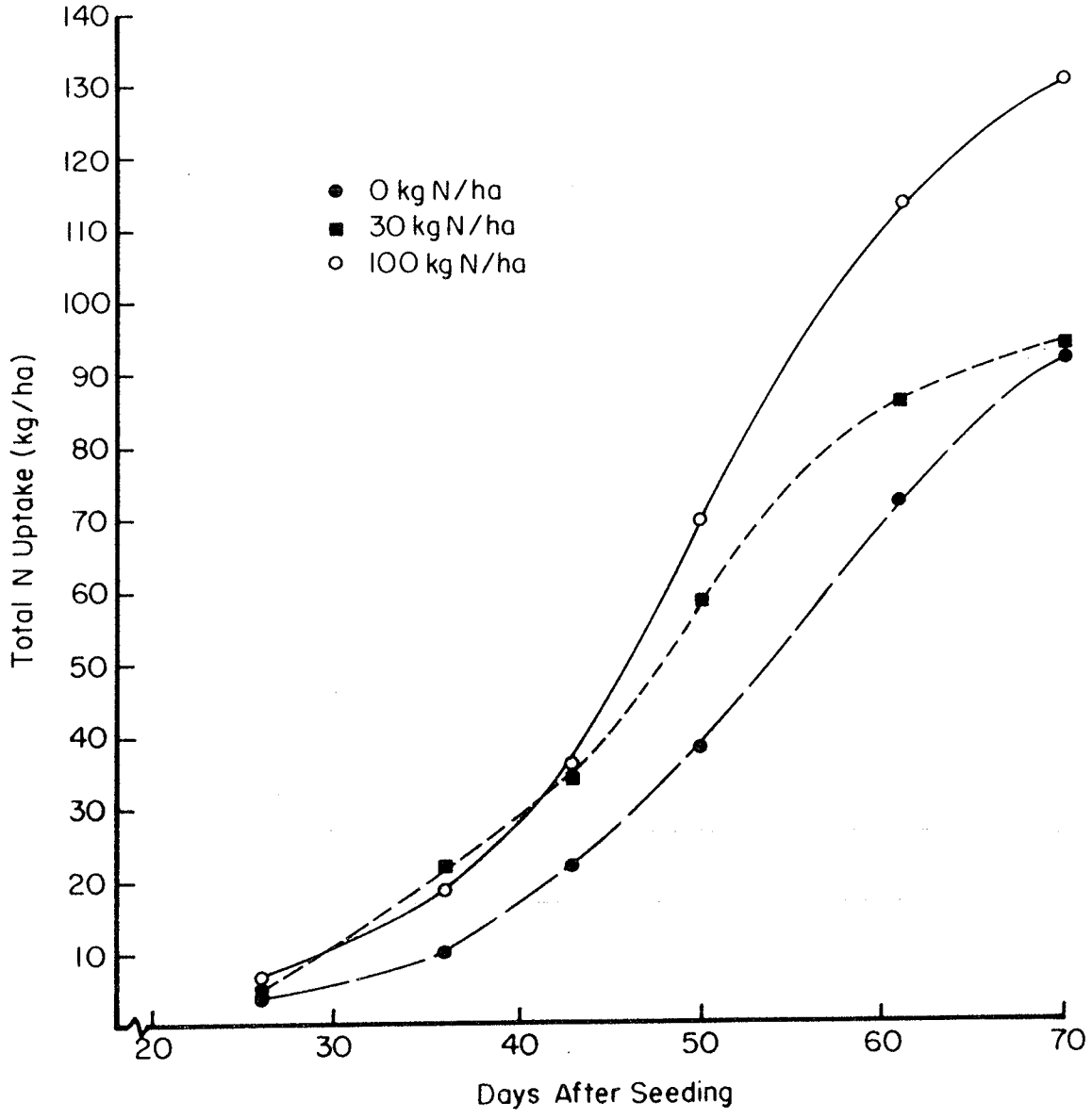

Figure 4. Effect of nitrogen application on total N uptake of aerial portions of soybeans throughout the growing season (Enns).

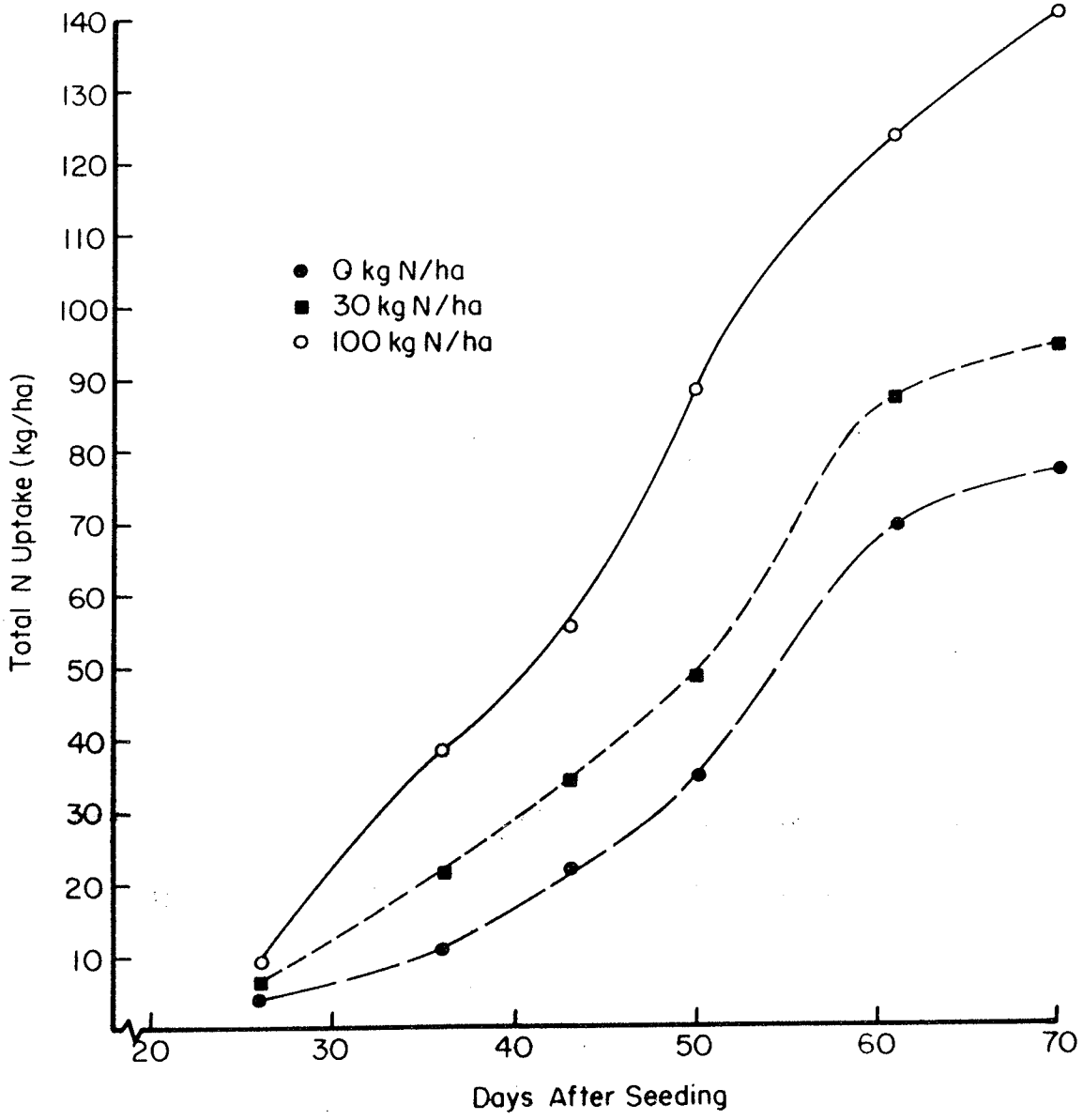

Figure 5. Effect of nitrogen application on total N uptake of aerial portions of soybeans throughout the growing season (Toews).

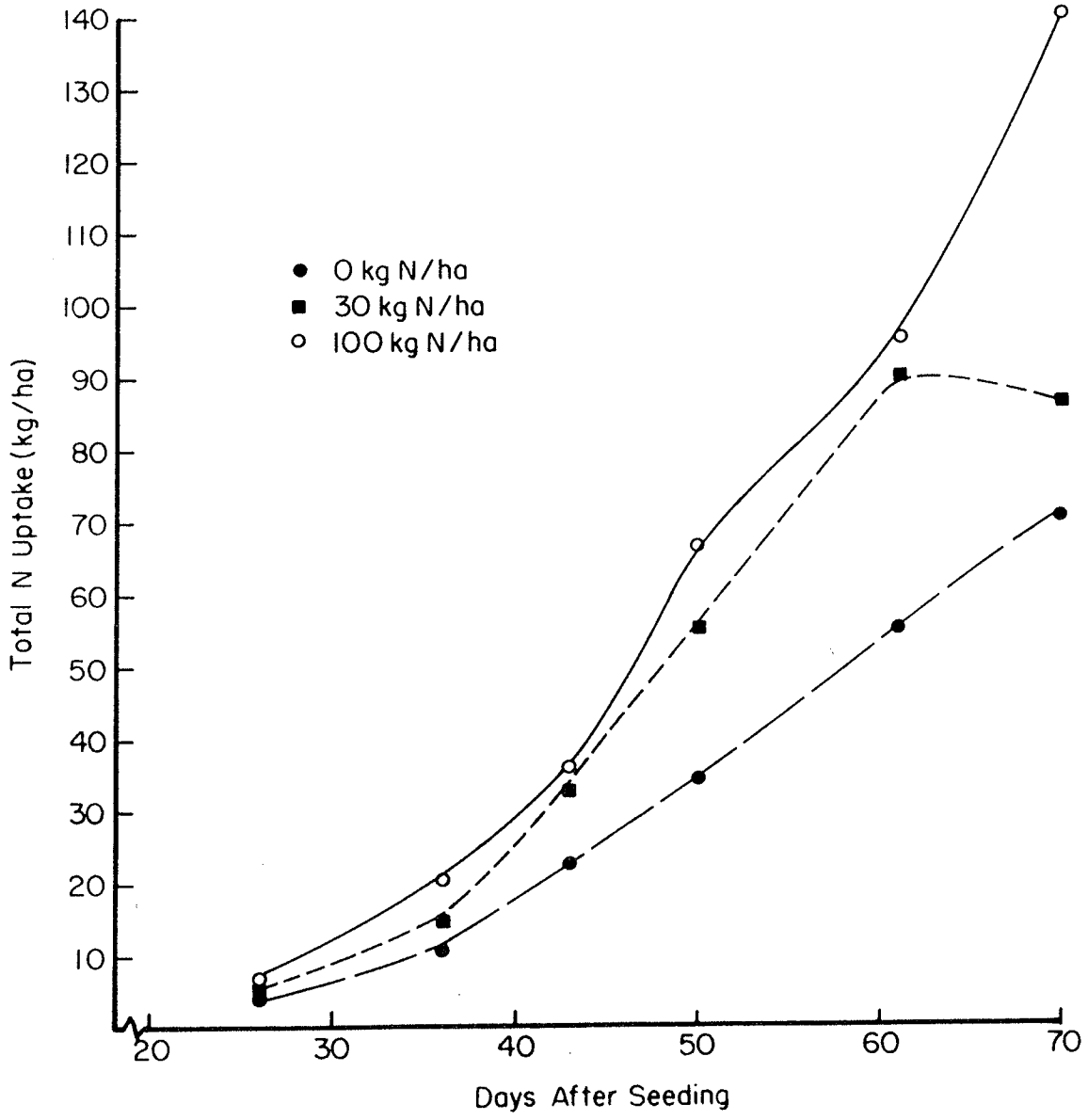

Figure 6. Effect of nitrogen application on total N uptake of aerial portions of soybeans throughout the growing season (Nikkel).

Fertilizer nitrogen uptake and percent utilization of fertilizer were determined quantitatively on plants sampled 70 days after seeding through the use of tracer ^{15}N (Table 14 and 15). Fertilizer uptake between the nodulating soybeans the non-nodulating soybeans and the fababeans was quite consistent for a given rate of fertilizer. The efficiency of fertilizer utilization was similar to values reported by Regitnig (1979), Diebert et al. (1979) and Rennie et al. (1978). All values were similar among different crops and with different fertilizer applications to the same crop. The treatments applied had no significant effect on the percent utilization of fertilizer. Diebert et al. (1979) and Rennie et al. (1978) both reported that the percent utilization of fertilizer of nodulating and non-nodulating soybean isolines grown in the field increased or remained constant with increasing increments of nitrogen. Regitnig (1979) showed decreases in percent utilization of fertilizer with increasing rates of nitrogen on a high nitrogen soil. Diebert et al. suggested that observations of increasing utilization of fertilizer with increased rates of addition in soybeans as compared to a normal decrease in utilization with cereals may result from the soybeans high demand for nitrogen during seed formation. They suggest soybeans utilize proportionately more nitrogen at later growth stages than other crops.

TABLE 14

EFFECT OF NITROGEN APPLICATION ON FERTILIZER N UPTAKE OF AERIAL PORTIONS OF SOYBEANS AND FABABEANS

	Nitrogen Applied (kgN/ha)	Fertilizer Nitrogen Uptake (kg/ha)		
		Enns	Toews	Nikkel
Non-Nodulating Soybeans	30	13 a*	15 a	16 a
	100	46 b	52 b	43 b
Nodulating Soybeans	30	15 a	17 a	19 a
	100	46 b	66 c	54 b
Uninoculated Soybeans	30	14 a	17 a	19 a
Fababeans	30	15 a	15 a	11 a

* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

TABLE 15

PERCENT UTILIZATION OF FERTILIZER

	Nitrogen Applied (kgN/ha)	Percent Utilization of Fertilizer		
		Enns	Toews	Nikkel
Non-Nodulating Soybeans	30	43 a*	51 a	52 a
	100	46 a	52 a	43 a
Nodulating Soybeans	30	50 a	57 a	63 a
	100	46 a	66 a	54 a
Uninoculated Soybeans	30	48 a	55 a	63 a
Fababeans	30	48 a	49 a	35 a

* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

4.1.4 Symbiotic Nitrogen Fixation Measurements in Maple Presto Soybeans

A number of methods were used in these experiments to evaluate symbiotic nitrogen fixation in Maple Presto soybeans. The purpose was essentially two fold in that it was desireable to evaluate the plants nitrogen fixing capability as well as evaluate the methods for determining symbiotic fixation.

Nitrogen fixation at specific times throughout the growing season was examined using the acetylene reduction technique (Table 16, Figures 7-9). This technique appeared to give a good indication of the occurence of N_2 fixation at a point in time as well as a comparison of nitrogen fixation among treatments. An examination of the micromoles of ethylene produced indicated that fixation was indeed occurring at each of the sites and that the amount of fixation was reduced by the addition of nitrogen fertilizer. Addition of 100 kgN/ha reduced the occurence of fixation to a relatively small amount at all sites examined. This reduction appeared most significant in plants at Enns although the other sites also showed significant decreases. Symbiotic fixation was lowest at Nikkel which was likely the result of higher soil nitrogen at this site. Soybeans at Enns showed relatively greater symbiotic fixation without added fertilizer than plants at Toews. An explanation for this may be that plants at Toews were in a more nitrogen deficient state prior to rhizobium infection and this had an effect on nodule formation and viability. Vincent (1965) indicated that greater supply of photosynthate to nodules as well as higher total N in vegetative plant parts promoted increased nodule formation and viability. With nitrogen fertilization N_2 fixation was comparable between plants at Enns and Toews.

TABLE 16

EFFECT OF NITROGEN APPLICATON ON THE ETHYLENE PRODUCTION OF ROOT NODULES OF SOYBEANS

Location and Nitrogen Rates (kgN/ha)		µmoles/plant-hr of Ethylene Produced at Different Sampling Times (days after seeding)					
		26 (1)	36	43	50	61	70
Enns	0	0.11 a*	2.04 a	3.01 a	5.02 a	9.57 a	5.28 a
	30	0.08 a	0.84 b	1.95 b	2.51 b	5.91 b	3.77 b
	100	0.04 a	0.04 c	0.09 c	0.10 c	0.48 c	0.65 c
Toews	0	0.12 a	1.41 a	2.43 a	3.58 a	5.18 a	4.54 a
	30	0.15 a	0.58 b	1.78 ab	3.12 a	3.17 a	4.18 a
	100	0.15 a	0.08 b	0.20 b	0.36 b	2.27 a	0.95 b
Nikkel	0	0.11 a	0.61 a	1.13 a	1.58 a	1.83 a	2.22 a
	30	0.06 a	0.22 b	0.52 ab	1.21 ab	2.05 a	0.86 ab
	100	0.05 a	0.08 b	0.07 b	0.08 b	0.16 a	0.11 b

* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05 (Statistical determinations were done separately for each site and harvest).

(1) Developmental Stages: 26 = 4-5 trifoliate leaf stage, 36 = early flowering, 43 = flowering, 50 = early pod formation, 61 = pod filling, 70 = early leaf senescence.

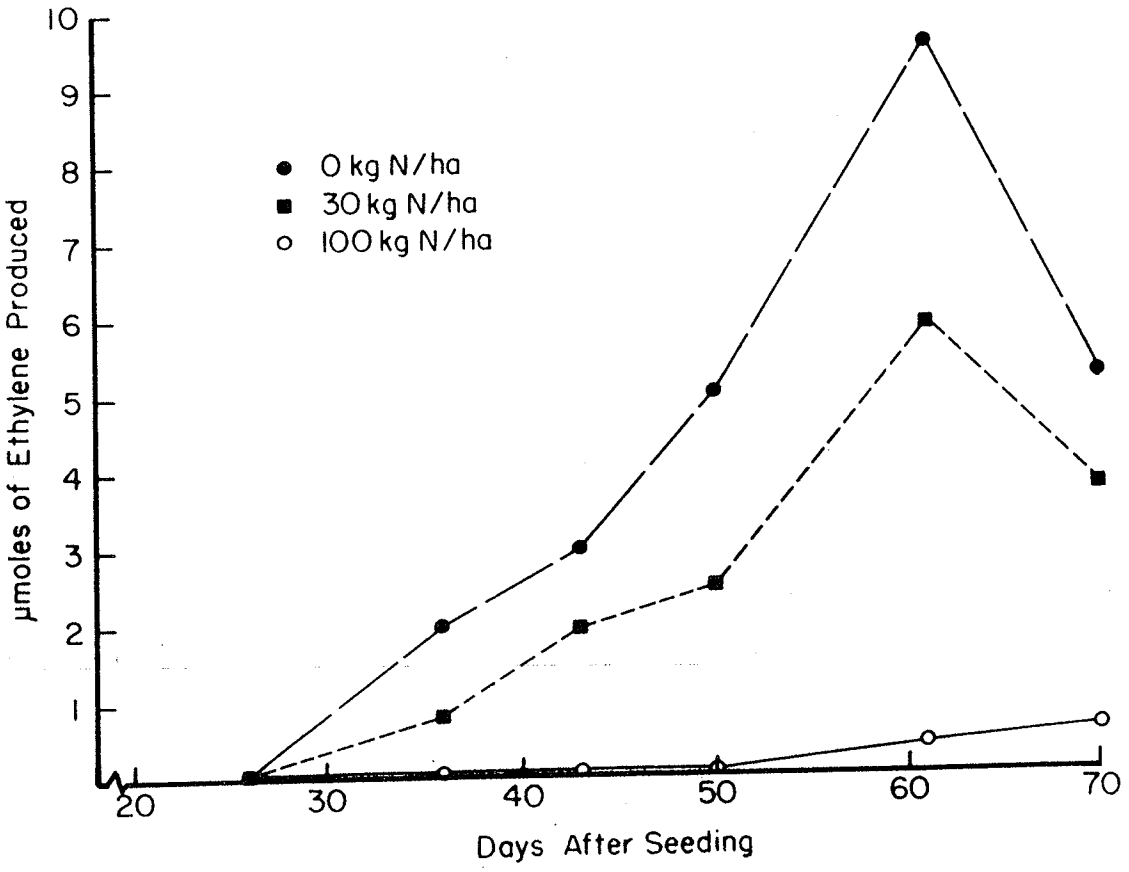

Figure 7. Effect of nitrogen application on the ethylene production of root nodules of soybeans (Enns).

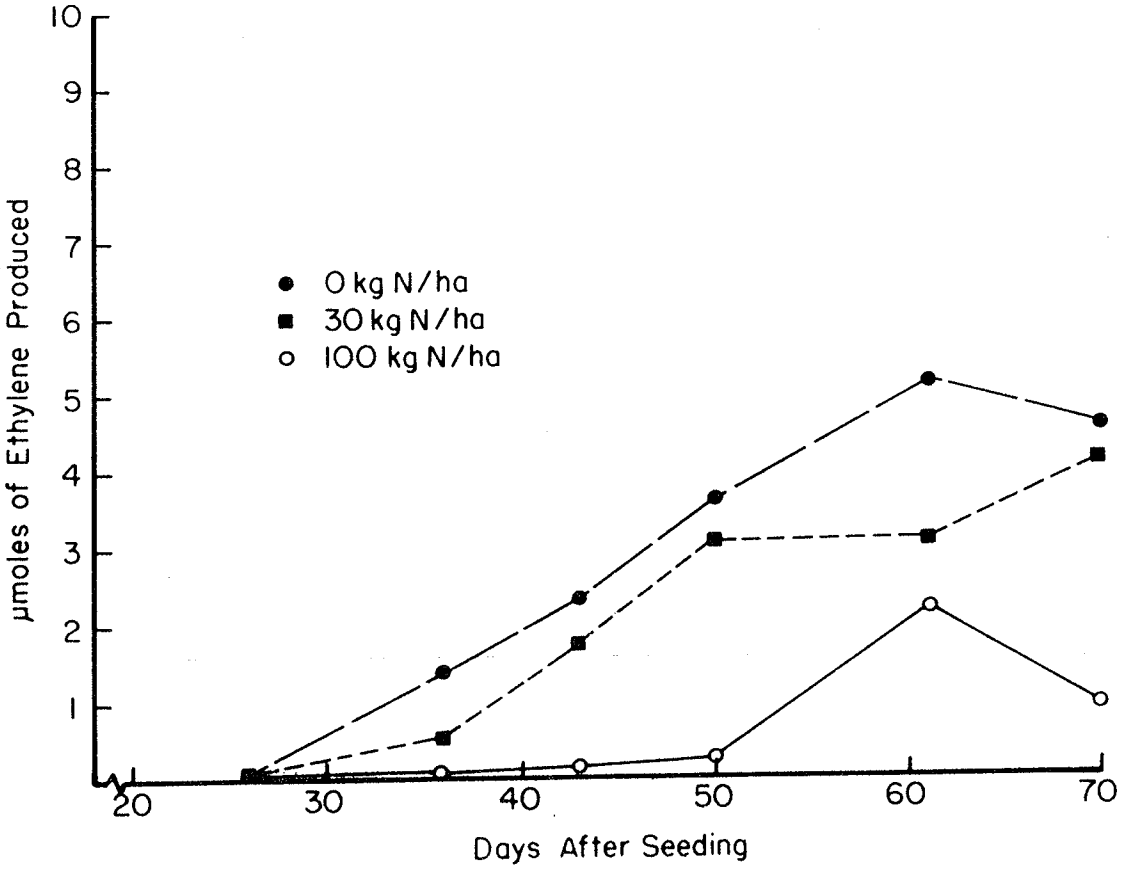

Figure 8. Effect of nitrogen application on the ethylene production of root nodules of soybeans (Toews).

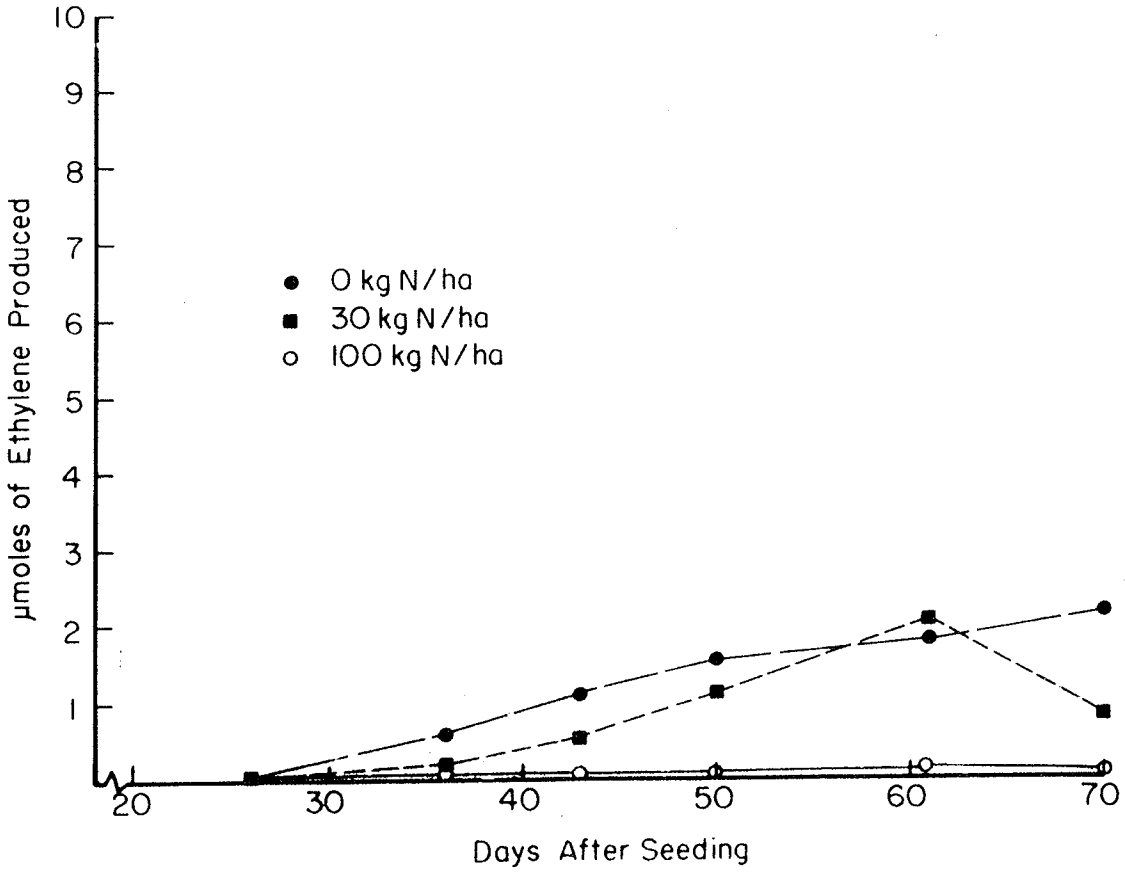

Figure 9. Effect of nitrogen application on the ethylene production of root nodules of soybeans (Nikkel).

Ethylene production was observed to be the greatest at 61 days after seeding for most of the treatments examined. This corresponded to the stage when pod filling was occurring. Considerable ethylene production occurred between flowering and the completion of pod filling approximately 70 days after seeding. Much of the N_2 fixation therefore occurred during the reproductive stages of development rather than during the stages when strictly vegetative growth was occurring. This may in part explain the previous observation that symbiotic fixation had a more significant effect on increasing seed yield as opposed to vegetative yield in the nodulated soybeans. The significant increases in protein content as a result of symbiotic fixation may also have been influenced by the fact that this nitrogen was supplied largely during reproductive stages of growth when the plant had its maximum nitrogen requirements. These statements must be qualified in that ethylene production at any of these points during the growing season may have been reduced or exaggerated due to environmental conditions present.

Quantitative determinations of nitrogen fixation throughout the season were not reported for the acetylene reduction technique. It was evident from environmental data collected at sampling times during the season that there was a wide variation of light intensity, surface soil moisture and soil temperature depending on the day of sampling or even the sampling time during the day. Sprent and Bradford (1977) indicated that environmental factors such as these affect acetylene reduction by field legumes. In light of this, it seemed undesirable in these experiments to extrapolate six one hour incubations over an entire growing season. With variable weather conditions other researchers suggest that even with more frequent samplings this technique may not be accurate for

quantitative measurement (Phillips and Bennett, 1978). Quantitative measurements of seasonal fixation would also dictate that the technique be made more cumbersome accounting for such things as H_2 evolution in the absence of acetylene (Peterson and Burris, 1976), an experimentally determined C_2H_2/N_2 conversion factor (Hardy et al., 1972) and the fact that N_2 is assimilated for protein synthesis while C_2H_2 makes no contribution to the plants metabolism (Rennie et al., 1978). Root volume sampled and nodule distribution between tap root and laterals would also have to be defined. Rodd([1]) (personal communication, 1983) indicated lateral roots of soybeans in the growth chamber exhibited significant nodulation. It would appear that the best way to use the acetylene reduction technique for examining legume fixation in the field is for a relative measurement of fixation at a point in time in which these factors do not have a major effect. The degree to which this statement is true will depend on how cumbersome one is prepared to make the technique when applying it to a field situation.

Nodule rating was carried out at each acetylene reduction sampling period (Table 17). This procedure was an observation of the degree of nodulation of randomly selected plants. General trends of decreased nodulation with increasing rates of nitrogen addition were observed at all sites although significant differences were only observed in a few instances. Nodulation appeared to be less on plants at Nikkel than on plants at Enns or Toews. Nodule counts on plants at Enns also appeared to increase over counts on plants at Toews at approximately 43 days

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([1]) V. Rodd., MSc. Graduate Student, Department of Soil Science, University of Manitoba

TABLE 17

NODULE RATING

Location and Nitrogen Rates (kgN/ha)		Nodule Rating (1) at Different Sampling Times (days after seeding)					
		26 (2)	36	43	50	61	70
Enns	0	0.8 a*	1.7 a	2.8 a	3.3 a	3.6 a	3.8 a
	30	0.6 a	1.6 a	2.0 a	2.5 a	2.9 a	2.8 b
	100	0.4 a	0.9 b	0.8 b	0.8 b	0.9 b	0.9 c
Toews	0	1.1 a	2.1 a	2.1 a	2.3 a	2.8 a	2.9 a
	30	0.8 a	1.6 a	1.6 a	1.9 ab	1.5 a	2.6 a
	100	0.6 a	0.8 b	0.9 a	1.1 b	1.4 a	1.2 b
Nikkel	0	0.6 a	0.9 a	1.1 a	1.7 a	1.3 a	1.3 a
	30	0.4 a	0.7 a	0.8 a	1.4 a	1.2 a	1.2 a
	100	0.3 a	0.5 a	0.8 a	0.6 b	0.4 b	0.7 a

(1) Nodule Rating Scale: 0 = no evidence of nodulation, 1 = 1-4 nodules, 2 = 5-8 nodules, 3 = 9-12 nodules, 4 = 13-16 nodules, 5 = 17 or more nodules.

* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05 (Statistical determinations were done separately for each site and harvest).

(2) Developmental Stages: 26 = 4-5 trifoliate leaf stage, 36 = early flowering, 43 = flowering, 50 = early pod formation, 61 = pod filling, 70 = early leaf senescence.

after seeding when no nitrogen was applied. Nodules were generally small and located on the tap root until forty three to fifty days after seeding. After this point increasingly more nodules were observed on the lateral roots and the size of nodules was larger than that observed earlier in the season. Some nodules began to turn from pink to green at seventy days after seeding, indicating loss of N_2 fixing activity. Observed nodulation on plant roots compared well with observed ethylene production. Increased ethylene production was closely associated with increases in the number of nodules as well as the size of the nodules. Based on these observations it appears that nodule rating is a fairly consistent method of indicating relative symbiotic nitrogen fixation between different treatments of Maple Presto soybeans.

Seasonal nitrogen fixation was determined at 70 days after seeding when nodule activity appeared to be terminating and leaves were beginning to senesce. Two methods were used in determining symbiotic fixation, the first of these being the ^{15}N "A" value method. Calculated "A" values are reported in Table 18. The values for non-nodulating and uninoculated soybeans represent the available nitrogen in the soil in terms of the fertilizer standard added. Values calculated for nodulating soybeans and fababeans include nitrogen derived from symbiotic fixation as well as soil nitrogen, these again being in terms of the fertilizer standard. A comparison between the non-nodulating soybeans and uninoculated soybeans showed similar "A" values were calculated for each. The non-nodulating soybeans were used as a measure of soil available nitrogen in these experiments.

TABLE 18

EFFECT OF NITROGEN APPLICATION ON THE "A" VALUE

	Nitrogen Applied (kgN/ha)	"A" Values (Fertilizer Equivalent Units)		
		Enns	Toews	Nikkel
Non Nodulating Soybeans	30	157 a*	72 a	103 a
	100	173 a	100 ab	139 ab
Nodulating Soybeans	30	171 a	136 b	110 a
	100	190 a	113 ab	166 b
Uninoculated Soybeans	30	150 a	96 ab	101 a
Fababeans	30	230 a	241 c	257 c

* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

The "A" values determined for the non-fixing crops tended to increase with higher rates of nitrogen although these increases were not significant. Fried and Broeshart (1975) stated that the "A" value is normally rate independent. Fried and Broeshart based their whole rationale for using the "A" value to calculate fixation on the fact that the technique was rate independent and different fertilizer rates could be used on the test and control crops. Therefore, small nitrogen additions can be made to the test crop so as not to greatly inhibit symbiotic fixation while larger additions can be made to the control crop to promote healthy development.

Significant differences were observed in the "A" values for the fixing crops over those of the non fixing crops. Since these values included nitrogen derived from symbiotic fixation in terms of the fertilizer standard it was evident from these results that fixation was occurring in these treatments.

Seasonal symbiotic fixation as calculated by the ^{15}N "A" value technique is reported in Table 19. The results show that symbiotic fixation was occurring in Maple Presto soybeans at Toews when 30 kgN/ha was applied. Symbiotic fixation was essentially reduced to zero with the addition of 100 kgN/ha at this site. Fixation was also reduced by the higher nitrate nitrogen levels at the other two sites. Inorganic soil nitrogen and fertilizer nitrogen therefore both served to decrease symbiotic nitrogen fixation in soybeans.

Calculated values for symbiotic fixation using the "A" value technique also show that fababeans were significantly better nitrogen fixers than Maple Presto soybeans with concurrent applications of 30 kgN/ha at

TABLE 19

SYMBIOTIC NITROGEN FIXATION AS CALCULATED BY THE ^{15}N "A" VALUE METHOD

	Nitrogen Applied (kgN/ha)	Symbiotic Nitrogen Fixation (kgN/ha)		
		Enns	Toews	Nikkel
Nodulating Soybeans	30	7 a*	36 b	4 a
	100	8 a	8 a	15 a
Fababeans	30	35 a	83 c	54 b

* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

two of the three sites studied. Soybeans were terminating fixation at 70 days after seeding whereas fababeans were still at a stage of active fixation and possibly would have continued this for a significant length of time due to their longer time to maturity. Differences in total fixation of soybeans and fababeans may have been greater if fababeans were taken to maturity. This would have been consistent with observations reported by Richards (1977) who stated that fababeans were capable of fixing considerable quantites of nitrogen in their later stages of growth.

Richards and Soper (1982) reported that fababeans grown in the field fixed an average of 54% of their nitrogen requirements. They calculated that the total seasonal fixation was between 53 and 112 kgN/ha when no supplemental nitrogen was applied. These values confirm that the symbiotic fixation calculated for fababeans at each of the sites in 1979 was reasonable.

Seasonal symbiotic fixation to 70 days after seeding was also calculated by the ^{15}N assisted difference method where fertilizer was applied and the classical difference method in the absence of fertilizer addition (Table 20). Non-nodulating soybeans were used to measure the amount of soil nitrogen which was available to the nodulating soybeans and fababeans (Table 21). Soil nitrogen uptake was significantly increased by nitrogen additions of 100 kgN/ha to plants at Enns and Toews. Increased soil nitrogen accumulation may have resulted from an increased capacity of these plants to take up nitrogen due to a more extensive root system. Higher soil nitrogen uptake may also have resulted from a priming effect of the fertilizer on mineralization of

TABLE 20

SYMBIOTIC NITROGEN FIXATION AS CALCULATED BY THE DIFFERENCE METHOD

	Nitrogen Applied (kgN/ha)	Symbiotic Nitrogen Fixation (kgN/ha)		
		Enns	Toews	Nikkel
Nodulating Soybeans	0	47 b*	51 a	18 a
	30	19 ab	43 a	16 a
	100	10 a	22 a	28 a
Fababeans	30	30 ab	82 b	39 a

* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

TABLE 21

EFFECT OF NITROGEN APPLICATION ON SOIL N UPTAKE OF AERIAL PORTIONS OF NON NODULATING SOYBEANS

Nitrogen Applied (kgN/ha)	Soil Nitrogen Uptake (kgN/ha)		
	Enns	Toews	Nikkel
0	45 a*	26 a	54 a
30	60 ab	35 a	51 a
100	74 b	52 b	58 a

* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

soil organic matter. Increases in soil nitrogen uptake as a result of fertilizer application did not appear to take place on soils with moderate to high amounts of nitrate nitrogen. This was shown by results from the Nikkel site and from Regitnig (1979) who worked on soils high in NO_3-N. Values calculated for soil nitrogen were subtracted from the total nitrogen along with any fertilizer nitrogen which was determined through the use of ^{15}N and symbiotically fixed nitrogen was determined. Fixation in the Maple Presto soybeans occurred to the greatest extent at the Toews site when no nitrogen application was made. Substantial fixation also occurred in soybeans at this site when 30 kgN/ha was applied. Results from plants at Enns showed a significant decrease in fixation when 100 kgN/ha was applied as compared to the zero treatment. These results are consistent with the acetylene reduction assay and nodule counts discussed previously which indicated that symbiotic fixation decreased with applied nitrogen. Substantial fixation did not appear to occur in soybeans at the Nikkel site. Soil nitrogen reduced fixation to a relatively small amount.

Fixation appeared to be occurring at each experimental site when 30 kgN/ha was applied to fababeans. Toews was the only site at which fababeans fixed significantly more nitrogen that soybeans with the same fertilizer application.

The percent of total above ground nitrogen derived from symbiotic fixation using the ^{15}N "A" value and difference techniques are reported in Table 22. Unfertilized soybeans are shown to derive up to 66% of their aerial nitrogen from fixation up to 70 days after seeding on a low nitrogen soil (Toews). Increased soil nitrogen at Enns and Nikkel pro-

gressively reduced the percent fixed nitrogen in the plant from that observed at Toews. The addition of nitrogen fertilizer decreased the amount of nitrogen symbiotically fixed by soybeans while yields remained constant or increased and therefore, the percent of total above ground nitrogen derived from symbiotic fixation was reduced to a relatively small amount. Symbiotic fixation accounted for between 6 and 20% of the total nitrogen with additions of 100 kgN/ha. At this application rate the percent of total nitrogen derived from fixation was similar for all sites in spite of the different levels of soil nitrogen present. At the 30 kgN/ha rate it was evident that differences in soil NO_3-N still had an effect on the percent symbiotic nitrogen in the plant. Soybeans fixed a maximum of 46% of their nitrogen when 30 kgN/ha was applied. Fababeans averaged about 47% of their nitrogen from symbiotic fixation over the three sites. This was similar to the 54% reported by Richards and Soper (1982).

Results indicate that both the ^{15}N "A" value method and ^{15}N difference method are useful in determining relative fixation between different treatments. However, the acetylene reduction procedure appears to give a better comparison between treatments at a point in time and would be recommended over the other two methods for this purpose. In terms of measuring actual amounts of fixation over the entire growing season the use of tracer ^{15}N techniques appear to give reasonable results. Values obtained using the "A" value technique and the difference technique were similar which was expected since the only difference was the first technique used N ratios rather than total N uptakes which were used in the second technique.

TABLE 22

PERCENT OF TOTAL ABOVE GROUND NITROGEN DERIVED FROM SYMBIOTIC FIXATION AS CALCULATED BY THE ^{15}N "A" VALUE AND ^{15}N ASSISTED DIFFERENCE TECHNIQUES

	Nitrogen Applied (kgN/ha)	Percent of Aerial Nitrogen Derived from Symbiosis					
		^{15}N "A" Value Technique			^{15}N Assisted Difference Technique		
		Enns	Toews	Nikkel	Enns	Toews	Nikkel
Non-Nodulating Soybeans	0	-	-	-	51	66	25
	30	7	38	5	20	46	19
	100	6	6	11	8	16	20
Fababeans	30	34	63	54	29	62	39

Results from these methods do appear to confirm that on low NO_3-N soils Maple Presto soybeans are involved in symbiotic nitrogen fixation. The amount fixed may be as high as 51 kgN/ha. Symbiotic nitrogen fixation in these soybeans is reduced significantly by nitrogen fertilizer addition or increased available soil nitrogen. Yield and protein responses to added fertilizer would indicate that high levels of soil nitrogen or fertilizer nitrogen addition are required for optimum growth of Maple Presto soybeans and that symbiotic nitrogen fixation alone will not supply the plants nitrogen requirements if optimum yield and protein are to be achieved in the field.

4.2 GROWTH CHAMBER EXPERIMENTS

Two growth chamber experiments were carried out to examine the yield, protein and symbiotic nitrogen fixation potential of Maple Presto soybeans. In studying this variety of soybeans it was desirable to examine their complete range of response to added nitrogen in a controlled environment. The two studies undertaken were similar with respect to design and treatments. Soils for both growth chamber experiments were obtained from field site locations in the Morden-Winkler area of southern Manitoba. Chemical and physical properties of the soils used in the growth chamber studies are outlined in Table 23 and 24.

4.2.1 THE EFFECT OF NITROGEN ADDITION ON DRY MATTER YIELD AND PROTEIN CONTENT OF MAPLE PRESTO SOYBEANS IN GROWTH CHAMBER STUDIES

The addition of supplementary nitrogen was found to be an important factor in increasing dry matter yield of Maple Presto soybeans at the pod filling stage of development in growth chamber studies. In experiment A a significant increase in dry matter yield was realized with 100 ppm and 200 ppm additions of nitrogen (Table 25). Applications of less than 100 ppm nitrogen also resulted in dry matter increases although these were not statistically significant. Successive levels of N were not significantly different in terms of dry matter yield, however, increases in dry matter with applications of up to 200 ppm N indicated that the plants may not have reached their maximum capacity for nitrogen uptake. Rates of 200 ppm did not appear toxic to plant growth and continued increases in dry matter may have resulted from higher application rates.

TABLE 23

Characteristics of Soil Used In Growth Chamber Experiment A

Soil Series	Hockfeld
Subgroup	Orthic Black
Textural Class	Fine Sandy Loam
pH	7.6
Conductivity (dS/m)	0.3
NO_3-N (ppm)	7.0
$NaHCO_3$ extractable P (ppm)	8.2
NH_4OAc extractable K (ppm)	135
$\%CaCO_3$ equivalent	0.57
Moisture Content at Field Capacity (48 Hrs.)	22%

TABLE 24

Characteristics of Soil Used In Growth Chamber Experiment B

Soil Series	Reinland
Subgroup	Gleyed Carbonated Rego Black
Textural Class	Very Fine Sandy Loam
pH	7.2
Conductivity (dS/m)	0.2
Carbonate Content	Very low
NO_3-N (ppm)	13.6
$NaHCO_3$ extractable P (ppm)	14.6
NH_4OAc extractable K (ppm)	150
$CaCl_2$ extractable SO_4-S (ppm)	3.2
Moisture Content at Field Capacity (48 hrs.)	27.3%

TABLE 25

EFFECT OF RATE AND TIME OF NITROGEN APPLICATION ON DRY MATTER YIELD AND PROTEIN CONTENT OF SOYBEANS IN A GROWTH CHAMBER (1) (EXPERIMENT A)

Nitrogen Applied (ppm)	Time of Application	Dry Matter Yield (grams)	Protein Content (%)
0	Seeding	14.2 cd*	15.4 f
20	Seeding	14.3 cd	14.0 def
40	Seeding	15.3 cde	11.2 bcd
60	Seeding	15.7 de	9.8 abc
100	Seeding	17.6 ef	12.1 cde
200	Seeding	18.5 f	12.8 de
60	7 weeks	16.3 de	13.0 def
60	Early flower	14.5 cd	14.1 ef
60	Early pod fill	11.8 ab	14.8 ef
0 (2)	Seeding	11.0 a	8.3 ab
60 (2)	Seeding	13.5 bc	8.1 a

(1) Soybeans were harvested at the mid pod fill stage of development.
(2) Uninoculated Soybeans
* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

Supplemental nitrogen was also shown to significantly increase dry matter yield of soybeans which did not receive inoculum. It was shown, however, that uninoculated soybeans had significantly lower dry matter yields than inoculated soybeans with concurrent fertilizer rates. This indicated that symbiotic nitrogen fixation was supplying nitrogen for dry matter production in the inoculated soybeans and that this was still occurring with additions of 60 ppm N.

Late applications of 60 ppm nitrogen at 7 weeks and early flowering had no significant effect on increasing or decreasing dry matter yields as compared to the same application made at seeding. Applications of 60 ppm nitrogen at early pod fill resulted in dry matter yields being significantly lower than yields for 60 ppm application at seeding, 7 weeks or early flower. Dry matter yields for the early pod fill application were also significantly lower than yields for the treatment where no nitrogen was applied at seeding. This shows that the nitrogen applied at early pod fill was not utilized to a large extent for dry matter production. Soil and symbiotic nitrogen sources were more beneficial by themselves (0 treatment) in terms of dry matter yield than an application of 60 ppm at early pod fill. It might be suggested that this late fertilizer application inhibited symbiotic nitrogen fixation without equivalent amounts of fertilizer nitrogen being taken up by the plant.

Percent protein in the dry matter was significantly decreased with nitrogen additions and this was observed to the greatest extent with an application of 60 ppm N. Applications of 100 and 200 ppm N increased the protein content of the plants over that observed with 60 ppm N but did not increase it to the levels observed when no nitrogen was applied.

Supplemental nitrogen did not increase the protein content of soybeans which did not receive inoculum. Protein contents of these plants were 8.3 and 8.1 for the 0 and 60 ppm N applications and this was significantly lower than inoculated treatments which received no nitrogen and similar to treatments which had 60 ppm N added at seeding. Added nitrogen, therefore, increased dry matter production rather than the concentration of nitrogen in the tissue of uninoculated soybeans.

Applications of 60 ppm nitrogen at different growth stages were observed to increase the protein content of plants over an identical application at seeding. Significant differences in protein content were not observed among late applications or between late applications and the zero treatment at seeding. Late applications of 60 ppm nitrogen were not as inhibitory to plant protein content as the same applications made at seeding.

Nitrogen fertilizer addition did not have a beneficial effect over the control treatment at any rate when both yield and protein content were considered. Significant yield increases over the control as in the case of the 100 and 200 ppm applications were accompanied by corresponding decreases in protein content. Symbiotic nitrogen fixation in the absence of fertilizer addition supplied enough nitrogen to the plant to realize a significant yield and protein increase over that observed when supplemental and symbiotic nitrogen sources were not present.

Growth chamber experiment A was grown for 28 days at temperatures simulating mean annual spring temperatures for Brandon, Manitoba. It was found that these temperatures were too low and resulted in slow plant development. Although temperatures were increased, growth stages were delayed for the duration of the experiment. This delay was

observed to be approximately three weeks when compared to occurence of flowering and early pod fill in growth chamber experiment B. These poor growing conditions made it desirable to repeat a similar experiment under more favorable conditions. Higher rates of nitrogen were added to this second experiment to determine the level at which maximum dry matter yield and protein content occurred.

Dry matter yields at the mid pod fill stage of development in growth chamber experiment B were much higher than those observed for experiment A (Table 26). This was most likely a direct result of a more favorable temperature for plant growth in experiment B. Significant increases in dry matter yield occurred with applications of 40, 60, 120 and 240 ppm nitrogen applied at seeding. Applications up to 60 ppm N exhibited a linear dry matter response. The maximum dry matter yield of 30.4 g. was obtained with an application of 120 ppm N, rates above this resulting in lower dry matter yields. The yield of dry matter with an application of 360 ppm N was very similar to that observed when no nitrogen was applied indicating significant nitrogen toxicity.

A significant increase in dry matter yield also occurred with an application of 100 ppm N at flowering. Dry matter yields for this treatment were comparable to those obtained when 120 ppm N was applied at seeding indicating that nitrogen application had its greatest effect on dry matter production after flowering had been initiated. This was consistent with the 1979 field experiment which showed that rapid dry matter accumulation and nitrogen uptake in Maple Presto soybeans were initiated at the flowering stage of development. It is apparent that the healthier plants in experiment B had clearer dry matter responses than those in experiment A.

TABLE 26

EFFECT OF RATE OF NITROGEN APPLICATION ON DRY MATTER YIELD AND PROTEIN CONTENT OF SOYBEANS IN A GROWTH CHAMBER (1) (EXPERIMENT B)

Nitrogen Applied (ppm)	Dry Matter Yield (grams)	Protein Content (%)
0	18.1 a*	9.0 c
20	21.9 abc	8.7 c
40	25.3 bcd	8.7 c
60	28.1 cd	9.1 cd
120	30.4 d	10.8 de
240	26.4 cd	11.9 e
360	16.7 a	15.4 f
100 (2)	30.2 d	12.2 e
100 (3)	18.4 ab	16.8 f
0 (4)	19.3 ab	3.8 a
60 (4)	38.8 e	4.7 a
120 (4)	45.1 f	6.7 b

(1) Soybeans were harvested at the mid pod fill stage of development.
(2) Nitrogen added at flowering
(3) Nitrogen added at early pod fill
(4) Barley
* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

Barley dry matter was harvested at the soft dough stage, 59 days after seeding. Dry matter yields were significantly increased with nitrogen additions of 60 and 120 ppm N and this was primarily a result of greater tillering. Treatments averaged 6, 11 and 17 tillers for the 0, 60 and 120 ppm N applications respectively. Dry matter increases in the Maple Presto soybeans corresponded to visual differences between treatments at time of harvest. Soybeans with no nitrogen applied appeared smaller and more chlorotic than other treatments. Applications of 20 and 40 ppm N improved this condition, plants being less chlorotic and slightly larger than the zero treatment. The 60 ppm N treatment appeared to be a transition between plants which appeared unhealthy and plants receiving 120 and 240 ppm N which appeared quite vigorous. The application of 360 ppm nitrogen resulted in soybeans which were somewhat stunted in size. Late applications of 100 ppm N at flowering and early pod fill appeared comparable in color and size with plants to which 120 and 240 ppm N were applied at seeding.

Nitrogen addition also resulted in differences in pod formation. Large pods were found on soybeans to which no nitrogen was applied and on soybeans to which 20 and 40 ppm nitrogen was applied. Very few pods were observed on plants with nitrogen applications of 60 ppm or greater. Large pods were, however, observed on plants when 100 ppm nitrogen was applied at flowering or early pod fill. Nitrogen applications of 60 ppm or greater at seeding delayed maturity of the soybeans as evidenced by the delay in this developmental stage. Delayed nitrogen application eliminated this problem and this occurred with no adverse

effects on dry matter yield and protein content when the nitrogen was applied at flowering.

The trends observed in dry matter yield and protein content in experiment B indicated that added nitrogen increased dry matter yield with no corresponding protein increase until maximum yields were approached. At maximum dry matter yield a significant protein response was observed. As nitrogen was applied after this point yields decreased with a corresponding increase in protein content.

Nitrogen applications at flowering and early pod fill also resulted in significant increases in protein content over applications of 60 ppm N or less at seeding. At flowering this protein increase occurred with the significant dry matter increase already discussed. An adequate supply of available nitrogen at flowering, therefore, seems to be essential in maintaining high dry matter yields and protein content. Maple Presto soybeans do not require these same amounts of nitrogen at earlier stages of development in order to realize yield and protein increases later in the season. In terms of yield and protein increases per fertilizer added, the 100 ppm N application at flowering was the best treatment. The significant protein increases observed with 100 ppm N applied at early pod fill occurred in the absence of a significant dry matter response. At this developmental stage the entire benefit of the fertilizer application was realized in increased plant protein content.

Yield and protein results from experiment B appeared to correlate more closely with the 1979 field experiments than did results from experiment A. This was likely the result of plant growth in experiment B being more comparable to what was observed in the field. Both field

and growth chamber studies show that Maple Presto soybeans require supplemental nitrogen to maximize yield and protein content. They also suggest that added nitrogen will increase yield with little corresponding protein increase until maximum yields are approached.

4.2.2 THE EFFECT OF NITROGEN ADDITION ON NITROGEN UPTAKE BY MAPLE PRESTO SOYBEANS IN GROWTH CHAMBER STUDIES

A significant depression and rebound in total nitrogen uptake occurred as nitrogen was added to Maple Presto soybeans in experiment A (Table 27). The maximum extent of depression occurred with 60 ppm N. Since plants in this treatment were supplied with symbiotic, soil and fertilizer nitrogen it might be suggested that the fertilizer inhibited the symbiotic fixation process from functioning to its full capacity and then was not able to supply a corresponding amount of nitrogen equal to that forfeited when fixation was reduced. It was apparent that supplemental nitrogen additions up to 60 ppm did not add to or even equally replace the symbiotic and soil nitrogen present in plants in the 0 treatment. Fertilizer supplied 13, 24 and 33 mg N/pot while the total nitrogen uptake was reduced by 32, 79 and 103 mg N/pot for the 20, 40 and 60 ppm N treatments respectively. Additions of 100 and 200 ppm N resulted in total nitrogen uptake levels which were similar to those observed when no nitrogen was added. The fertilizer nitrogen supplied in these treatments was therefore similar to that which was lost from soil and symbiotic sources. The increase in fertilizer uptake to 200 ppm suggests that soybeans may not have reached their maximum capacity to take up fertilizer nitrogen in this experiment.

Nitrogen additions of 60 ppm at early flower and early pod fill had no significant effect on total nitrogen uptake when compared to the same treatment applied at seeding, however, additions at 7 weeks significantly increased the total nitrogen uptake from that observed at seeding. Significantly more fertilizer nitrogen was used by the plant when it was

TABLE 27

TOTAL NITROGEN AND FERTILIZER NITROGEN UPTAKE BY SOYBEANS IN A GROWTH CHAMBER (EXPERIMENT A)

Nitrogen Applied (ppm)	Time of Application	Total Nitrogen Uptake (mgN/pot)	Fertilizer Nitrogen Uptake (mgN/pot)
0	Seeding	352 de*	-
20	Seeding	320 cde	13 a
40	Seeding	273 cd	24 ab
60	Seeding	249 bc	33 bc
100	Seeding	339 de	109 f
200	Seeding	378 e	189 g
60	7 weeks	340 de	47 de
60	Early flower	328 cde	56 e
60	Early pod fill	282 cd	13 a
0 (1)	Seeding	146 a	-
60 (1)	Seeding	174 ab	35 bcd
0 (2)	Seeding	104 a	-
60 (2)	Seeding	132 a	42 cd

(1) Uninoculated Soybeans
(2) Barley
* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

applied at 7 weeks or early flowering as opposed to seeding. Late additions of nitrogen had no significant effect on total nitrogen uptake when compared to the 0 treatment.

Nitrogen additions of 60 ppm N to the barley and uninoculated soybeans did not result in significant increases in total nitrogen uptake although increasing trends were observed. Trends between the two crops showed that the total nitrogen uptake in the uninoculated soybeans was higher than the barley for both rates of fertilizer applied, these increases again being nonsignificant. Fertilizer nitrogen uptake was similar between the two crops indicating that slight differences in total nitrogen uptake may be the result of the uninoculated soybeans fixing small amounts of nitrogen due to contamination with viable rhizobium. The occurence of fixation in Maple Presto soybeans which had no inoculum added was observed by Smith (1980) suggesting the real possibility of its occurence in this experiment as well. Nodules were not observed on the tap roots of uninoculated plants in this experiment, however, uncertainty regarding the occurence of fixation led to the rejection of this standard for calculating symbiotic nitrogen fixation. In light of these results it is recommended that future experiments refrain from using uninoculated soybeans as a standard.

The percent utilization of fertilizer was very low for all the treatments in experiment A (Table 28). Significant differences were observed in the percent utilization of fertilizer between treatments in spite of the low values. Applications of 20, 40 and 60 ppm nitrogen at seeding were all very similar with respect to the utilization of fertilizer by barley and soybeans. 100 and 200 ppm N additions were, however, utilized to a significantly greater extent than the lower applications.

TABLE 28

PERCENT UTILIZATION OF FERTILIZER IN GROWTH CHAMBER EXPERIMENT A

Nitrogen Applied (ppm)	Time of Application	Utilization of Fertilizer (%)
20	Seeding	13 bc
40	Seeding	12 bc
60	Seeding	11 b
100	Seeding	22 e
200	Seeding	19 de
60	7 weeks	16 cd
60	Early Flowering	19 de
60	Early Pod Fill	4 a
60 (1)	Seeding	14 bc
60 (2)	Seeding	12 b

(1) Uninoculated Soybeans
(2) Barley
* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

The low overall percent utilization of fertilizer and the higher utilization at high application rates may suggest that the soil was chemically fixing or immobilizing a large amount of the nitrogen added. This was more likely than losses through denitrification since the soil moisture level was carefully monitored so anaerobic conditions would be avoided. If soil used in the growth chamber chemically fixed or biologically immobilized nitrogen it also must be considered that a constant soil volume was used and that high rates of fertilizer may have exceeded the capacity of the soil to tie up nitrogen, more nitrogen being available to the plants. If this was true, large nitrogen applications would be required before toxic effects on yield would be observed, possibly explaining why no such observations were made in this experiment.

Percent utilization of fertilizer was greater when 60 ppm N was added at 7 weeks and at early flowering than when the same applications were made at seeding. This may have been a function of the rate at which plants utilized nitrogen fertilizer after it was applied. If competition between chemical fixation or biological immobilization and plant uptake occurred then proportionately more nitrogen would have been tied up through soil processes when plants were at early stages of development and did not have large nitrogen requirements than when plants had a larger nitrogen demand at the 7 week or early flowering stage of development. Soybeans at the more mature developmental stages would have utilized a greater amount of fertilizer nitrogen before it was tied up. The very low utilization of fertilizer when it was applied at early pod fill may have been caused in part by the short time period

of one week between this application and harvest of the plants. There was a visual cessation of growth during this time period, although no dry matter measurements were obtained to support this. This apparaent reduction in growth rate would explain a decrease in the utilization of fertilizer.

Significant increases in total nitrogen uptake were shown to occur with added fertilizer in experiment B (Table 29). These increases were in uniform intervals and reached a maximum at rates of 120 ppm N applied at seeding. Decreases from this maximum occurred at rates of 240 and 360 ppm N with the total nitrogen uptake at 360 ppm N being significantly lower than that which occurred at 120 ppm N. Rates of 240 and 360 ppm N, therefore, resulted in toxic responses in terms of dry matter accumulation and total nitrogen uptake. Since fertilizer nitrogen was placed away from the seed in this experiment it likely did not effect germination or early seedling development. Toxicity to root development probably occurred as the plants were established and matured, this being evident in the 360 ppm N application by a visual dwarfing in plant growth as the plants moved into the stages of greater dry matter accumulation.

Fertilizer nitrogen uptake when the fertilizer was applied at seeding, was maximized with applications of 120 ppm N corresponding to the application which maximized total nitrogen uptake. Each addition of nitrogen up to 120 ppm N showed a significant increase in fertilizer uptake. Fertilizer nitrogen supplemented the plants nitrogen supply in excess of any losses which were incurred as a result of reduced symbiotic fixation. This was evidenced by the increases in total nitrogen

TABLE 29

TOTAL NITROGEN AND FERTILIZER NITROGEN UPTAKE BY SOYBEANS IN A GROWTH CHAMBER (EXPERIMENT B)

Nitrogen Applied (ppm)	Total Nitrogen Uptake (mgN/pot)	Fertilizer Nitrogen Uptake (mgN/pot)
0	260 b*	-
20	302 bcd	68 a
40	349 cde	153 b
60	409 ef	222 c
120	521 gh	428 e
240	492 fgh	338 d
360	399 def	309 d
0 (1)	117 a	-
60 (1)	292 bc	196 bc
120 (1)	478 fg	454 e
100 (2)	582 h	430 e
100 (3)	493 fgh	290 d

(1) Barley
(2) Nitrogen Applied at flowering
(3) Nitrogen Applied at early pod fill
* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

observed with added fertilizer. The significant decreases in fertilizer uptake from the maximum when 240 and 360 ppm N were applied showed that plants were inhibited from taking up available nitrogen into aerial parts.

Nitrogen added at different growth stages had the same total nitrogen uptake as additions of 120 ppm N at seeding. Fertilizer uptake when nitrogen was applied at flowering was also the same as that observed with 120 ppm applied at seeding. This indicates that most of the fertilizer nitrogen was required at or past the flowering stage of development. Fertilizer nitrogen uptake from nitrogen application at early pod fill was significantly reduced from that observed at flowering, however, it was still greater than the total nitrogen accumulation over the entire growing period when no nitrogen was applied. This indicates that plants were still actively growing at this stage of development and contrasts experiment A which had a cessation of growth and little fertilizer uptake after early pod fill. Differences in growth conditions and resulting differences in plant development between the two experiments were likely the causes of this. Soybeans in experiment B appeared to maintain more vigorous growth in the later stages of plant development than those in experiment A.

Significant increases in total nitrogen uptake of barley were shown to occur with added nitrogen. The fertilizer nitrogen uptake with these additions of 60 and 120 ppm N was very similar to that of the soybeans at the same rate of nitrogen additions. This indicates that the two crops had a similar capacity to utilize the available nitrogen applied and, therefore, lends confidence to the use of barley as a standard for measuring soil nitrogen in the soybean plant.

The percent utilization of fertilizer in experiment B was significantly increased over that which occured in experiment A (Table 30). The apparent loss of fertilizer in experiment A was therefore not a problem in experiment B. The utilizaton of fertilizer was essentially the same with nitrogen additions of 20 to 120 ppm at time of seeding, values ranging from 68 and 77 percent. This utilization was significantly reduced between applications of 120 and 240 ppm N and further decreased by applications of 360 ppm N. It was therefore, apparent that plants had reached their limit for efficient utilization of fertilizer with applications of 120 ppm nitrogen. It must, however, be remembered that with these high applications absolute uptake was also less, indicating a toxicity as well as a simple efficiency problem.

The percent utilization of 100 ppm N applied at flowering was significantly better than that of any other nitrogen application. This was likely a reflection of the soybeans immediate need for nitrogen at ths stage of growth. Losses of fertilizer nitrogen in this treatment had less chance of occurring prior to plant utilization of the applied nitrogen than they did when nitrogen was applied at seeding. The decreased utilization of 100 ppm N applied at early pod fill was an indication that plants were harvested before they could fully utilize this application.

The percent utilization of fertilizer for barley in experiment B was similar to that of the soybeans with similar rates of fertilizer addition. Differences in utilization of fertilizer between the barley treatments was shown, a possible explanation for this being that the ratio of shoot to root nitrogen increased with the large application.

TABLE 30

PERCENT UTILIZATION OF FERTILIZER IN GROWTH CHAMBER EXPERIMENT B

Nitrogen Applied (ppm)	Utilization of Fertilizer (%)
20	68 de*
40	77 e
60	74 de
120	71 de
240	28 b
360	17 a
60 (1)	65 cd
120 (1)	76 e
100 (2)	86 f
100 (3)	58 c

(1) Barley
(2) Nitrogen Applied at flowering
(3) Nitrogen Applied at early pod fill
* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

Residual nitrates left in the soil after the dry matter was harvested further demonstrated the extent to which Maple Presto soybeans utilized applied nitrogen (Table 31). Essentially all the nitrate nitrogen from soil and fertilizer sources was utilized by the soybeans in treatments receiving 20 to 120 ppm N at seeding. This was also true of applications made to the barley. At applications of 120 ppm N to the soybeans, a small increase in residual nitrate appeared to occur. Larger additions resulted in highly significant increases in nitrate left in the soil. The amounts left in the soil with these high applications were very similar to the fraction applied at seeding over and above 120 ppm N. These residual amounts were 127.3 and 274.0 ppm for the 240 and 360 ppm applications respectively. Nitrogen losses in this experiment therefore seemed to be minimal and it was evident that the soybeans in this experiment did not require nitrogen applications greater than 120 ppm.

When applications of 100 ppm N were made at flowering very low residual nitrates were observed in the soil. This further confirmed that soybeans obtained a large percentage of their fertilizer requirements after the flowering stage of development. A significant increase in residual nitrates left in the soil was observed when 100 ppm N was applied at early pod fill as compared to flowering. As with utilization of the fertilizer this result was likely caused by the short time period the plants were exposed to the applied fertilizer.

Results indicate that the maximum nitrogen requirement of Maple Presto soybeans in the growth chamber was 120 ppm N or 600 mg N applied per pot plus whatever available nitrogen came from the soil. This was

TABLE 31

RESIDUAL SOIL NITRATE NITROGEN IN GROWTH CHAMBER EXPERIMENT B

Nitrogen Applied (ppm)	Time of Application	Residual Nitrate Nitrogen (ppm)
0	Seeding	4.9 ab*
20	Seeding	5.3 ab
40	Seeding	3.9 ab
60	Seeding	4.3 ab
100	Seeding	12.0 b
240	Seeding	127.3 d
360	Seeding	274.0 e
0 (1)	Seeding	3.3 ab
60 (1)	Seeding	0.9 a
120 (1)	Seeding	0.7 a
100	Flowering	4.1 ab
100	Early pod fill	33.5 c

(1) Barley
* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

the plants requirment up to the mid pod fill stage of development and resulted in 521 mg of actual nitrogen being taken up in the aerial dry matter per pot (from Table 29). Since there were two plants per pot the total uptake per plant was 261 mg N. Field observations of plant populations indicated approximately 48 plants per 3 metres of row with 61 cm row spacing. Aerial portions of field soybeans would require approximately 68 kgN/ha up to early pod fill based on the total nitrogen uptake of 261 mg N/plant in the growth chamber. Observations of total N uptake of aerial portions of soybeans in the field at this stage of development concur with this estimate, values ranging from 67 to 88 kgN/ha when 100 kgN/ha was applied. Yield and protein content of field grown soybeans did not appear to be limited at this stage of growth when 100 kgN/ha was applied. These observations suggest a very loose comparison can be made between 100 kgN/ha in the field and optimum rates in the growth chamber. Soybeans in the field were shown to have a total nitrogen uptake of 130 to 140 kgN/ha at the stage when leaf senescence was occurring with applications of 100 kgN/ha. If plants in the growth chamber were grown to maturity the optimum nitrogen application may have exceeded 120 ppm. These results all indicate that apart from added nitrogen Maple Presto soybeans do not have the capacity to produce high yield and protein content.

4.2.3 Measurements of Symbiotic Nitrogen Fixation in Growth Chamber Studies

Symbiotic nitrogen fixation in the growth chamber was evaluated by qualitative and quantitative methods. Qualitative measurements of nitrogen fixation were carried out in the form of nodule rating after aerial portions of plants were harvested (Table 32). Nodulation generally appeared more abundant in the two growth chamber experiments than it did under field conditions. This may have been the result of a more optimum soil moisture regime in the growth chamber. Optimum soil moisture has been shown to be very important in maximizing nodulation in soybeans (Lyons and Early, 1952; DeMooy et al., 1973).

Nodulation was not inhibited in either growth chamber experiment with nitrogen applications of 120 ppm or less at seeding. Only with an application of 200 ppm N was nodulation observed to be substantially decreased in Experiment A. In experiment B nodules began to decrease in size as well as number with applications of 240 ppm N. These high rates were toxic to plant growth, therefore it seemed that as long as nitrogen applications did not exceed the plants need a large number of nodules were present.

Nodules appeared viable at time of harvest as indicated by the leghaemoglobin observed when they were dissected. Values calculated for symbiotic nitrogen fixation in these experiments must therefore be viewed as nitrogen fixed up to the mid-pod fill stage of development and not the total capacity of the plant to fix nitrogen. If symbiotic fixation profiles in the growth chamber were similar to those observed in the field experiments, then the soybeans were harvested at the stage of development when the greatest amounts of nitrogen were being fixed.

TABLE 32

NODULE RATING IN GROWTH CHAMBER STUDIES

EXPERIMENT A			EXPERIMENT B		
Nitrogen Applied (ppm)	Time of Application	Nodule Rating (1)	Nitrogen Applied	Time of Application	Nodule Rating (1)
0	Seeding	5	0	Seeding	5
20	Seeding	5	20	Seeding	5
40	Seeding	5	40	Seeding	5
60	Seeding	5	60	Seeding	5
100	Seeding	4	120	Seeding	5
200	Seeding	2	240	Seeding	4
60	7 weeks	5	360	Seeding	2
60	Flowering	5	100	Flowering	5
60	Early Pod Fill	5	100	Early Pod Fill	5

(1) Nodule Rating Scale: 0 = no evidence of nodulation, 1 = 1-4 nodules, 2 = 5-8 nodules, 3 = 9-12 nodules, 4 = 13-16 nodules, 5 = 17 or more nodules.

Based on the ethylene curves observed from the field experiments 68% of the total fixation calculated had occurred to the pod filling stage of development. The fixation values calculated for different treatments in the growth chamber must therefore be qualified in that the extent of calculated differences between treatments at maturity is not known.

Symbiotic nitrogen fixation was measured by two quantitative methods in the growth chamber. These methods were the ^{15}N "A" value method and the difference method or ^{15}N assisted difference method where fertilizer was applied. Calculated "A" values used in the ^{15}N "A" value method are reported in Table 33.

Significant differences occur in "A" values among different nodulating soybean treatments in both experiment A and B. "A" values for these plants represent both soil and fixed nitrogen in terms of the fertilizer standard. Differences among them represent differences in symbiotic fixation, soil nitrogen being constant. Decreasing "A" values with nitrogen applications of 20 to 120 ppm in both growth chamber experiments indicate fixation was depressed with nitrogen fertilizer addition.

The "A" values for the barley treatments represented soil nitrogen in fertilizer equivalent units. Significant differences between the soybeans and barley should therefore reflect the actual symbiotic nitrogen fixation which occurred. The "A" value for barley in experiment B appeared to decrease when 120 ppm N was added as compared to the 60 ppm application although this was not significant. "A" values are expected to remain constant with variable fertilizer additions, this being a major reason for using this method of calculation (Fried and Broeshart,

TABLE 33

THE EFFECT OF NITROGEN APPLICATIONS ON CALCULATED "A" VALUES IN GROWTH CHAMBER STUDIES

EXPERIMENT A			EXPERIMENT B		
Nitrogen Applied (ppm)	Time of Application	"A" Value (NH_4NO_3 Equivalent Units)	Nitrogen Applied	Time of Application	"A" Value (NH_4NO_3 Equivalent Units)
20	Seeding	481 c*	20	Seeding	359 d*
40	Seeding	415 bc	40	Seeding	258 cd
60	Seeding	392 bc	60	Seeding	251 cd
100	Seeding	210 ab	120	Seeding	135 abc
200	Seeding	204 ab	240	Seeding	542 e
60	7 weeks	384 bc	360	Seeding	528 e
60	Flowering	290 abc	60 (1)	Seeding	150 abc
60	Early Pod Fill	1237 d	120 (1)	Seeding	31 a
60 (1)	Seeding	168 a	100	Flowering	105 ab
60 (2)	Seeding	187 ab	100	Early Pod Fill	208 bc

* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

(1) Barley

(2) Uninoculated Soybeans

1975). A large amount of nitrogen can be added to the non-fixing plant to promote the growth of a healthy plant while a small amount of nitrogen can be added to the fixing plant so as not to greatly inhibit fixation. The reason for a reduction in the "A" value for barley in experiment B is not apparent.

In experiment A a high "A" value was reported when 60 ppm N was added at early pod fill. This large amount of available soil nitrogen calculated was a result of the decreased nitrogen derived from fertilizer. Since the fertilizer nitrogen was applied at a late stage and utilization was very low the assumption that soil and fertilizer nitrogen were utilized by the plant in proportion to their respective availabilities was not valid. When this assumption was satisfied the time of application did not have a significant effect on the "A" value as shown by values calculated for 60 ppm N applied at 7 weeks and flowering. Proportional uptake of fertilizer and soil nitrogen in the growth chamber is not a problem as long as all the available nitrogen is utilized by the plant.

High calculated "A" values were reported in experiment B when 240 and 360 ppm N were added at seeding. It was expected that decreased nodulation would result in decreased symbiotic fixation and, therefore, lower "A" values relative to the treatments with lower amounts of added nitrogen. The high values cannot be explained by nitrogen loss since essentially all the fertilizer nitrogen was accounted for by residual soil nitrates. The fertilizer in these treatments was toxic to plant growth and fertilizer nitogen uptake was reduced relative to the 120 ppm

N application. Fertilizer nitrogen was also applied to the bottom half of the soil volume. It is possible that the roots stayed away from the fertilizer nitrogen creating a difference in the availability of soil and fertilizer nitrogen. This situation would cause an increase in the "A" values calculated.

When calculating symbiotic fixation by the difference method in experiment A, barley was used to measure the amount of soil nitrogen that was available to the nodulating soybeans (Table 34). As was discussed earlier, significant differences were observed between barley and uninoculated soybeans with respect to soil nitrogen uptake. This suggested that the higher values for the uninoculated soybeans were caused by contamination with viable rhizobium and they were consequently not used as a standard. Calculated values for symbiotic fixation in experiment A using both the ^{15}N "A" value method and the difference method are reported in Table 35. Symbiotic fixation was observed to occur, particularly when low amounts of fertilizer nitrogen were applied or when nitrogen application was delayed to 7 weeks or later. Fixation was observed to decrease significantly as nitrogen applications were increased. The high total nitrogen uptake discussed earlier for plants with no supplementary fertilizer nitrogen is further evidence to support the presence of symbiotic fixation in this treatment. This uptake decreased as nitrogen was applied in larger amounts indicating that fixation was being inhibited without a corresponding increase in fertilizer nitrogen uptake. This may explain why yields were not significantly increased and protein content was reduced with nitrogen additions of 20, 40 and 60 ppm.

TABLE 34

SOIL NITROGEN UPTAKE AS MEASURED BY UNINOCULATED SOYBEANS AND BARLEY IN GROWTH CHAMBER EXPERIMENT A

Nitrogen Applied (ppm)	Soil Nitrogen Uptake (mg/pot)
0 (1)	146 b*
60 (1)	131 b
0 (2)	104 a
60 (2)	97 a

(1) Uninoculated Soybeans
(2) Barley
* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

TABLE 35

SYMBIOTIC NITROGEN FIXATION AS CALCULATED BY THE DIFFERENCE METHOD AND THE ^{15}N "A" METHOD IN GROWTH CHAMBER EXPERIMENT A

Nitrogen Applied (ppm)	Time of Application	Aerial N from Fixation by the Difference Method (mg/pot)	Aerial N from Fixation by the "A" Value Method (mg/pot)
0	Seeding	206 c*	-
20	Seeding	161 bc	188 cd
40	Seeding	118 abc	137 cd
60	Seeding	85 ab	113 bc
100	Seeding	99 ab	28 ab
200	Seeding	58 a	15 a
60	7 weeks	162 bc	155 cd
60	Flowering	141 abc	96 abc
60	Early Pod Fill	138 abc	241 d

* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

It is interesting to note that although applications up to 60 ppm were observed to inhibit fixation, they were not observed to reduce the number of nodules present on the tap root. It would appear that at these rates, growth and functioning of the nodules was inhibited more than nodule formation.

Both methods of calculation resulted in similar values for the amount of nitrogen fixed when nitrogen applications between 20 and 60 ppm N were made. With applications of 100 and 200 ppm N the "A" value method was observed to produce lower values for symbiotic nitrogen fixation than the ^{15}N assisted difference method. The lower values calculated by the "A" value technique appear more reasonable in light of the reduced nodulation observed in these two treatments. It might be suggested that the possible fixation of fertilizer nitrogen by the soil discussed earlier may be a reason for this difference. As the fixing capacity of the soil was exceeded both soil and fertilizer uptake may have increased relative to the barley control which was fertilized with lower rates of nitrogen. This assumes that soil and fertilizer nitrogen are fixed in equal proportion. The result would be high values for symbiotic fixation when calculated by the difference method because this method is based on the total nitrogen uptake of the soybeans and barley. "A" values for the soybeans and barley would not change since only ratios of soil and fertilizer nitrogen are used in their calcalation.

Differences were also observed between the two methods when fixation was calculated at early pod fill. Fixation calculated by the "A"

value method was excessively high because the utilization of fertilizer was not proportional to the fertilizers availability since it was applied so close to harvest. The assumption that plants took up nitrogen in proportion to its availability was not valid. The difference method, however, involved total nitrogen uptake of the soybeans and produced reasonable values for symbiotic nitrogen fixation because the fertilizer uptake was subtracted from the total irrespective of how much the plant utilized. Fixation as calculated by the ^{15}N assisted difference method with 60 ppm N applied at early pod fill was 68 mg less than with no nitrogen addition. Fertilizer uptake by this treatment was only 13 mg (Table 27) showing the suggestion that decreased yields at early pod fill resulted from inhibition of fixation without corresponding fertilizer uptake may be a reasonable explanation of the results.

Barley was again used in experiment B to determine the amount of soil nitrogen that was available to soybeans which were actively fixing nitrogen (Table 36). Values were statistically the same when no nitrogen was applied or when 60 ppm was applied. As with the trends observed when calculating available soil nitrogen by the "A" value method, it is not apparent why soil nitrogen uptake was significantly less when 120 ppm N was added. Uptake would be expected to be the same as lower treatments since the plants nitrogen requirements were not exceeded and there is no reason why soil nitrogen would be lost from the system without corresponding fertilizer losses.

TABLE 36

SOIL NITROGEN UPTAKE AS MEASURED BY BARLEY IN GROWTH CHAMBER EXPERIMENT B

Nitrogen Applied (ppm)	Soil Nitrogen Uptake (mg/pot)
0	117 b*
60	96 b
120	24 a

* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

Calculated values for symbiotic nitrogen fixation in experiment B using the ^{15}N "A" value method and the difference method are reported in Table 37. Fixation was reduced with added fertilizer nitrogen although this response did not appear as significant as it did in experiment A. As in experiment A the number of nodules did not decrease until higher rates of nitrogen were added. This might be expected more so in experiment B since the nitrogen was applied away from the seed and only the tap root was sampled when counting nodules. Harper and Cooper (1971) observed this response when nitrogen was placed away from the seed. The significant increases observed in dry matter yield with added fertilizer were an indication that the nitrogen supplied by symbiotic fixation was not sufficient to meet the plants requirements. Both methods of calculation produced similar values for symbiotic nitrogen fixation. The values calculated for treatments where 240 and 360 ppm N were applied were perhaps higher than expected in light of the decreasing trends in fixation with added nitrogen and the fact that these plants were neither larger nor contained more total nitrogen than plants to which 120 ppm was applied. The possible reason for this was dealt with when discussing the high "A" values observed for these treatments. Late applications of nitrogen did not significantly decrease symbiotic nitrogen fixation when compared to treatments where no nitrogen application was made. This is important since it shows that nitrogen management in terms of time of application has an influence on efficient fertilizer uptake as well as efficient symbiotic fixation.

TABLE 37

SYMBIOTIC NITROGEN FIXATION AS CALCULATED BY THE DIFFERENCE METHOD AND THE ^{15}N "A" METHOD IN GROWTH CHAMBER EXPERIMENT B

Nitrogen Applied (ppm)	Aerial N from Fixation by the Difference Method (mg/pot)	Aerial N from Fixation by "A" Value Method (mg/pot)
0	143 b*	-
20	118 ab	141 a
40	100 ab	83 a
60	91 ab	75 a
120	70 a	74 a
240	130 ab	144 a
360	66 a	85 a
100 (1)	128 ab	64 a
100 (2)	179 b	102 a

(1) Nitrogen applied at flowering
(2) Nitrogen applied at early pod fill
* Duncan's Multiple Range: Numbers followed by the same letter are not significantly different at P = 0.05

The percent of total aerial nitrogen derived from fixation was generally higher in experiment A than experiment B (Table 38). This was a result of the much greater increases in total nitrogen uptake with added fertilizer in experiment B. In this experiment the nitrogen percentage derived from fixation rapidly decreased with added nitrogen. Late applications of nitrogen in both experiments resulted in a substantial percentage of the plants nitrogen coming from symbiotic fixation. Soybeans receiving no nitrogen fertilizer fixed 103 and 72 mg/plant or 59 and 55 percent of their N content respectively for experiment A and B.

Results from growth chamber studies indicated that soybeans were fixing significant amounts of nitrogen where little or no nitrogen fertilizer was added. It was evident that fixation did not supply adequate nitrogen to the plants to meet requirements for maximum dry matter yield and protein content. Maximum dry matter yield and protein content occurred with the addition of 120 ppm N at seeding at which point fixation supplied 14% of the plants nitrogen. Equally high yield and protein content were obtained when 100 ppm was applied at flowering and symbiotic fixation was increased to 22% of the total in the plant.

TABLE 38

PERCENT OF TOTAL AERIAL NITROGEN DERIVED FROM SYMBIOTIC FIXATION IN GROWTH CHAMBER STUDIES

Nitrogen Applied (ppm)	Time of Application	Experiment A	Experiment B
0	Seeding	59 (1)	55 (1)
20	Seeding	59	47
40	Seeding	50	24
60	Seeding	45	18
100	Seeding	8	-
120	Seeding	-	14
200	Seeding	4	-
240	Seeding	-	29
360	Seeding	-	21
60	7 weeks	48 (1)	-
60	Flowering	43 (1)	-
60	Early Pod Fill	49 (1)	-
100	Flowering	-	22 (1)
100	Early Pod Fill	-	36 (1)

(1) Percents were calculated using Aerial N from Fixation by the Difference Method. All other values used fixation as calculated by the ^{15}N "A" value method.

5. Summary and Conclusions

Maple Presto soybeans are an early maturing variety of soybeans which were developed specifically for cool climate conditions such as those found in Manitoba, which is considered to be the northern limit for soybean production. This early maturing variety growing under long day, cool climate conditions presents a situation which is different from many previous investigations carried out in the U.S.A.

Field and growth chamber work was undertaken with the objective of looking at yield, protein content and symbiotic nitrogen fixation of Maple Presto soybeans in response to the addition of nitrogen fertilizer. The work conclusively showed that the seed and dry matter yield of Maple Presto soybeans could be increased with the addition of nitrogen fertilizer. Growth chamber studies revealed that dry matter yields at the early pod fill stage of development increased with increasing amounts of nitrogen added until toxic levels of nitrogen were reached with applications of 240 ppm N. In field studies the highest applications of 100 kg N/ha continued to promote dry matter yield increases and did not induce nitrogen toxicity. Seed yield response was a reflection of dry matter increases during the growing season when nitrogen was added. Seed yield increases were highly significant with additions of 30 and 100 kg N/ha on soils low to moderate in NO_3-N. Increases were consistently in the order of 400 kg/ha with 100 kg/ha of added nitrogen, maximum production of seed being 1942 kg/ha. It is apparent from these results that Maple Presto soybeans require supplemental nitrogen to

produce maximum seed yields on soils low to moderate in NO_3-N, and that amounts as low as 30 kgN/ha will significantly increase seed yield.

Significant differences in dry matter protein content with increasing amounts of nitrogen added occurred at early stages of development, but these disappeared as the season progressed and yield differences became greater. Differences in protein content among treatments at later stages of development only occurred when sufficient nitrogen was applied to realize maximum yield as well as increase protein. The seed protein content of Maple Presto soybeans was increased in some cases as a result of nitrogen fertilizer addition. This was observed to occur when 100 kgN/ha was applied to a soil with moderate amounts of NO_3-N. On soils low in NO_3-N only seed yield and no protein content was increased with added nitrogen.

The seed protein content of Maple Presto soybeans has been reported to be below the standards required for processing in Manitoba. Experiments confirmed that seed protein levels were well below the acceptable processing level of 40% when nitrogen was not applied but the addition of 100 kgN/ha could increase protein content to this level on soil with moderate amounts of NO_3-N. Therefore, on soils low to medium in NO_3-N, nitrogen fertilizer is required in amounts greater than or equal to 100 kgN/ha for acceptable seed protein levels to be reached.

Maple Presto soybeans, inoculated with an effective Rhizobia strain, fixed up to 51 kgN/ha or 66% of their total nitrogen content. These soybeans fixed substantial amounts of nitrogen subsequent to the flowering stage of development, maximum fixation occurring at the mid pod fill stage of development. Approximatley 68% of the plant's total

fixation appeared to have taken place to the mid pod fill stage. The period of highest fixation activity corresponded with the period of greatest nitrogen and dry matter accumulation. Maximum fixation therefore seemed to correspond to the period of maximum nitrogen requirement for Maple Presto soybeans.

Experiments showed that the total nitrogen in the dry matter increased with added fertilizer nitrogen. The percent of this total which was symbiotically fixed nitrogen and the actual amount of nitrogen fixed decreased when nitrogen fertilizer was applied in larger increments. The amount fixed when no nitrogen fertilizer was applied was not enough to realize maximum yield and protein production by the soybeans. Applications of 100 kgN/ha reduced nitrogen from symbiotic fixation to a very small proportion of the plants total nitrogen.

The combination of methods used to evaluate symbiotic nitrogen fixation (^{15}N "A" value method, ^{15}N assisted difference method, classical difference method, acetylene - ethylene assay and nodule rating technique) gave a reasonable portrayal of symbiotic fixation in Maple Presto soybeans. Nodule rating evaluated the number of nodules on the tap root and appeared to be an effective indication of relative fixation among treatments. The acetylene - ethylene assay carried out on these same roots was a somewhat more precise determination of relative fixation among treatments, accounting for the total nodule activity. Results obtained from these two techniques produced seasonal symbiotic nitrogen fixation curves which decreased as increasing increments of nitrogen were added. The ^{15}N "A" value, difference and ^{15}N assisted difference

techniques produced quantitative values for the total amount of symbiotic nitrogen fixation. The values were reasonable in light of the yield and protein responses when soybeans were inoculated as opposed to uninoculated and when nitrogen was applied. The responses in themselves would lead to the expectation that symbiotic nitrogen fixation was occurring but was not adequate to meet the plant's requirements if maximum production was to be attained.

It is evident from this work that this early maturing variety of soybean is not capable of producing maximum seed yield or protein content apart from high soil nitrogen or fertilizer nitrogen addition. Nitrogen can be considered a limiting factor in producing this early maturing variety.

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