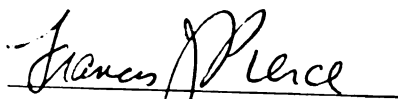




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Nitrogen dynamics in and under a fixing dry bean

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**N DYNAMICS IN AND UNDER A FIXING DRY BEAN
USING ^{15}N AS TRACER**

By

Olaf Erik Martinson

A THESIS

**Submitted to
Michigan State University
in partial fulfillment of the requirements
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ABSTRACT

N DYNAMICS IN AND UNDER A FIXING DRY BEAN USING ^{15}N AS TRACER

By

Olaf Erik Martinson

The dry bean (*Phaseolus vulgaris*) is a cash crop in Michigan, a source of protein around the world and a fixer of N_2 . It would be useful to understand N_2 fixation in the dry bean under the conditions in which it is cultivated. A field experiment was conducted to test the effects of N fertilization and tillage on dry bean (*Phaseolus vulgaris*) growth, soil physical properties, N_2 fixation, yield, and N dynamics. This was followed by a greenhouse study to confirm results and test the suitability of a mutant soybean and a mutant dry bean as controls. Isotope Dilution of ^{15}N was used to estimate N_2 fixation, which was unaffected by tillage or fertilizer. The average N derived from the atmosphere (N dfa) at harvest was found to be 35% of the N in the dry bean shoot, and 60% of shoot N dfa accumulated after pod-fill. The mutant soybean was found to be the better control due to similarity in maturation periods with the navy bean test crop. A scheme of N movement was proposed to describe N_2 fixation in well fertilized plots.

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Finally I'd like to thank the friend who convinced me to go try again.

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INTRODUCTION

Cultivation of the dry bean (*Phaseolus vulgaris*) accounts for over \$100 million per year of farm income in the state of Michigan (Kelly, 1992). Of the 140,000 ha of Michigan land under dry bean cultivation in 1992, 80% was for navy bean production (Kelly, 1992). Additionally, the dry bean is a major source of protein world-wide, combining desirable flavor with a high N content.

Trends within Michigan now demand cultivation of the dry bean with lower uses of fertilizer (Kelly, 1992). Likewise in developing countries, fertilizers are often limited or unavailable. Cultivation practices and cultivars that maximized N₂ fixation could fill a need both in Michigan and around the world.

In order to maximize fixation more knowledge is needed about the mechanisms and dynamics of a fixing dry bean. Any experiments done on N₂ fixation, however, must rest on the accuracy of one of the methods of estimating fixation.

Choice of N₂ fixation Measurement: The goal of increasing N₂ fixation should be to increase the total N fixed by the end of the season. It is insufficient to find the rate of

fixation on a given day. Fixation may change from day to day based on the matric potential (Prankhurst and Sprent, 1975), the maturation of the plants tested (Graham and Rosas, 1977; Harper et al., 1989) or on the temperature. What is needed is a measure of the accumulated N_2 fixation up through harvest.

Of the methods of measuring accumulated N_2 fixation (Total Nitrogen Difference, Isotope Dilution and "A" value) Isotope Dilution (ID) has been shown to be the most consistent and best suited to field measurements (Rennie and Rennie, 1983; Boddey et al., 1989; Rennie, 1984).

^{15}N Isotope Dilution: Estimates by ID involve enriching a fertilizer with ^{15}N . (For this reason ID can't measure N_2 fixation on a no-fertilization experiment.) The ^{15}N label found in the plant is diluted by soil and atmospheric nitrogen. Greater fixation yields greater dilution of the isotopic label.

The fertilizer is applied to a test crop (a fixing system or "fs") and to a control crop (a non-fixing system or "nfs"). The fertilizer and soil nitrogen combine to make one pool of nitrogen. Unlike "A" value measurements, ID has no need to estimate the ^{15}N ratio in this available N pool. Instead ID assumes that the fs and nfs crops each take up nitrogen with the same ^{15}N ratio. In effect the nfs control is the measure of the ^{15}N label on the soil-and-fertilizer nitrogen pool.

Because ^{15}N is a naturally occurring isotope, the ratio of the isotope to total N is not the important label. The label is the percentage of N as ^{15}N above the natural occurring amount (0.3663%). Each time the label is diluted it is diluted with more N at 0.3663% ^{15}N . All calculations must then use the percent of ^{15}N that is in excess of natural abundance. Rennie and Rennie (1983) use the following definition:

$$\text{atm}\%^{15}\text{N ex} = \text{atm}\%^{15}\text{N}(\text{sample}) - \text{atm}\%^{15}\text{N}(\text{natural}). \quad [1]$$

Within the fixing system, the percent nitrogen derived from the soil-and-fertilizer pool is thus the ratio of the label on the fs over the label in the pool. The nitrogen derived from the atmosphere would then be 100% minus that from the s-a-f pool. Finally, the label in the nfs control is the measure of the s-a-f label and can be substituted directly. As per Rennie (1984) this yields the following:

$$\% \text{Ndfa} = (1 - [\text{atm}\%^{15}\text{N ex}(\text{fs}) / \text{atm}\%^{15}\text{N ex}(\text{nfs})]) \times 100\% \quad [2]$$

where %Ndfa is the percent N derived from the atmosphere.

The drawback to the ID technique is in the control. Theoretically, the nfs plant draws its nitrogen from the same pool as the fs plant and in the same ratio of labeled to unlabeled nitrogen. The non-fixing system should have roots that explore the same soil and have a similar uptake pattern for nitrogen (Witty, 1983). Furthermore it must

have a similar maturation.

Differences in maturation can be minimized by applying a slow-release form of ^{15}N fertilizer well before planting (Giller and Witty, 1986). Labeled organic nitrogen or nitrate trapped in gypsum work well. Solutions of $(^{15}\text{NH}_4)_2\text{SO}_4$ work less well. Gypsum pellets seem to be one of the best sources of ^{15}N label (Giller and Witty, 1986). Enrichment in ^{15}N declines exponentially through the course of the season (Witty, 1983). Slower release forms of nitrogen assure that the control and test crops are more likely to access the same pool.

Regardless of the N source, the choice of the control will determine the end result. Best results are obtained by choosing many controls and averaging the end results (Boddey et al., 1989.)

When using ID, the exact amount of N_2 fixation is always suspect. Fortunately relative measurements are consistent. If treatment A shows a higher level of N_2 fixation than treatment B, then it will continue to do so no matter what control crop is used in the calculation. ID is thus ideal for rating the relative fixation of different treatments.

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CHAPTER 1

Introduction

Although a legume, the dry bean (*Phaseolus vulgaris* L.) has long been considered a negligible fixer of dinitrogen. This poor reputation may stem from measurements of Acetylene Reducing Activity (ARA), which apparently under estimates fixation rates in dry beans (Rennie and Kemp, 1984). In the white bean variety of *P. vulgaris*, Smith and Hume (1987) found that ARA measurements of N_2 fixation were one tenth as large as measurements by Isotope Dilution (ID). They postulated that dry bean may be particularly vulnerable to acetylene poisoning. In fact, ID experiments indicate that the dry bean fixes 45% (Smith and Hume, 1987), over 60% (Rennie and Kemp, 1984), 34% to 69% (Rennie, 1984) or between 40% and 60% (Ruschel et al., 1982) of the nitrogen needed for growth. Rennie and Kemp (1984) remarked that with dry bean, "good yields can be obtained without the addition of fertilizer N."

Nevertheless recommendations for "starter fertilizer" on bean crops range from 45 kg N ha⁻¹ by Copeland and Leep (1982) to 80 kg N ha⁻¹ by Schild and Newland (1988.) Starter

fertilizer is apparently added to enhance the rapid development of the root system, which should then allow for increased N_2 fixation.

A starter fertilizer effect would then be an increased rate of N_2 fixation in a fertilized crop during the early stages of plant growth. Vasilas and Ham (1983) observed this effect at one sampling date at one of their locations in soybean. This location was on a loamy fine sand where "N was severely limiting." The crop also had to be replanted in July due to a late frost. In the second year at the same site higher N fertilization led to decreased fixation. While the Rennie and Kemp (1984) data could arguably be attributed to a starter fertilizer effect in dry bean, this effect was only seen in one cultivar at one sampling date. Additionally, in the following year the trend was reversed and higher fertilization led to lower fixation. By harvest neither Vasilas and Ham (1983) nor Rennie and Kemp (1984) found any significant effect of fertilization on yield, dry matter accumulation, total N uptake or %N in plant tissue. Schild and Newland (1988) report that N fertilization visually extends maturity and increases yield in field trials. They made no test for significance.

Most N fertilizers are rapidly catabolyzed to NO_3^- . Nitrate has been shown to inhibit N_2 fixation in dry bean (Streeter, 1988). Whether starter fertilizer inhibits or encourages fixation in dry bean remains unproven.

Meanwhile dry bean production is changing. New cultivars are being bred for an upright, bushy characteristic. Future breeding is expected to derive cultivars with increased N_2 fixation capacity (Kelly, 1992.) Cultural practices for dry bean are changing rapidly as well. Interest is increasing in the use of conservation tillage and narrow rows (Kelly, 1992.) Data on the effect of these changing practices on N_2 fixation in the dry bean is scarce. Conventional and no till cultivation have been shown to make no significant effect on fixation in soybean (Rennie et al., 1989.)

The objective of this research is to determine how tillage and N fertilizer affect N_2 fixation and the growth and development of an upright, bushy cultivar of dry bean.

Materials and Methods

A long-term experiment was initiated in 1985 at the Michigan State University Saginaw Valley Bean and Beet Experiment Station to evaluate tillage and row spacing effects dry beans following corn in rotation. The soil is a Misteguay silty clay (Aeric Haplaquent, fine, mixed, calcareous, mesic) that is well tilled. Three tillage systems - conventional tillage (CT), no-tillage (NT) and ridge tillage (RT) - were established in the 1985 growing season in corn. CT consisted of fall moldboard plowing with spring secondary tillage consisting of a single pass of a harrow prior to planting. CT treatments received one cultivation during the growing season. RT consisted of planting on ridges formed during the last cultivation of the previous crop and included one additional cultivation. NT consisted of slot planting directly into untilled soil.

In 1989, an new experiment was superimposed on the tillage study to evaluate nitrogen fixation by dry beans as a function of tillage systems and N fertilization rate. One subplot in the center of each tillage plot was split into two sections, one for dry beans and one for soybeans. Within the dry bean subplot, four plots 2m by 2.5m in size were randomly assigned one of four N fertilizer rates - 0, 28, 56 and 84 kg N ha⁻¹. The soybean subplot was divided into

3 plots 1.5m by 2.5m in size and randomly assigned one of three fertilizer rates: 28, 56, and 84 kg N ha⁻¹. The experimental design was a randomized complete split-plot block design with tillage as the main plots and N fertilizer rate as the subplots within tillage.

Seeds were planted in four rows with a 71 cm spacing in each tillage, in accordance with recommendations by Copeland and Leep (1982). Shoots emerged around ten days after planting. The shoots in each row were thinned to four plants per 30 cm. Four days after emergence the inner two rows of NT and CT plots were hand-sprayed with a solution of 60 g N L⁻¹ as (NH₄)₂SO₄ enriched to 1% ¹⁵N. The outer rows of NT and CT, as well as all rows of the RT subplots were also fertilized with unenriched (NH₄)₂SO₄ at the appropriate rate. All plots were then sprayed with additional water to insure that all received 0.5 cm of water.

Three soil probes of 2.5 cm were taken from each unfertilized plot at increments of 0-5 cm, 5-15 cm, 15-30 cm, 30-60 cm and 60-90 cm in order to obtain a preplant profile of inorganic N content at planting. Three soil samples per plot were taken to a depth of 30 cm using hand probes 2.5 cm in diameter at the following dates: within the vegetative stage, 19 days after emergence; at 50% flowering, 49 days after emergence; and at the beginning of senescence, 84 days after emergence. Using the procedure described by Fernandez and Gepts (1984) these dates

correspond to V4, R6 and R7. At harvest (R9), 100 days after emergence, three soil probes per plot were taken to a depth of 90 cm using a 5 cm diameter Giddings probe. Harvest soil samples were divided into the same depths as the pre-fertilization samples.

The plant stages described by Fernandez and Gepts (1984) will be used in this thesis to describe the soybean. Fernandez and Gepts did not describe the stages in order to apply them to soybean. In this case a mutant soybean was chosen that would mature at the same rate as the dry bean. When the Mayflower dry bean was in V4, R6 or R8, so was the soybean. For simplicity only one system of designating the plant stage will be used for the two crops.

Soil samples were air dried for a week and ground to pass through a 2mm mesh. Available nitrogen was extracted in a 1 N KCl solution as per Bremner (1959). The solutions were then analyzed with an flow injection analyzer for $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$. Additionally the field moist and dried weights of the harvest Giddings probings were used to estimate the volumetric moisture content and bulk density of the soil of all treatments.

At harvest, soil mechanical resistance was measured with a recording cone penetrometer on each treatment. The same day, intact soil cores of 7.6 cm diameter and 7.6 cm height were sampled in triplicate at two depths on the CT and NT: 0-7.6 cm and 7.6-15.2 cm. For ridge tillage, three

depths were taken from halfway off the ridge: 0-7.6 cm, 7.6-15.2 cm and 15.2-22.8 cm. The cores were weighed for field moisture content, saturated from the bottom for 48 hours, and weighed again to determine porosity at saturation. Moisture retention for matric potentials of -1 and -6 kPa were determined by the blotter paper tension table procedure as described by Leamer and Shaw (1941). For one replication the moisture retention at -2, -3, -4 and -5 kPa matric potential was determined by the same method. Also in that replication, moisture retention was measured at -10, -33.3 and -100 kPa matric potential by using pressure plates according to Klute and Dirksen (1986). After oven-drying for 48 hours at 105°C, the cores were weighed again to determine bulk density.

Shoot samples of all subplots were taken again at the following dates: in the vegetative stage at V4, 21 days after emergence; at 50% flowering (R6), 49-52 days after emergence; at the beginning of senescence (R8), 84-85 days after emergence; and at harvest (R9), 100 days after emergence. At harvest six shoots were taken from each treatment plot: three consecutive plants from each of the two inner rows. At all other dates four shoots were taken: two from each inner rows. At least two plants in each row (so at least 15 cm) stood between each sampling area and the next. At least four plants in a row (at least 30 cm) stood between a sampling area and the edge of the treatment plot.

Shoots were prewashed in water, washed in 0.1 g L⁻¹ lauryl sulfate solution, and rinsed again three times in distilled water. They were then straightened and measured to find the distance from the ground to the topmost node. The number of nodes on each plant was recorded, counting the cotyledons as one node. The number of trifoliate per plant was also recorded and, where applicable, so were the numbers of seeds and pods. Seed moisture at harvest was determined. At senescence and harvest shoots were divided into their components and all samples were oven dried at 55°C. They were chopped in a coffee grinder and ground in a UDY Cyclone Sample Mill.

Samples that had received ¹⁵N enriched fertilizer were weighed out into two determinations of 2.5 mg each and analyzed on a mass spectrometer according to the method described by Harris and Paul (1989). Estimates of N dfa were calculated as described on pages 2-4 of this thesis. Plant samples from ridge tillage plots were weighed out for Kjeldahl digests (Bremner and Mulvaney, 1982) and NH₄-N determined by a flow injection analyzer.

Results and Discussion

Topic one: Harvest data.

The most important results are often those that concern the plant at harvest. The final condition of the soil is also important as it will influence future seasons. Mid-season data will be discussed later.

In this experiment the dry bean and soybean at harvest indicated that there was no effect from either tillage or fertilization rate. Fertilization caused no significant effect on yield in either the dry bean or the soybean crop (Figure A.1). Neither was there a relationship between tillage and the yield in the dry bean. Table a.1 summarizes average dry weight and moisture content of the seeds. There were also no significant effects on the dry weight of any portion of the dry bean or on total dry weight (Figure A.2). Neither fertilizer nor tillage treatment caused a significant effect on percent N content in the dry bean (Figure A.3).

The dry bean yield of 4.5 Mg ha^{-1} was higher than the cultivar trials that have reported yields of 2.7 Mg ha^{-1} (Kelly et al. 1989) or 3.9 Mg ha^{-1} (Nuland and Carlson,

Table a.1: Summary of average dry bean and soybean yield measures and the mean square error for the population average (s_y)

<u>Harvest</u>	<u>Dry Bean</u>	<u>s_y</u>	<u>Soy Bean</u>	<u>s_y</u>
Above ground biomass (Mg ha ⁻¹)	6.92	1.1	5.61	1.1
Seed yield (Mg ha ⁻¹)	4.54	0.72	2.90	0.57
Seeds per kg	5,076	60	7143	200
Seed % moisture	27.0	7.1	46.1	5.6

1988.) It is important to note, however, that the results of our experiment assume a perfect stand.

The standard explanation of this situation would be

Table a.2: N derived from the atmosphere in the dry bean at harvest.

<u>Plant part</u>	<u>N dfa (mg plant⁻¹)</u>	<u>s_y</u>	<u>N dfa (kg N ha⁻¹)</u>	<u>s_y</u>	<u>%N dfa</u>	<u>s_y</u>
stems	18.3	2.2	3.77	0.41	36.6 %	4.2%
Pods	19.6	2.4	3.62	0.44	33.2 %	3.5%
seeds	333.3	45.8	61.50	8.45	34.4 %	4.6%
Total	368.3	46.8	67.96	8.64	34.2 %	4.2%

that the fertilizer N inhibited fixation. With so much N in the soil already, there would seem to be no reason for the dry bean to fix more. However by harvest fertilization neither increased nor decreased the N₂ fixation (Table a.2).

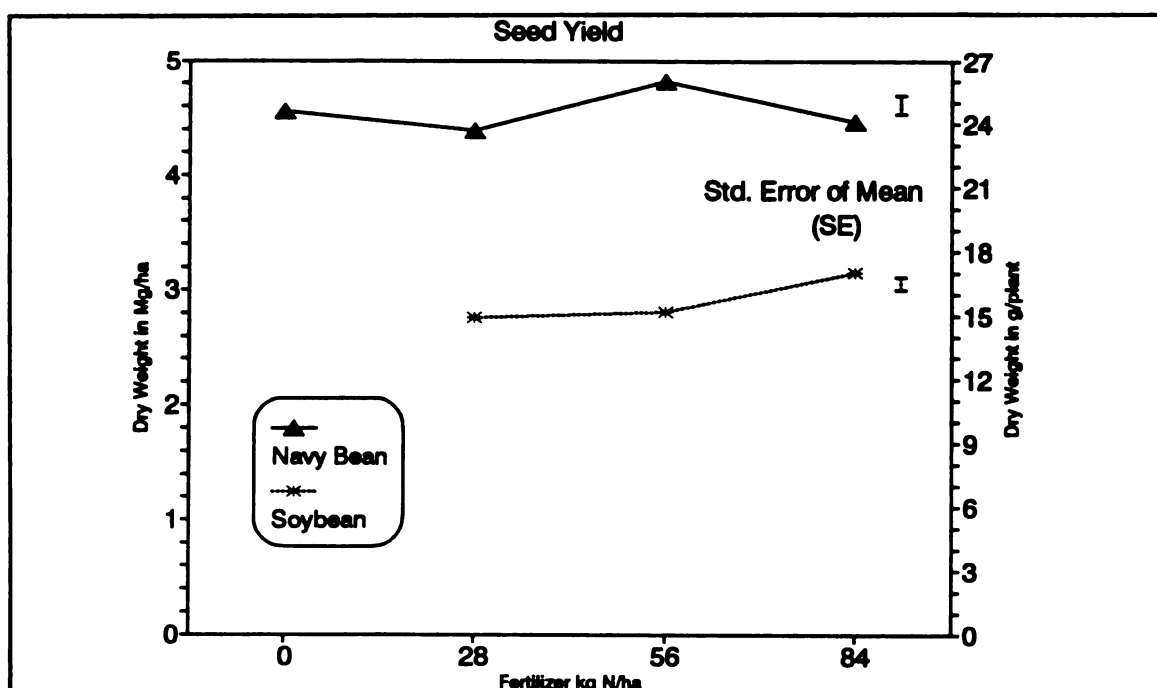


Figure A.1: Seed Yield in kg ha^{-1} for dry bean and soybean across fertilization rates of 0 to 84 kg N ha^{-1} .

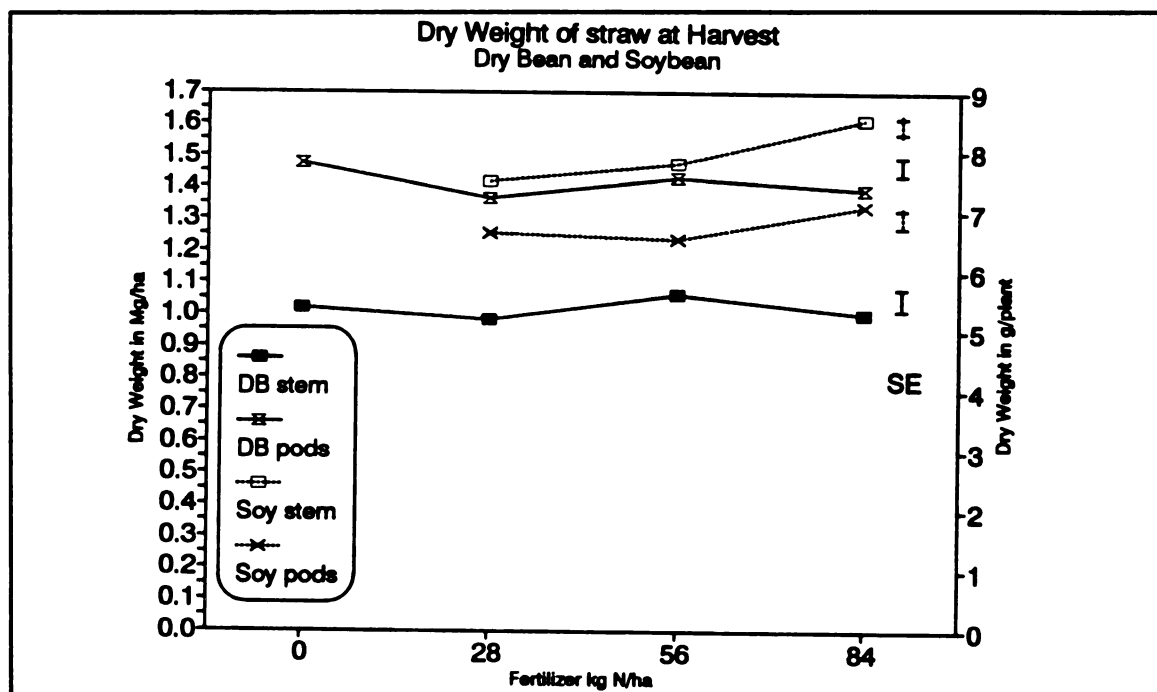


Figure A.2: Straw at harvest in kg ha^{-1} for dry bean and soybean across fertilization rates of 0 to 84 kg N ha^{-1} .

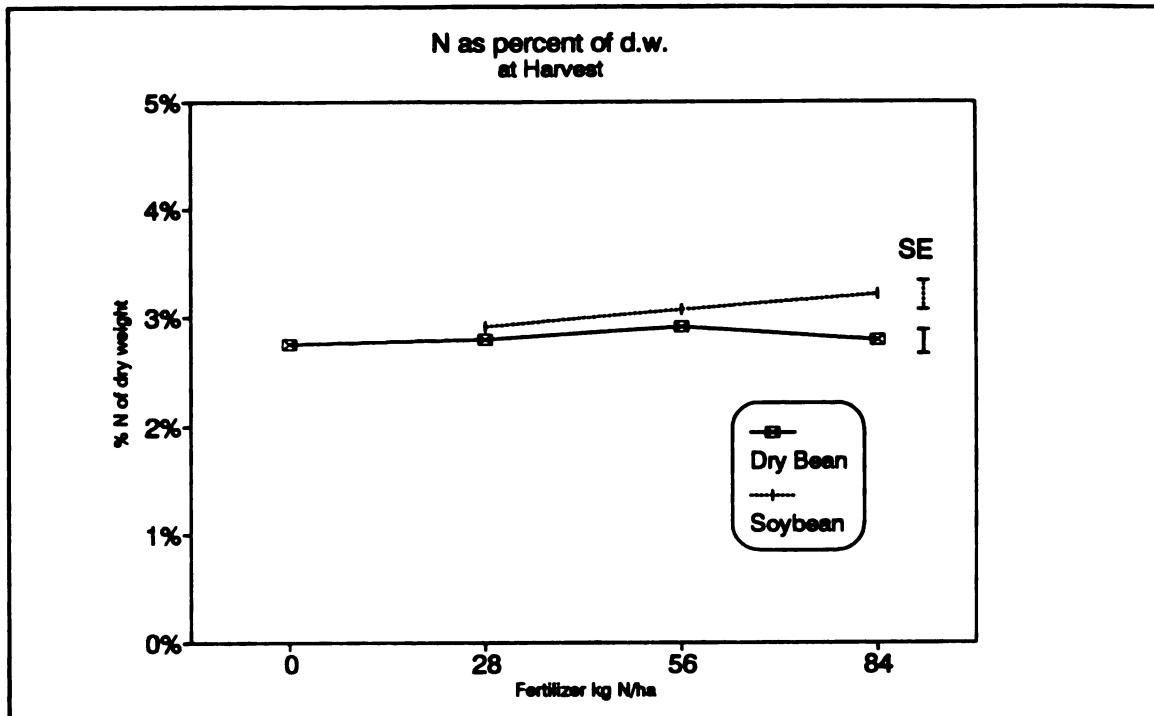


Figure A.3: Nitrogen concentration as a percentage of harvested dry bean and soy bean material.

The average share of N derived from the atmosphere was 34.2%. Other isotope dilution estimates of N_2 fixation in the dry bean include 44.4% for a white bean (Smith et al., 1987) and 40% to 60% for other cultivars (Rushel et al., 1982.) All these estimates contract sharply with the reputation dry beans have gained from acetylene reduction estimates of N_2 fixation. AR studies estimate that the dry bean fixes only 5% of its N (Rushel et al., 1982.)

The next possible conclusion is that the treatments failed to affect the root environment in this field and season. Perhaps the tillage treatments didn't affect the structure of the soil. Perhaps the fertilizer washed away

in the runoff. The following evidence leads me to believe that the treatments effectively modified the soil.

Fertilizer use: The fertilizer was incorporated into the plants and uptake of fertilizer was higher under the higher rates of fertilization (Figures A.4 and A.5.) Fertilizer use efficiency is approximately 30% in the dry bean and 45% in the soybean. In each fraction (seeds, stems, pods) of each crop, incorporation of fertilizer is linearly dependent on the rate of fertilizer applied.

Tillage also affected the percentage N derived from the fertilizer in the stems and pods of both crops at harvest (Figure A.6). NT plants consistently had a higher percentage of N dff.

Fertilizer effects on soil N: The higher percentage of N dff in no-till plants may be due to an increased availability of fertilizer in NT soils. Tillage and fertilizer had a interaction effect on NO_3 in the top 5 cm of the soil under the dry bean (Figure A.7). The significant cross effect in NH_4 at 30 to 60 cm under dry bean was caused by high levels in a single replication (Figure A.7). By the end of the season there were no more fertilizer effects on either NO_3 or NH_4 in the soil. Tillage had only one significant effect on soil N: between 15 and 30 cm deep in the soil NT had a higher quantity of NO_3 (Figures A.8 and A.9).

Tillage effects on soil: Tillage also affected the

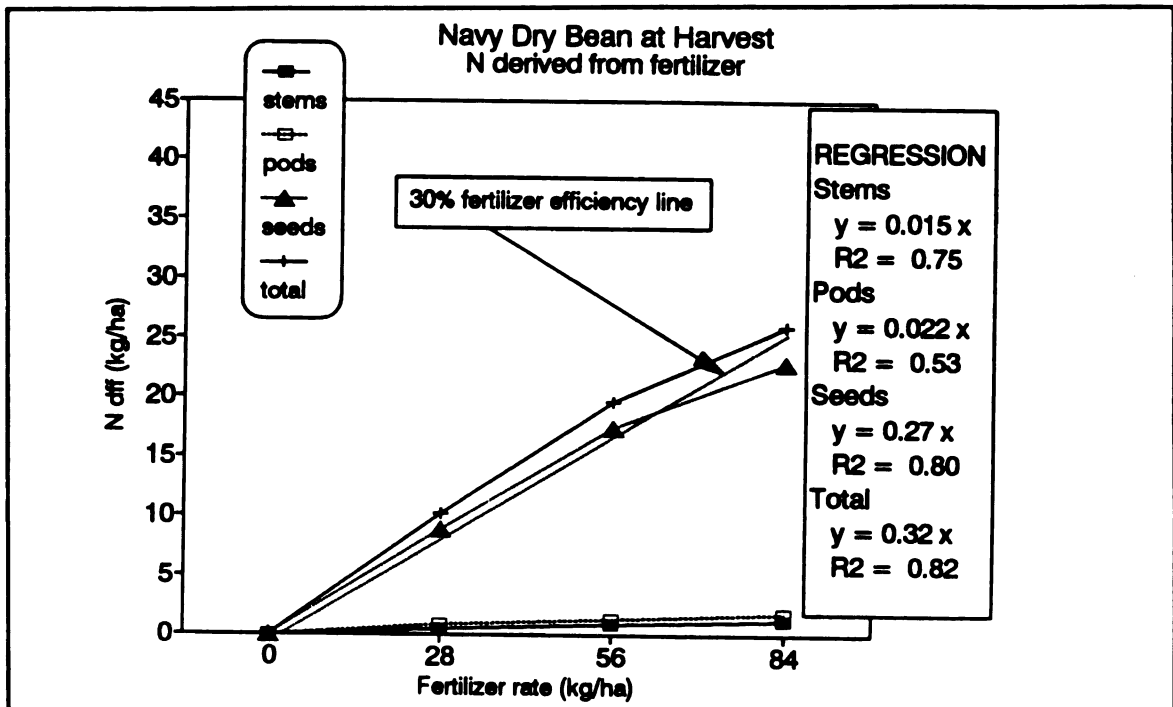


Figure A.4: N fertilizer incorporated into dry bean fractions at harvest. A line for 30 % fert. use efficiency is included.

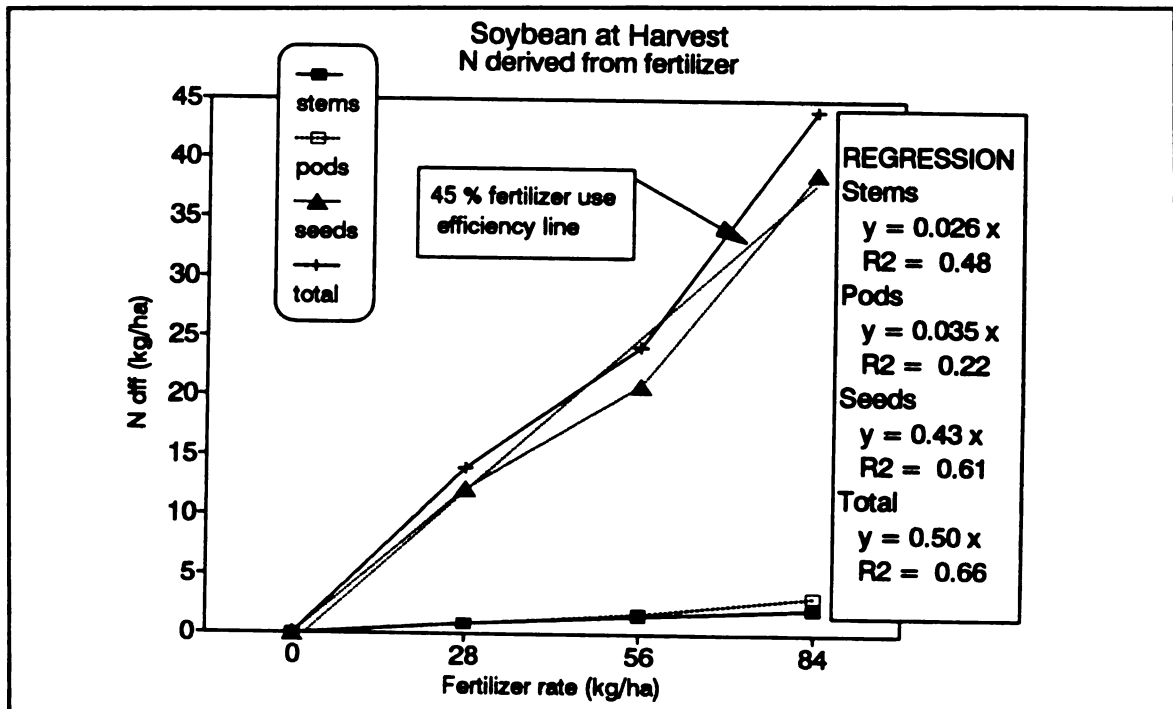


Figure A.5: N fertilizer in soybean at harvest.

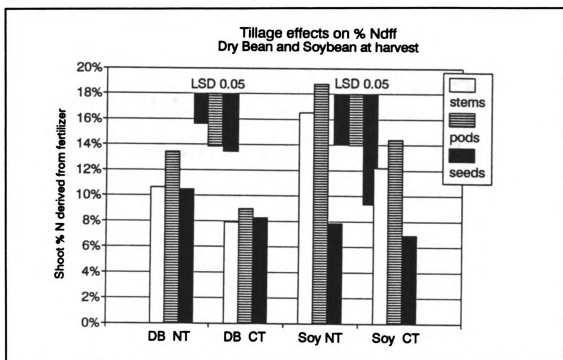


Figure A.6: Tillage effects on the share of N from fertilizer at harvest in Dry Bean (DB) and Soybean (Soy).

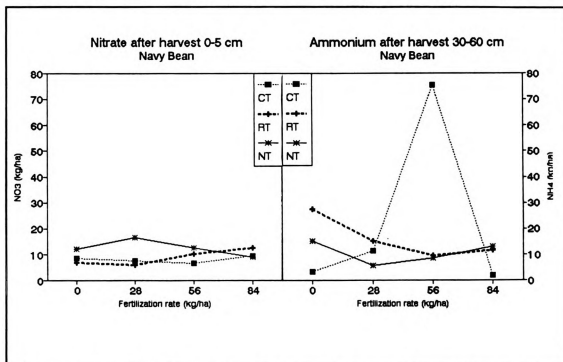


Figure A.7: Tillage-Fertilizer interaction effects on N under the dry bean at harvest.

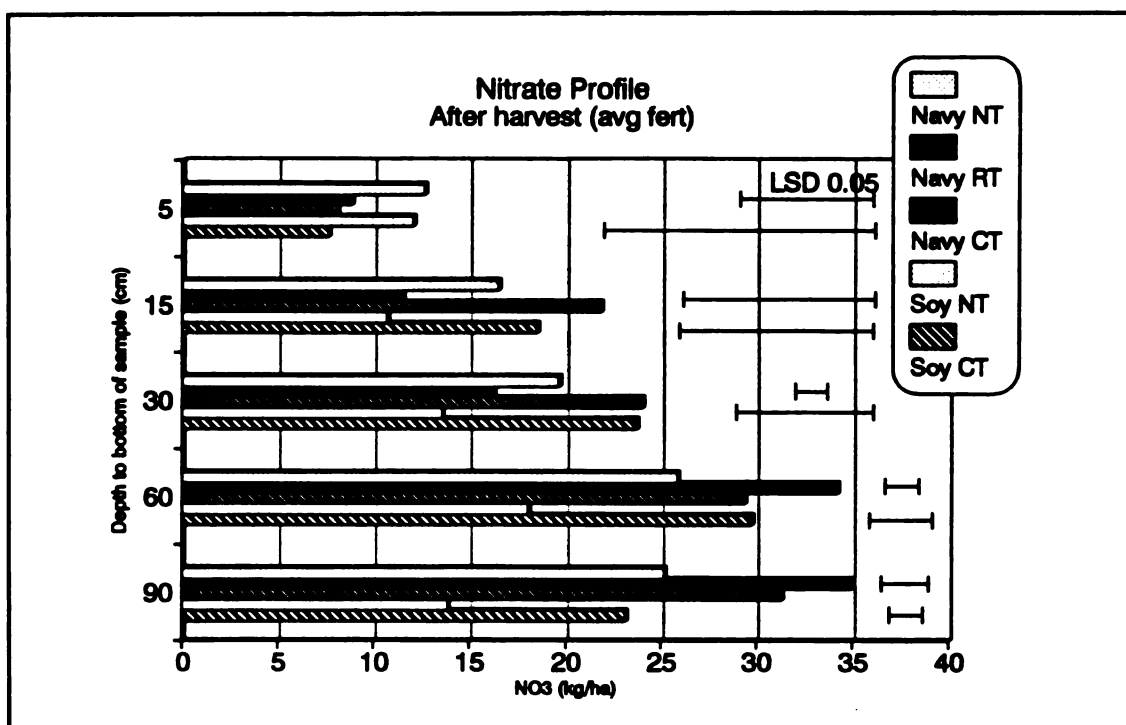


Figure A.8: Profile of the NO₃ quantity under soybean and dry bean by depth, averaged over fertilizer treatment.

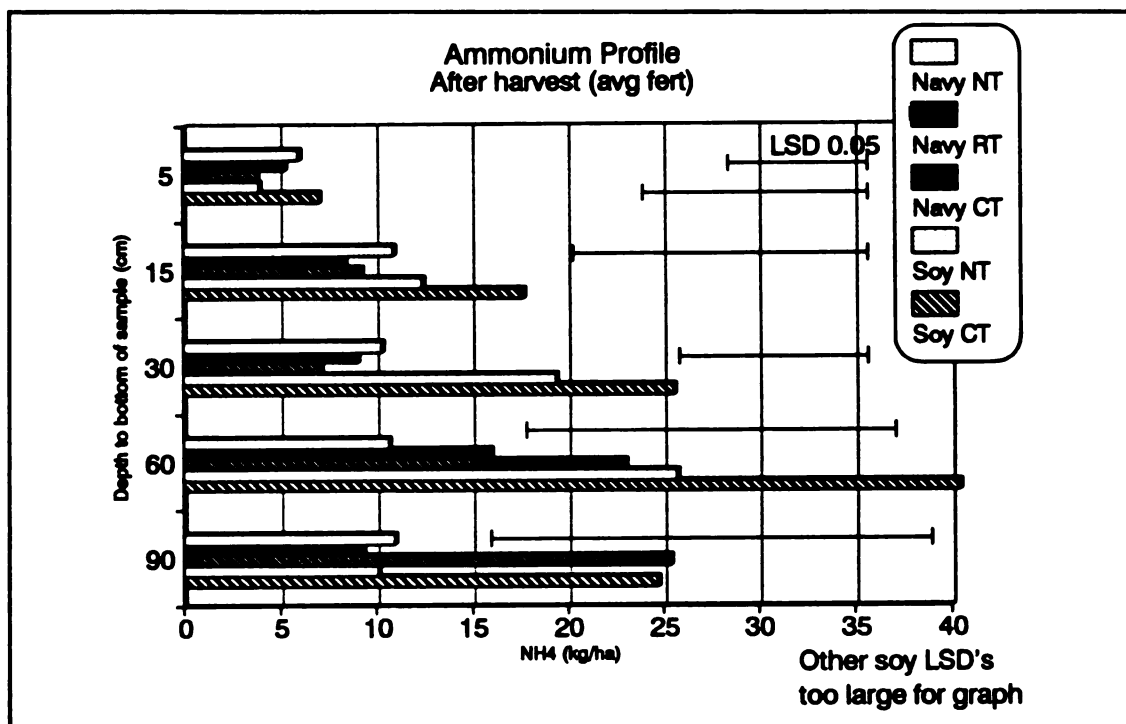


Figure A.9: Profile of the NH₄ quantity under soybean and dry bean by depth, averaged over fertilizer treatment.

physical state of the soil. Soil penetrometer resistance measurements taken at harvest show a higher mechanical resistance in the top 20 cm of no-till than conventional till plots (Figure A.10). RT resistances fell between the other two, mirroring no-till more closely than conventional till. The average volumetric moisture content at the time of resistance measurements were high but not different for the tillage treatments (Table a.3).

Table a.3: Data taken from soil cores for plots of No-Till (NT), Ridge Till (RT), Conventional Till (CT) and Ridge Till at the next depth (RT').

Tillage Treatments	Bulk Density Mg g ⁻¹	Moisture m ³ m ⁻³	Porosity as %
0-7.5 cm			
NT	1.15	0.398	54.7
RT	1.12	0.378	56.5
CT	1.36	0.414	46.5
RT'	1.30	0.429	50.0
LSD.05	0.21	n.s.	7.5
7.5-15 cm			
NT	1.33	0.442	48.8
RT	1.30	0.429	50.0
CT	1.44	0.445	46.1
RT'	1.37	0.442	47.3
LSD.05	0.07	n.s.	2.9

In the soil surface layer under the dry bean the bulk density of CT plots was significantly higher than either NT or RT plots (Table a.3). Correspondingly, the porosity of

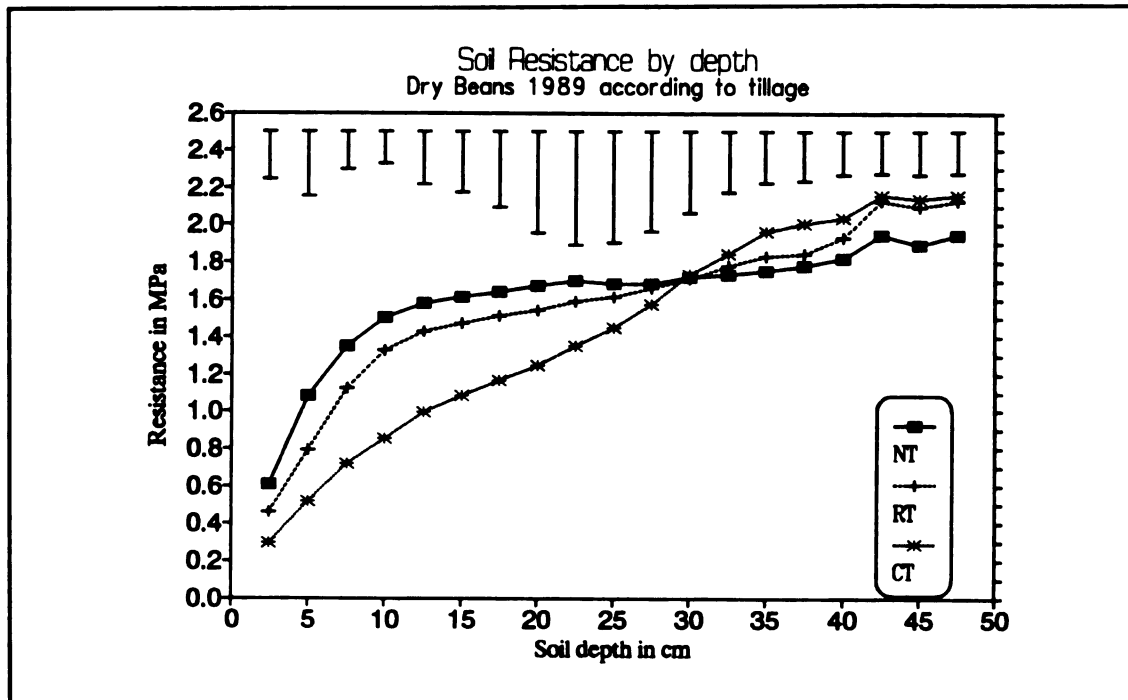


Figure A.10: Penetration Resistance of a Mistequay clay as affected by tillage treatments.

the CT plots was significantly lower than NT or RT. In the top 8 cm, NT plots show a significantly higher porosity at each increment of pore size up to 72 μm (Figure A.11). In the 8-15 cm depth both RT and NT showed significantly higher porosities at each increment of pore size up to 144 μm (Figure A.12).

Nitrogen Budgets and "Lost" Nitrogen: Examining the fate of the N fertilizer and all other sources of N leads to an interesting enigma. Table a.4 outlines the sources of N as well as the pools of N at harvest in the soybean crop. Soil organic N was not measured and appears nowhere on the budget. Soil Residual N measurements represent only

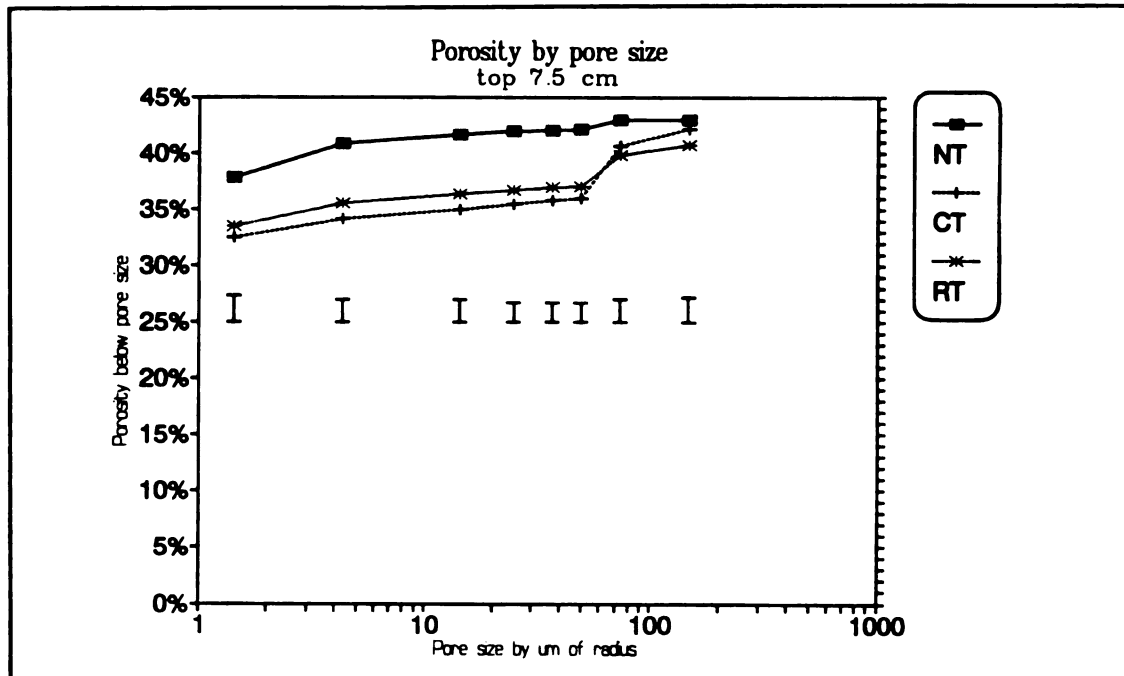


Figure A.11: Soil Porosity by pore size (percentage of volume occupied by pores of smaller or equal radius to that listed on the graph.)

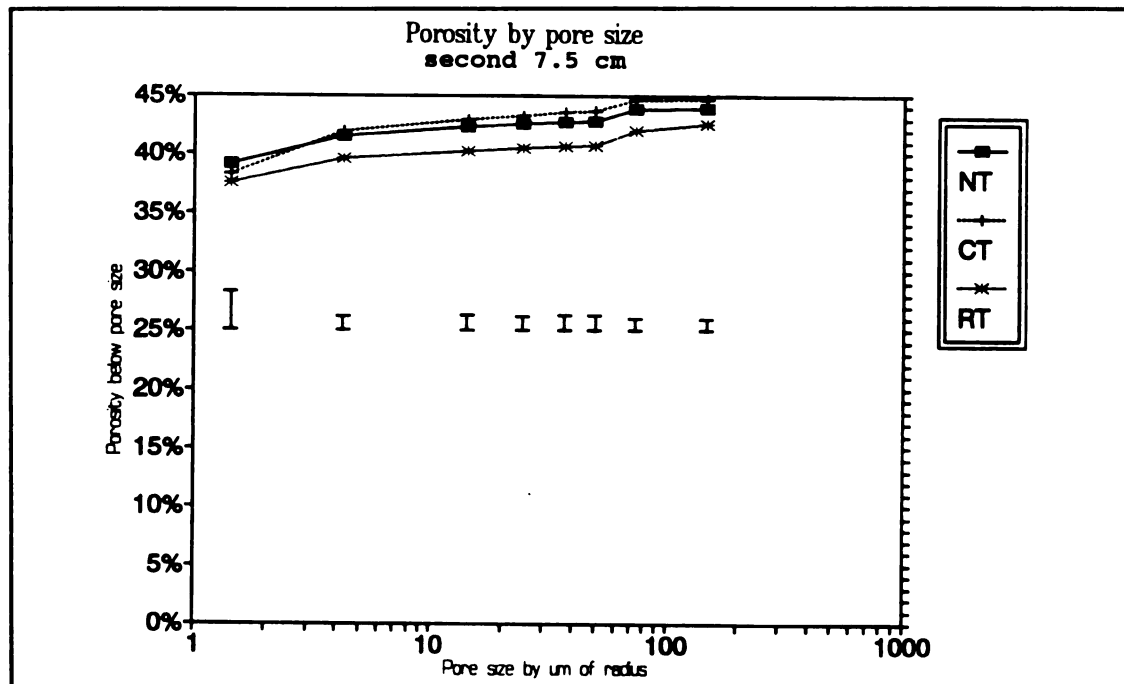


Figure A.12: Porosity by pore size

Table a.4: Inorganic and Plant N Budget in the Soybean.

<u>Nitrogen Sources</u>	Fertilization Rate		
	28	56	84
	(kg N ha ⁻¹)		
Fertilizer N	28	56	84
Soil N 0-30 cm	43	43	43
30-90 cm	86	86	86
Seed N	1	1	1
Fixed N in shoots	0	0	0
Total	157	185	213
<u>Nitrogen Pools at Harvest</u>	(kg N ha ⁻¹)		
Seed N	138	147	168
Straw N	18	21	26
Soil N 0-30 cm	100	73	83
30-90 cm	78	81	120
Total	334	321	397
Additional Inputs	177	135	183
i.e. net from mineralization, etc.			

available NO₃ and NH₄ as determined by KCl extracts. Of course the mutant soybean does not fix N. The total sources of N in the soybean comprise about 160 kg ha⁻¹ more N than the pools do. The increase in N must be due to net mineralization of soil organic N, plus mobilization of NH₄ fixed in the clay, less losses due to leaching, run off and denitrification.

The factors that led to the net mineralization of N in the soybean crop should have led to the same mineralization in the navy bean crop. There's a water table 80 to 100 cm below the surface of the field, so the soybean can tap no N

Table a.5: Inorganic and Plant N Budget for Navy Bean.

<u>Nitrogen Sources</u>	Fertilization Rate			
	<u>0</u>	<u>28</u>	<u>56</u>	<u>84</u>
	(kg N ha ⁻¹)			
Fertilizer N	0	28	56	84
Soil N 0-30 cm	43	43	43	43
30-90 cm	86	86	86	86
Seed N	1	1	1	1
Fixed N in shoots est.	68	64	76	64
Net Mineralization	165	165	165	165
Total	363	387	426	443
<u>Nitrogen Pools at Harvest</u>	(kg N ha ⁻¹)			
Seed N	172	168	192	172
Straw N	20	19	19	17
Soil N 0-30 cm	80	63	66	70
30-90 cm	94	79	109	86
Total	366	329	386	345
Additional Pools	-4	58	41	98
"Lost" Nitrogen				

source below the sampled layers. Using the estimate of mineralization on this soil for the dry bean N budget (Table a.5) gives a more complete approximation of the N inputs. Since the mineralization does not appear to depend on the rate of fertilization, an average of the soybean values is the best estimate to use on the dry bean budget. Because the amount of N₂ fixed showed no correlation to fertilization in this study, an average of fixation for the other fertilization rates is combined for the zero fertilization column.

Once again there is a discrepancy between the total

Sources and Pools of N. In the dry bean N budget there is a missing pool of N. The amount of N in this "lost" pool closely resembles the amount of N in the initial fertilizer (Figure A.13). Any N that left the system through leaching, denitrification, etc. should be accounted for in the "net mineralization" term. The only uncounted pool is in the organic matter of the soil and roots.

The experimental treatments did effectively modify the soil. They just didn't affect the yield. Any additional N due to the addition of fertilizer appears to have ended in the soil or root organic matter. Understanding the fate of the "lost" N pool depends on understanding of the sequence of events before harvest.

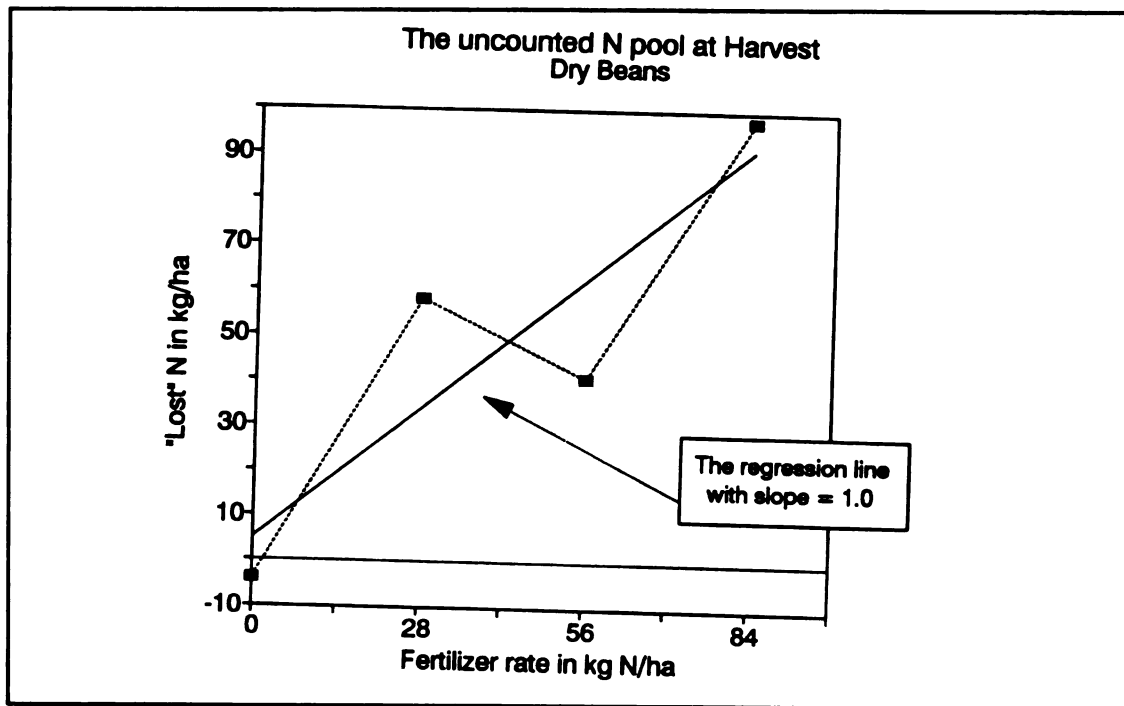


Figure A.13: Unaccounted N in Navy Bean Budget.

Topic two: Season's Sequence.

Throughout the season neither fertilization nor tillage treatments had a significant effect on the number of trifoliates, on the distance from root to top node or on the dry weight of any fraction of the dry bean. Average values summarized over tillage and fertilizer treatments are given in Table b.1 for seasonally measured plant parameters. While plant biomass was not effected by treatments, the dynamics of N in the soil and plant were.

Leading to mid-season, V4: At the end of the vegetative stage (V4) the N fertilizer was still present as NH_4 (Figure B.1). The zero fertilization treatment still has less NH_4 than the other treatments, but the other treatments are not different from each other and they exceed the zero treatment by less than the amount of fertilizer applied. Instead some of the fertilizer has clearly increased the concentration of soil nitrate (Figure B.2.) Soil microflora had already begun to nitrify fertilizer N.

Only a small amount of the fertilizer N entered the dry bean by V4. Only a small fraction of the fertilizer have entered either the dry bean or the soybean crops (Figures

Table b.1: Average development measurements for a Navy dry bean and a mutant soybean on a Mistequay clay with the standard deviation, $s_{\bar{y}}$ of the population mean.

Crop averages per plant	plant stage	Dry Bean	$s_{\bar{y}}$	Soy bean	$s_{\bar{y}}$
Distance to top node in cm	V4	6.4	0.9	9.0	1.6
	R6	41.0	7.3	46.3	5.5
	R8	55.8	11.3	80.0	6.3
Number of nodes	V4	6	1.3	6	0.6
	R6	36	6.0	31	6.2
	R8	45	9.0	34	5.9
Number of trifoliate	V4	4	0.9	5	0.6
	R6	28	4.8	24	4.6
	R8	42	8.2	31	5.8
No. pods	R8	25	5.5	54	12.5
No. seeds	R9	125	18.7	112	19.1
Dry weight in g	V4	0.7	0.2	0.9	0.2
	R6	7.3	1.7	11.3	2.9
	R8	42.8	8.5	43.7	8.0
	R9	37.5	5.8	30.4	5.7
Stem dry wt. in g	R8	7.8	1.5	14.4	2.6
	R9	5.4	1.2	8.0	1.7
Trifoliate dry weight	R8	7.3	1.9	9.9	2.4
Pod dry weight in g	R8	27.6	6.1	19.4	4.1
	R9	7.5	1.4	6.8	1.3
Seed dry weight in g	R9	24.6	3.9	15.7	3.1

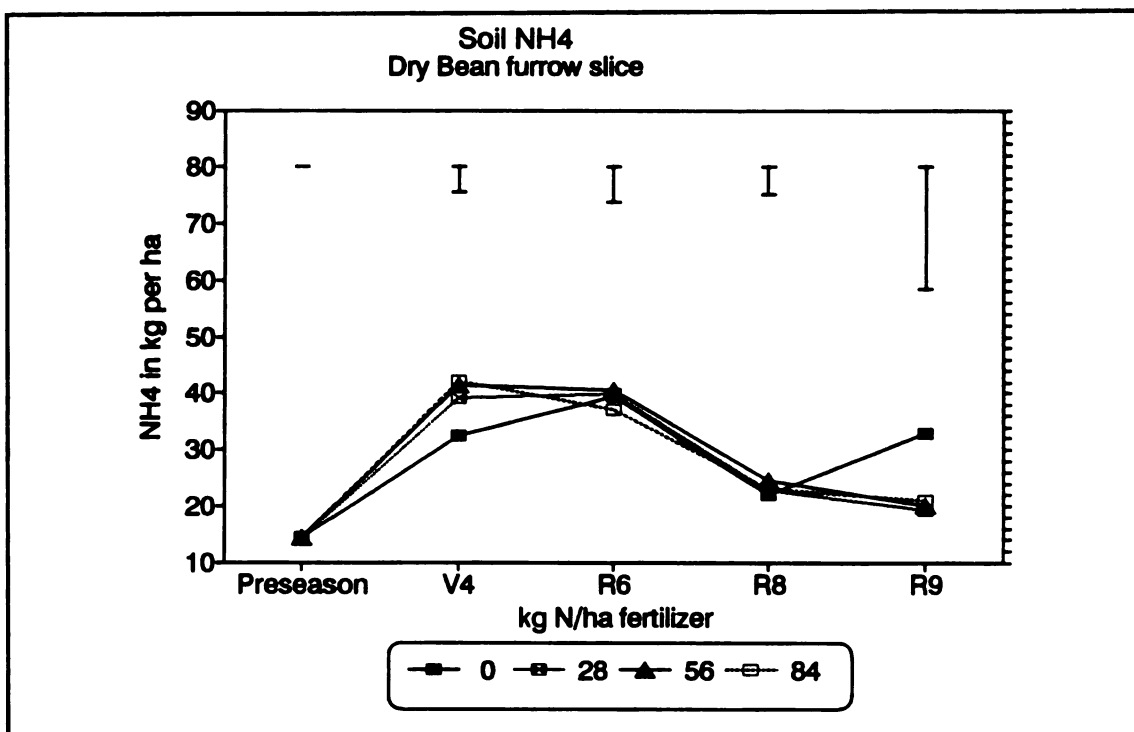


Figure B.1: Average Soil Ammonium concentrations in the top 30 cm at each sampling date on a Mistequay clay, 1989.

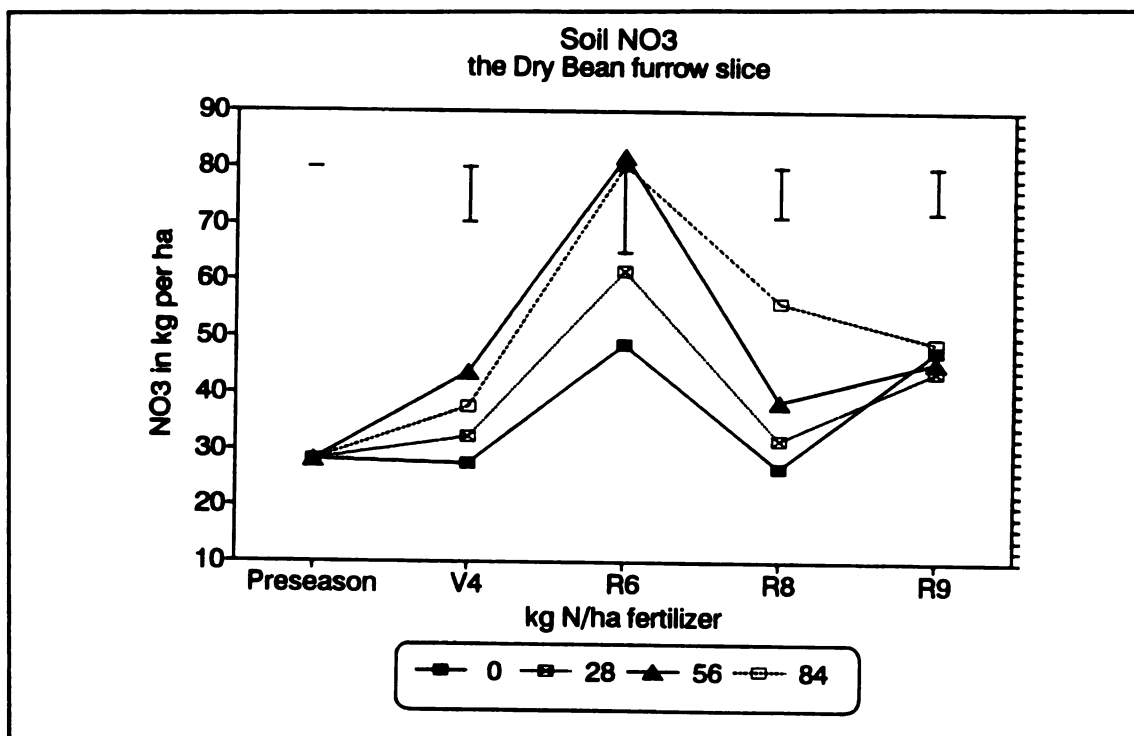


Figure B.2: Average Soil Nitrate concentrations in the top 30 cm at each sampling date on a Mistequay clay, 1989.

B.3 and B.4) by V4. At V4 the plants rely solely on soil and seed N. Considering the fact that the fertilizer treatment has no effect on the dry weight (Figure B.5), the dry bean seems to start the season with no advantage from the "starter" fertilizer. The lack of a difference in the dry weight of the soybean confirms the ineffectiveness of the fertilizer at this stage (Figure B.6).

Unfortunately the range of values for N_2 fixation at this stage is too wide to report the averages responsibly. The calculated percentages of N derived from the atmosphere were as low as -300% and as high as 120%. The absolute amount of ^{15}N taken up by either the dry bean or the soybean is still low at this stage; small differences in access to the labeled fertilizer lead to large calculation errors. The soybean also grows more vigorously than the dry bean until flowering (Figure B.7). If the soybean roots had grown to a source of residual soil N that the dry bean roots hadn't reached yet, the ^{15}N would be diluted, making the fixation estimate negative.

Flowering, R6: The NO_3 quantity in the top 30 cm of the soil under the dry bean rose between V4 and R6 (Figure B.2). The quantity of NO_3 was also more strongly correlated to the initial fertilization than at V4. Evidently NO_3 continued to mobilize from the microflora. Ammonium concentration was unchanged from V4, except for the zero fertilization treatment (Figure B.1). The soybean had

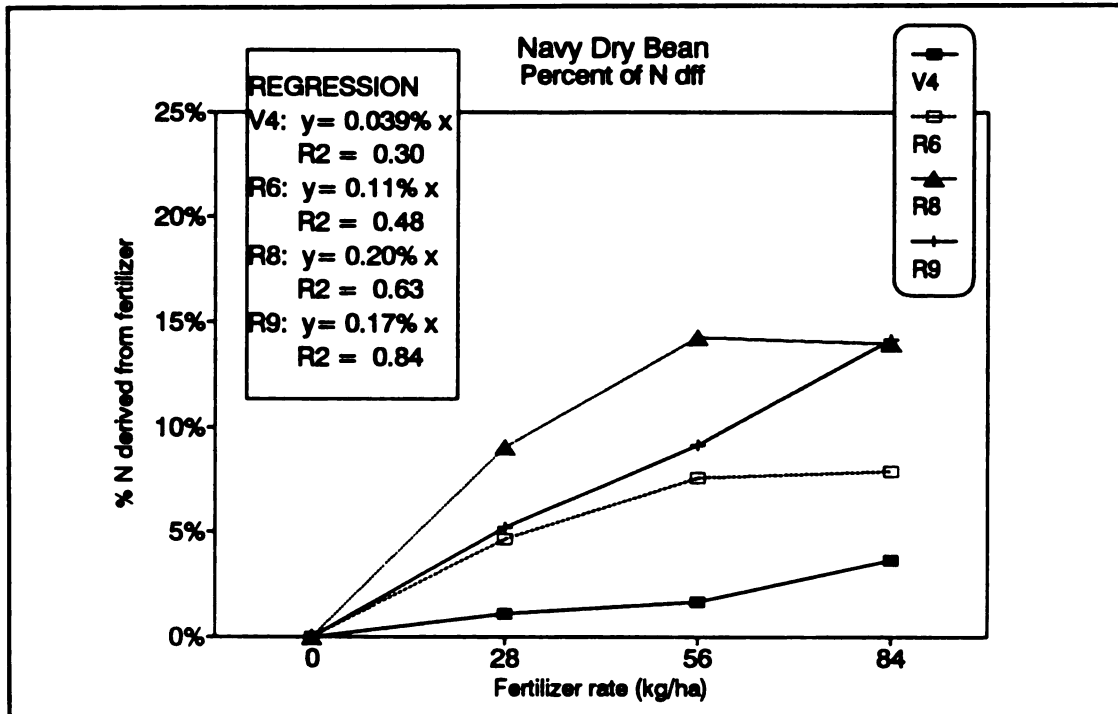


Figure B.3: Share of N derived from fertilizer in the dry bean shoot, averaged across tillages.

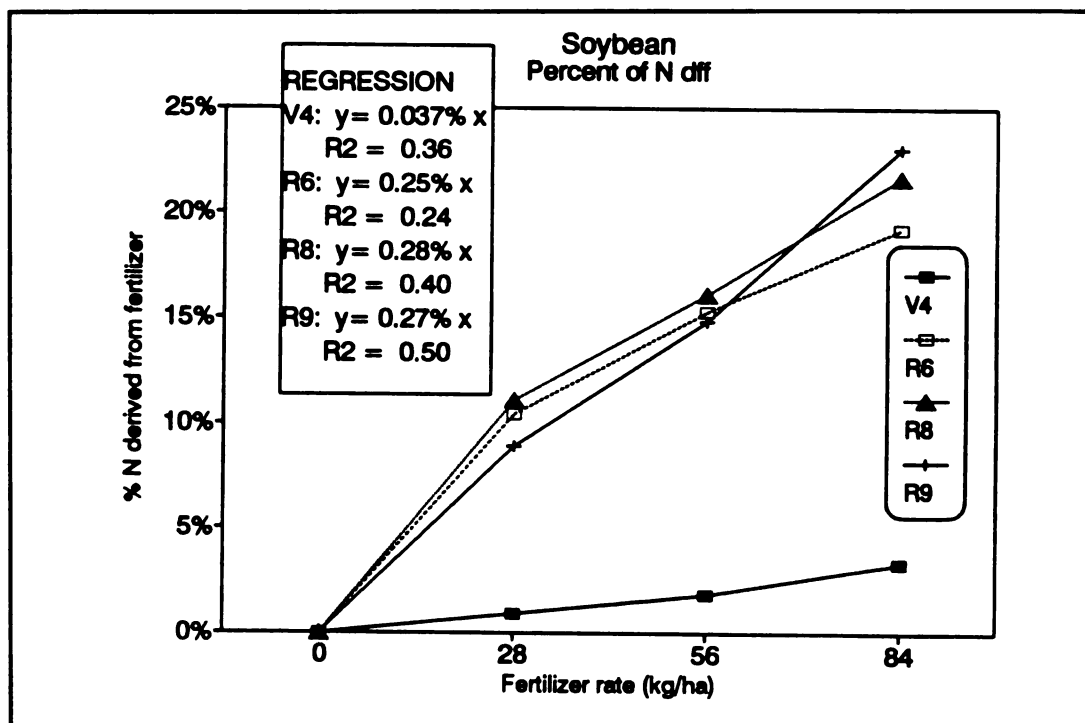


Figure B.4: Share of N derived from fertilizer in the soybean shoot, averaged across tillages.

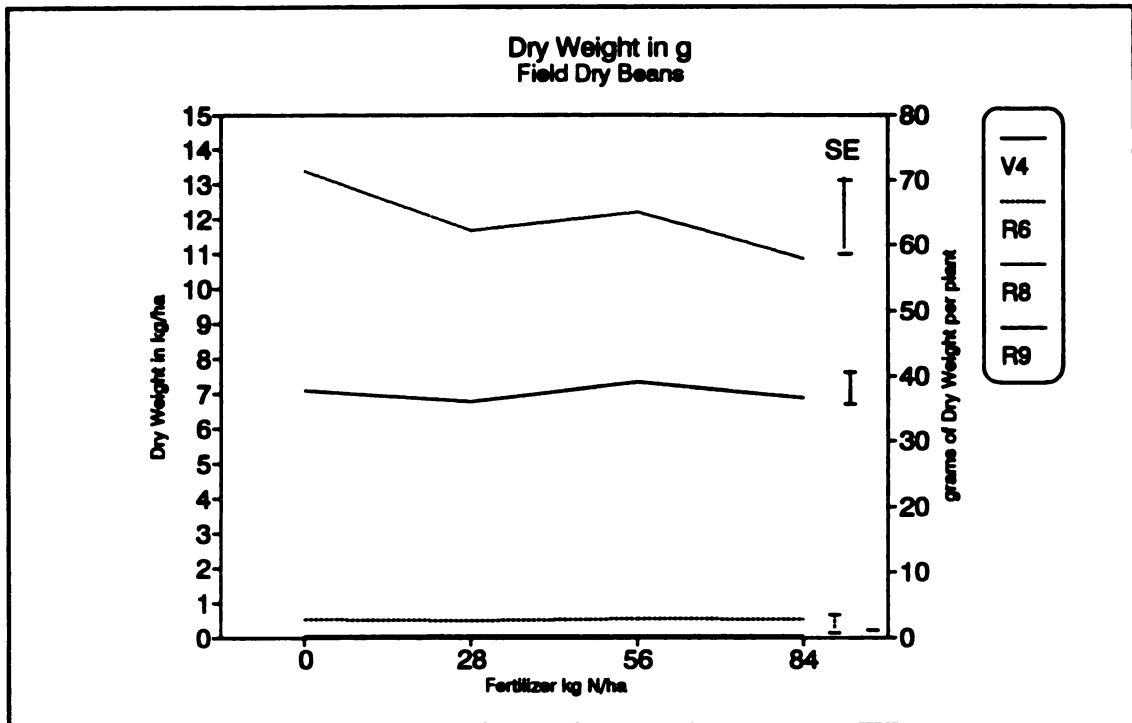


Figure B.5: Fertilizer effects on the total dry weight of the dry bean shoot, by plant stage.

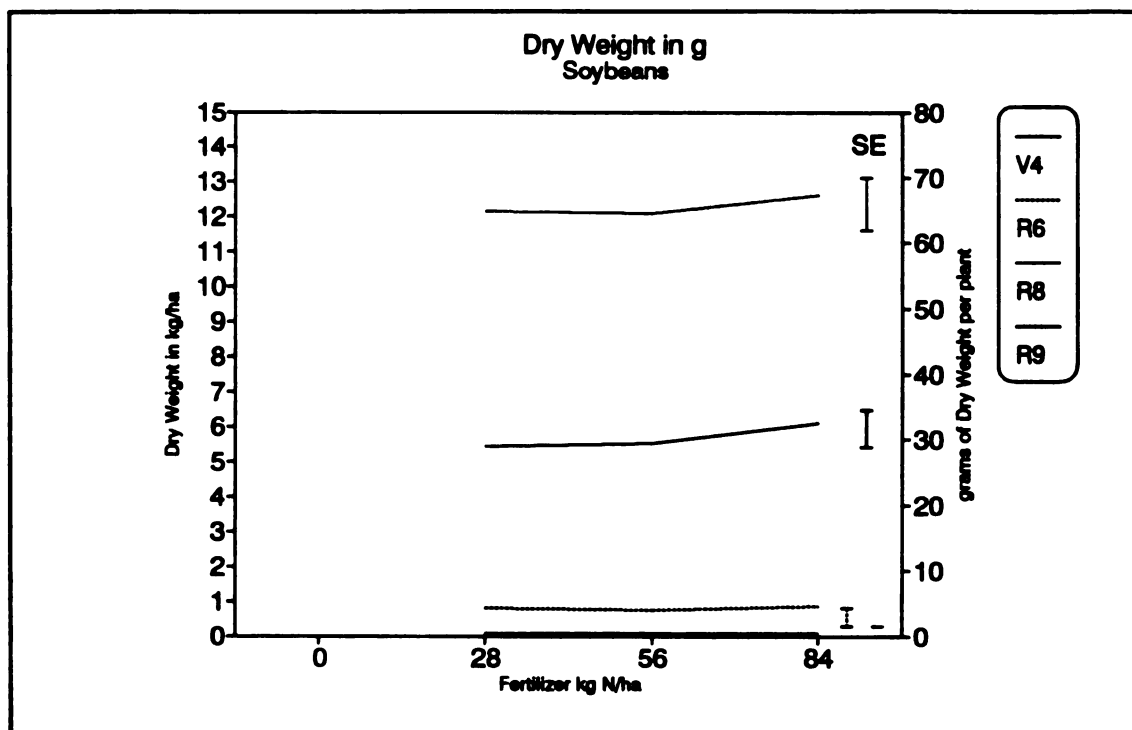


Figure B.6: Fertilizer effects on the total dry weight of the soybean shoot, by plant stage.

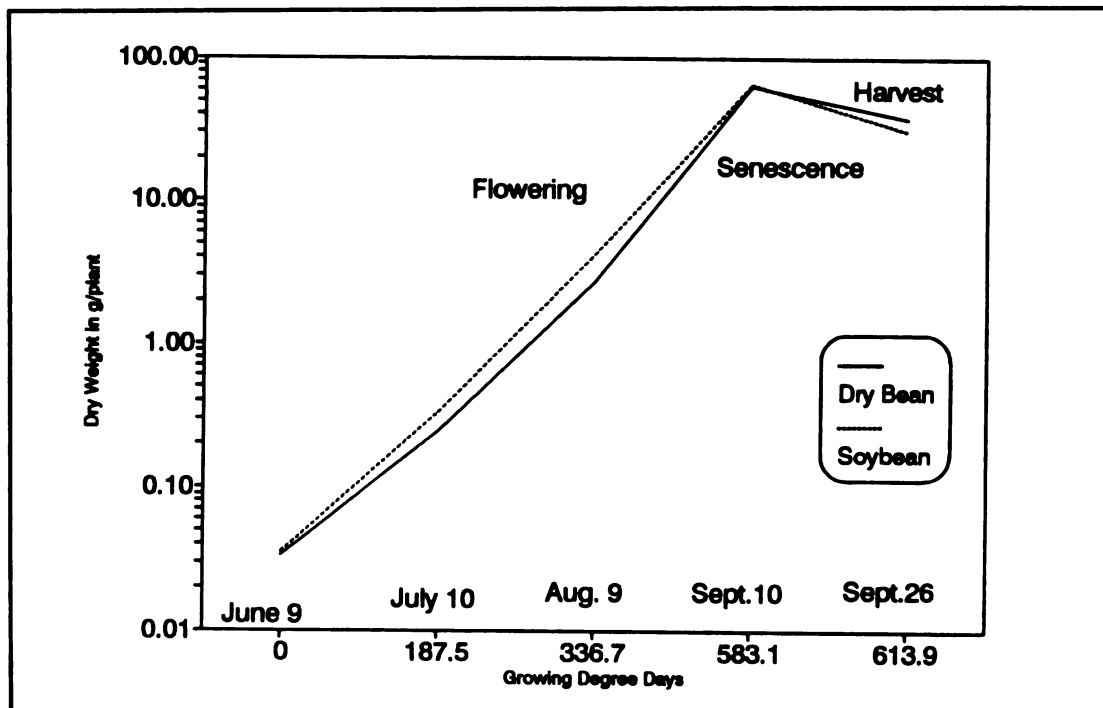


Figure B.7: Comparative dry weight accumulation curve for the shoots of the Mayflower Navy dry bean and a mutant soybean.

scavenged as much of the NH_4 as the dry bean did (Figure B.8). Soil NO_3 concentrations under the soybean were not only lower than those under the dry bean, they also were influenced less by the fertilizer (Figure B.9).

Evidently soil NO_3 was less influenced by fertilizer because the soybean had begun to incorporate the fertilizer (Figure B.4). In soybean the share of N derived from fertilizer already reached the percentage found at harvest. Presumably, soybean roots already extended to recoverable sources of soil N. The dry bean hadn't reached such a high percentage of N dff (Figure B.5). The difference may be due to fixation on the part of the dry bean.

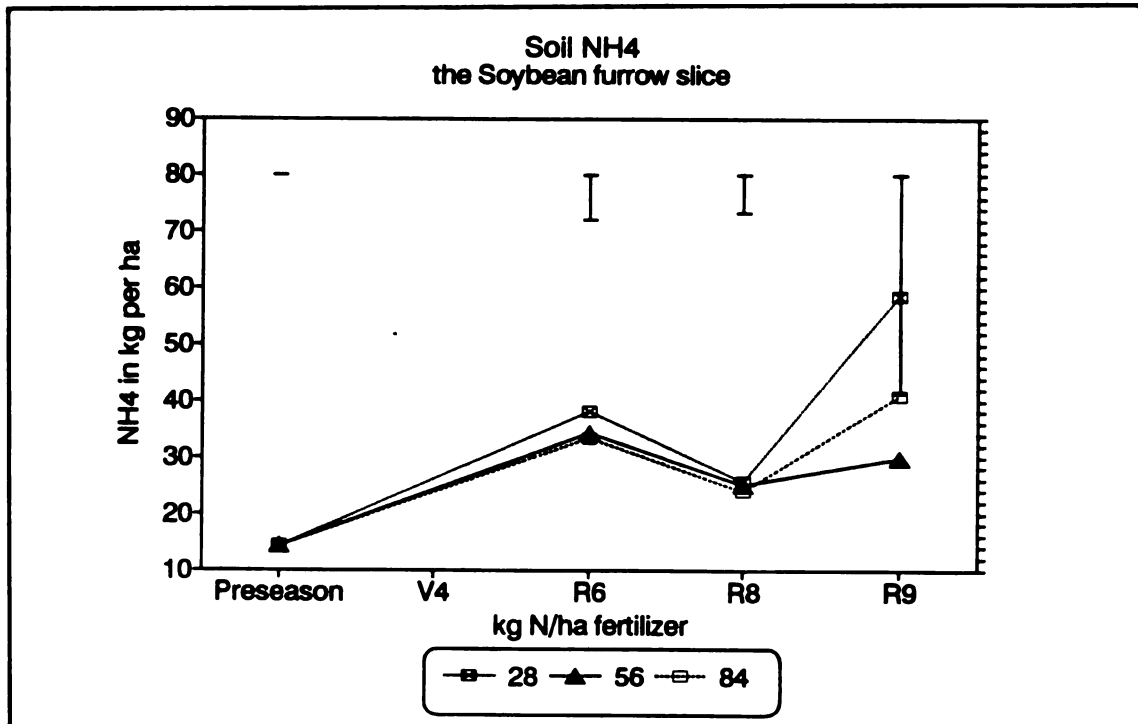


Figure B.8: Seasonal NH₄ concentrations in the top 30 cm of soil under the soybean, displayed by sampling date.

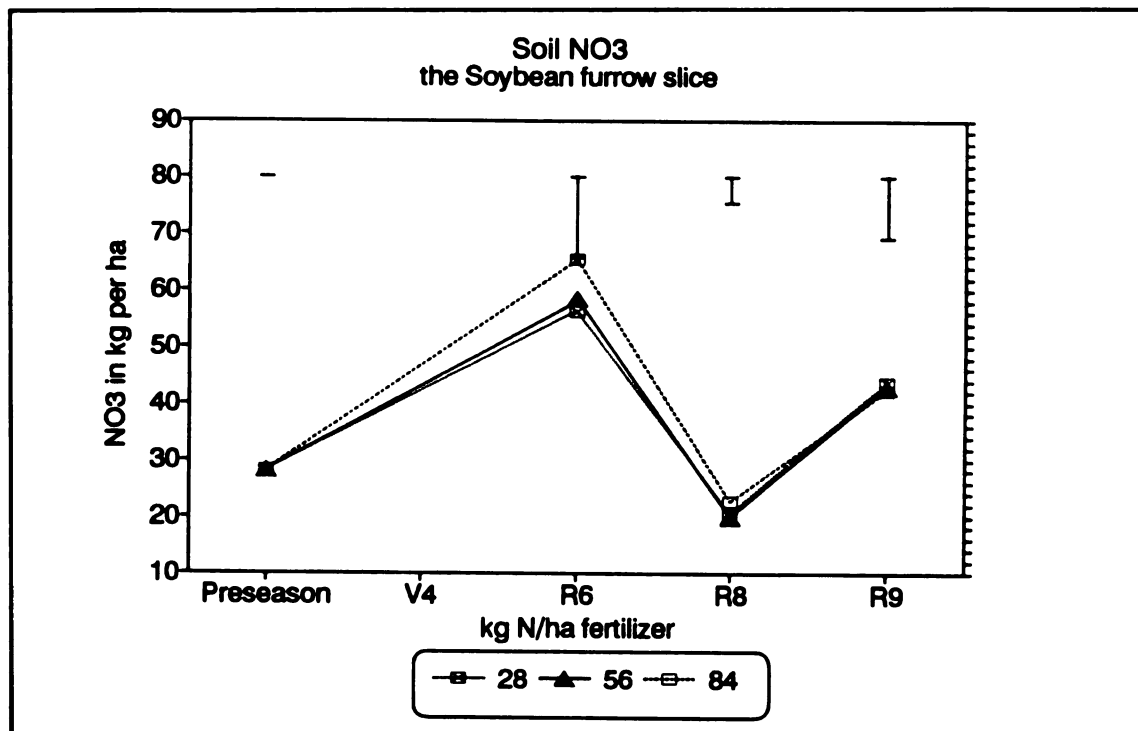


Figure B.9: Seasonal NO₃ concentrations in the top 30 cm of soil under the soybean, displayed by sampling date.

According to Wolyn et al. (1989) R6 is the time of highest N_2 fixation rates. Rennie and Kemp (1984) found that R6 was the midpoint of fixation. Each of these determinations was made using Acetylene Reduction Assays. The share of N derived from the atmosphere would seem to agree with the assays (Figure B.10). The amount of N dfa that had collected in the shoot, however, was still low (Figure B.11). The dry bean is apparently fixing N_2 actively and not accumulating that fixed N into the shoot.

Vegetative growth does not end when the flowers open (Figure B.12). At R6 stems and leaves had about a tenth of

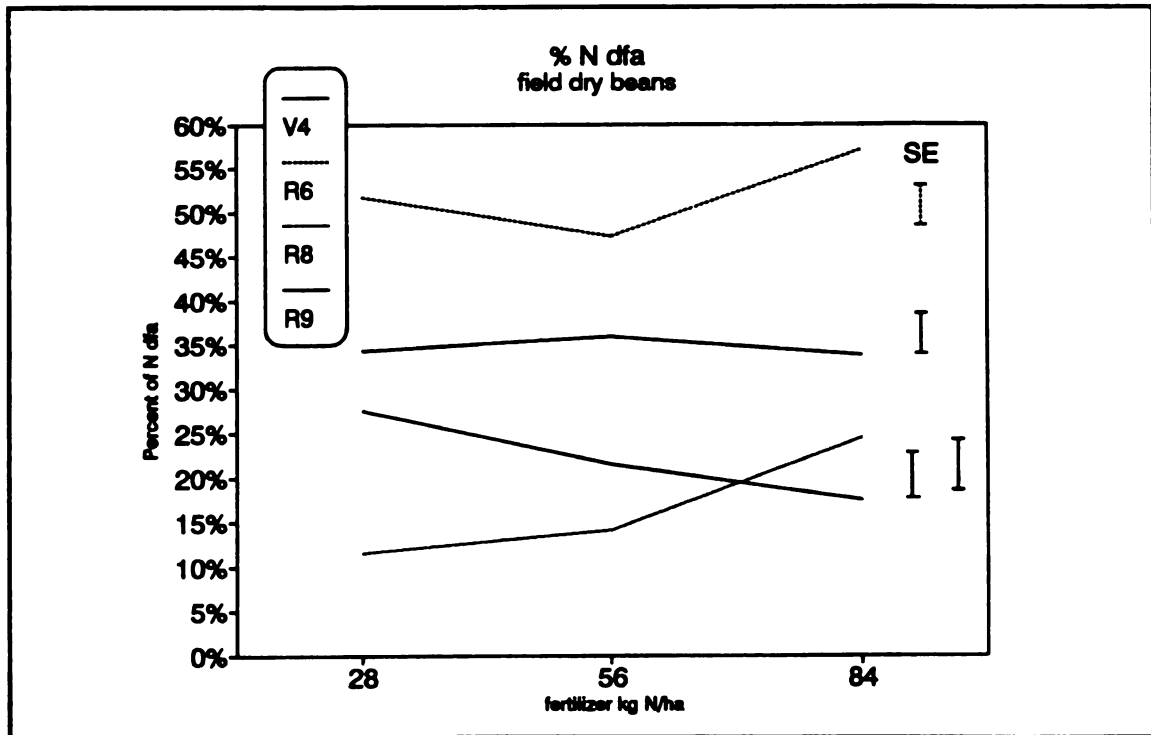


Figure B.10: N fixed as the percentage of N in the shoot of the dry bean.

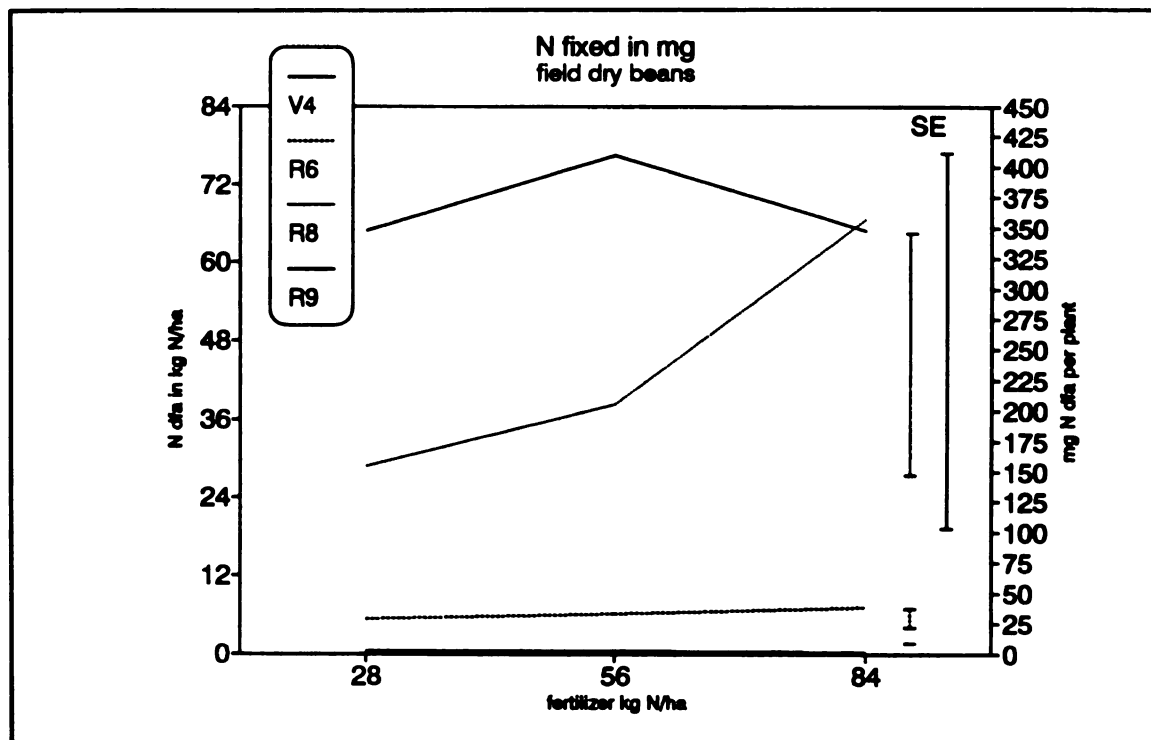


Figure B.11: N fixed as the amount collected in the shoot of the dry bean.

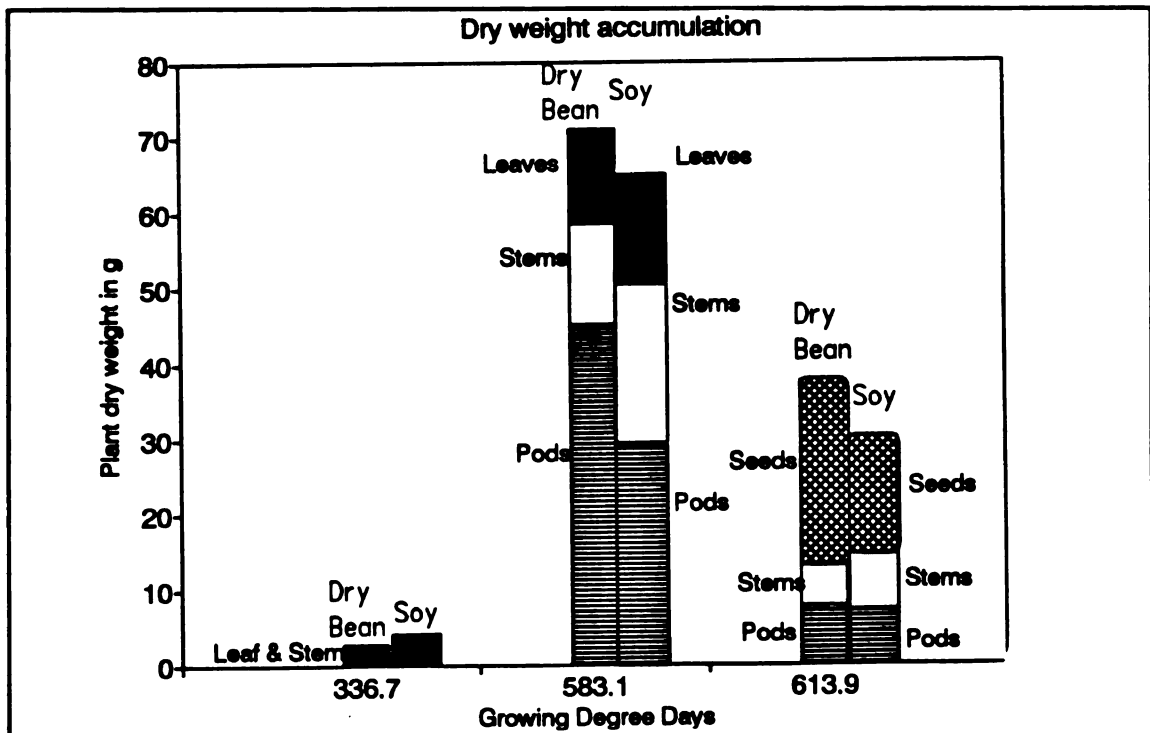


Figure B.12: Average dry weight per plant of each fraction of the shoot for dry bean and soybean.

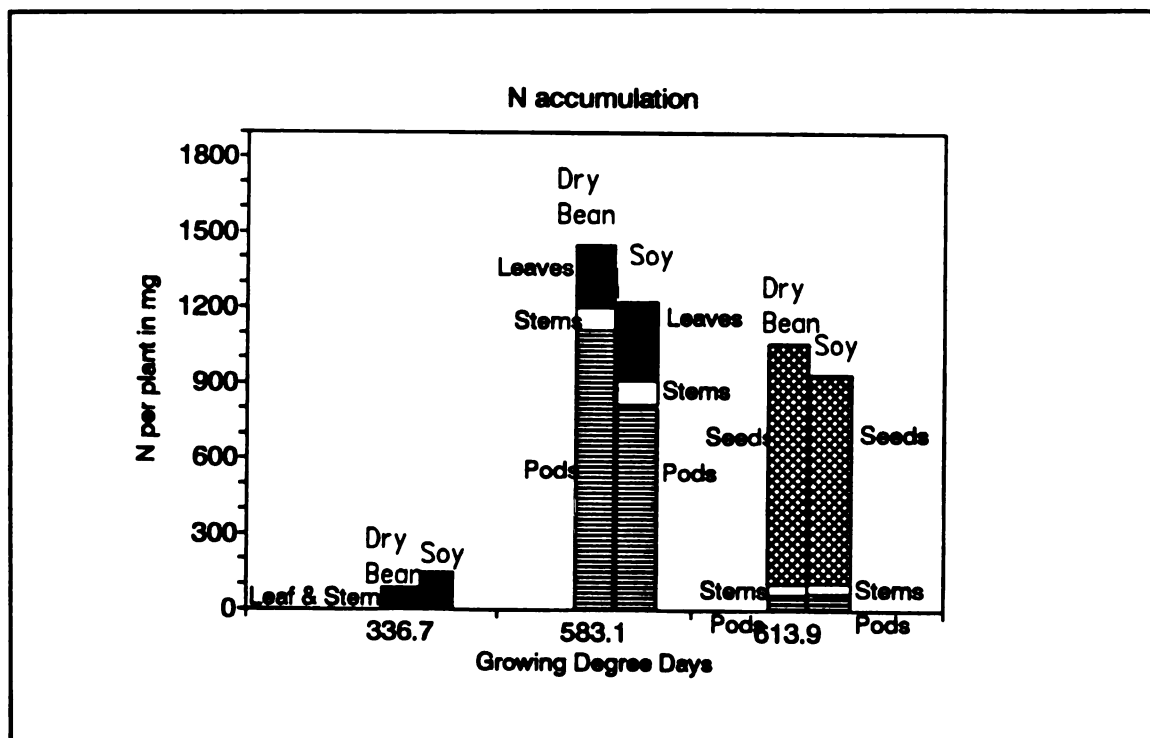


Figure B.13: Average N content per plant of each fraction of the shoot for dry bean and soybean.

the dry weight they did at R8. Shoot N increased proportionately to the dry weight (Figure B.13).

Leaf senescence and pod fill, R8: In each tillage-fertilizer treatment and in each crop, the NH_4 concentration in the top 30 cm is depleted to 20-25 kg N ha^{-1} (Figures B.1 and B.8). The dry bean also takes up 30 kg N ha^{-1} of inorganic NO_3 , regardless of how much is available (Figure B.2). The soybean, on the other hand, depletes NO_3 in all treatments down to 18-20 kg N ha^{-1} (Figure B.9).

The crops need the soil N at this stage because they are growing rapidly. Between R6 and R8, the dry weight of the shoot increases by a factor of ten (Figures B.5 and B.6).

Visual observations indicate that the development of the dry bean and soybean are dissimilar (Figures B.14 and B.15). Soon after R6 the soybean stopped growing taller. Stems may have thickened, but most stem growth stopped. No new leaves formed but old leaves grew broader and longer. The result was that the soybean formed first stems and leaves, then only leaves, then pods and, later, seeds.

Dry bean growth varied widely from plant to plant. Generally the plants were still short at R6. First one plant, then all its neighbors sent out feelers. Stems and leaves developed quickly along the feeler. Usually one or more additional stems would branch off at the first or second trifoliate. Growth along the new stem would follow

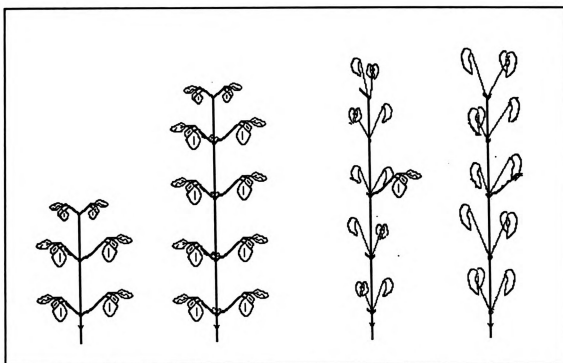


Figure B.14: Stages in the growth of a soybean plant.

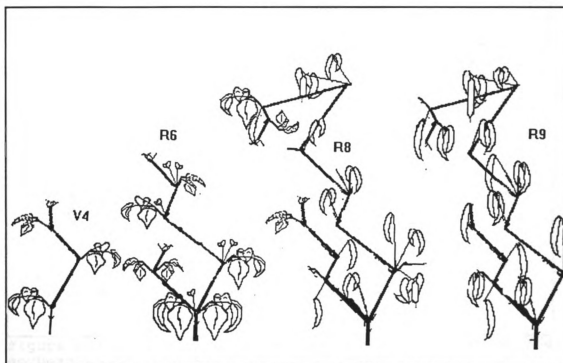


Figure B.15: Stages in the growth of a dry bean plant.

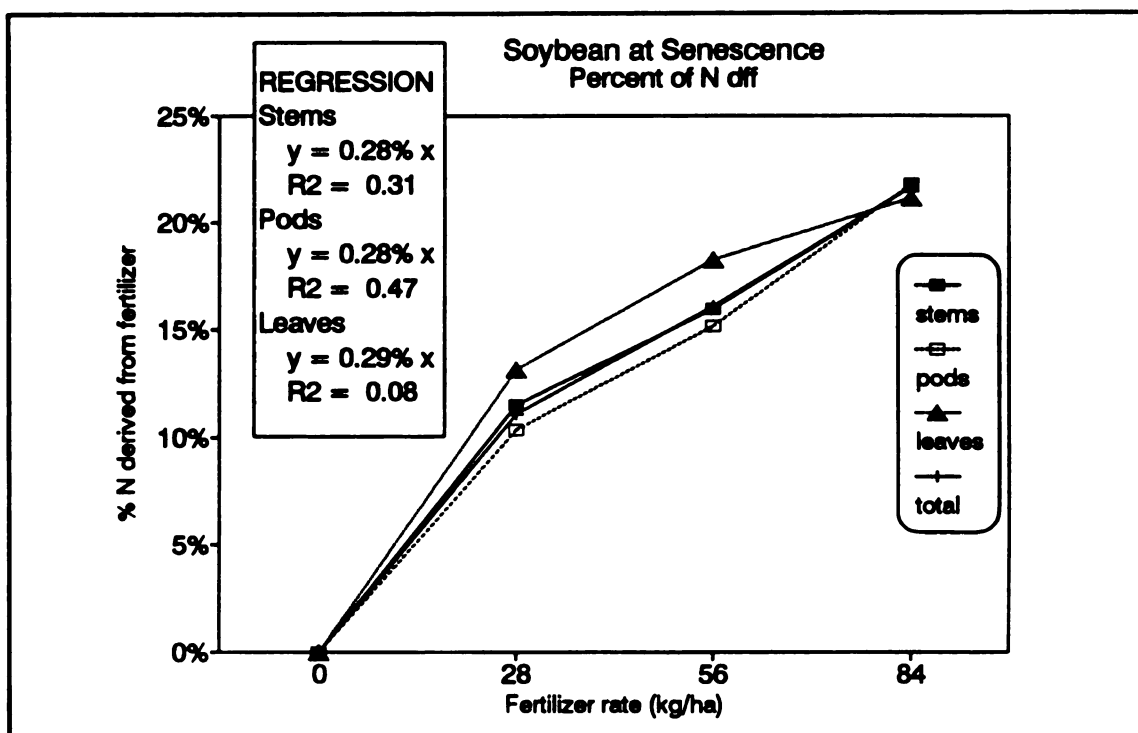


Figure B.16: Share of N derived from fertilizer by soybean plant part at R8.

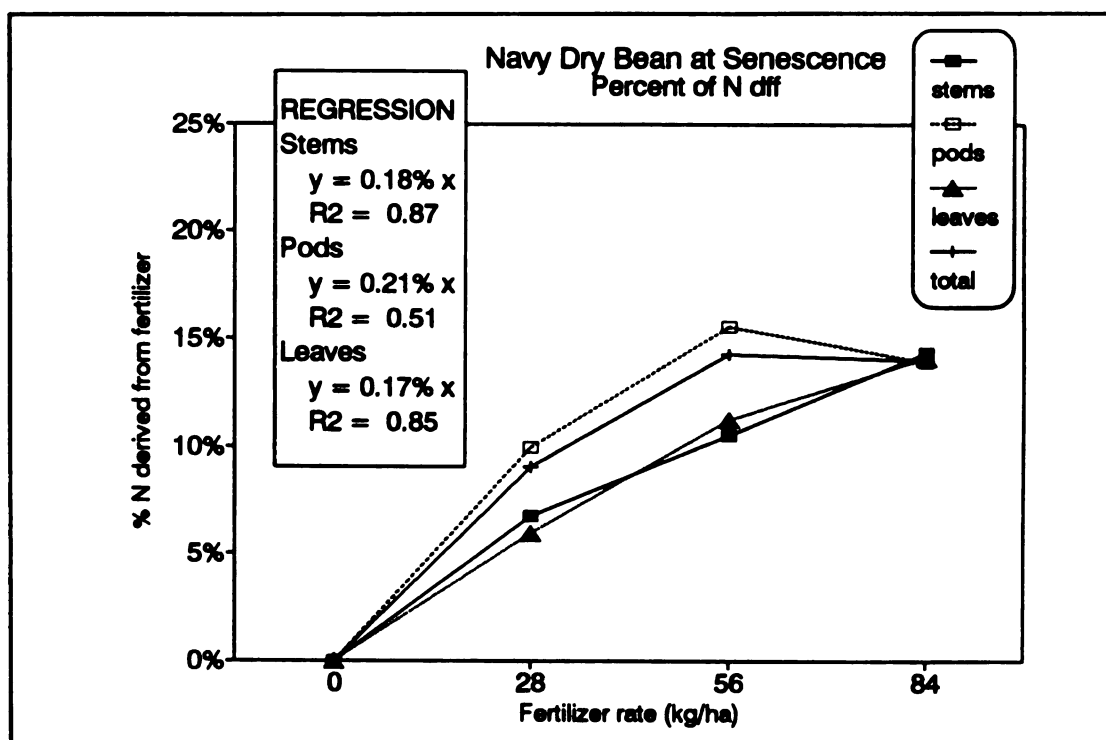


Figure B.17: Amount of N derived from fertilizer in the soybean shoot, averaged across tillages.

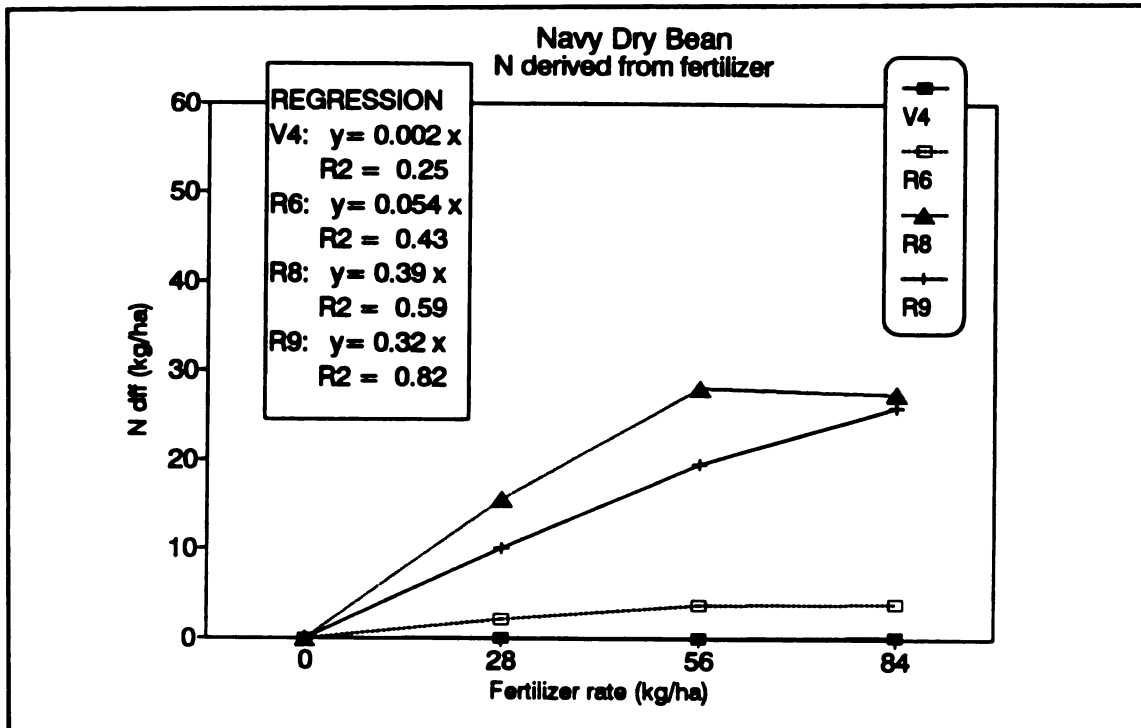


Figure B.18: Amount of N derived from fertilizer in the dry bean shoot, averaged across tillages.

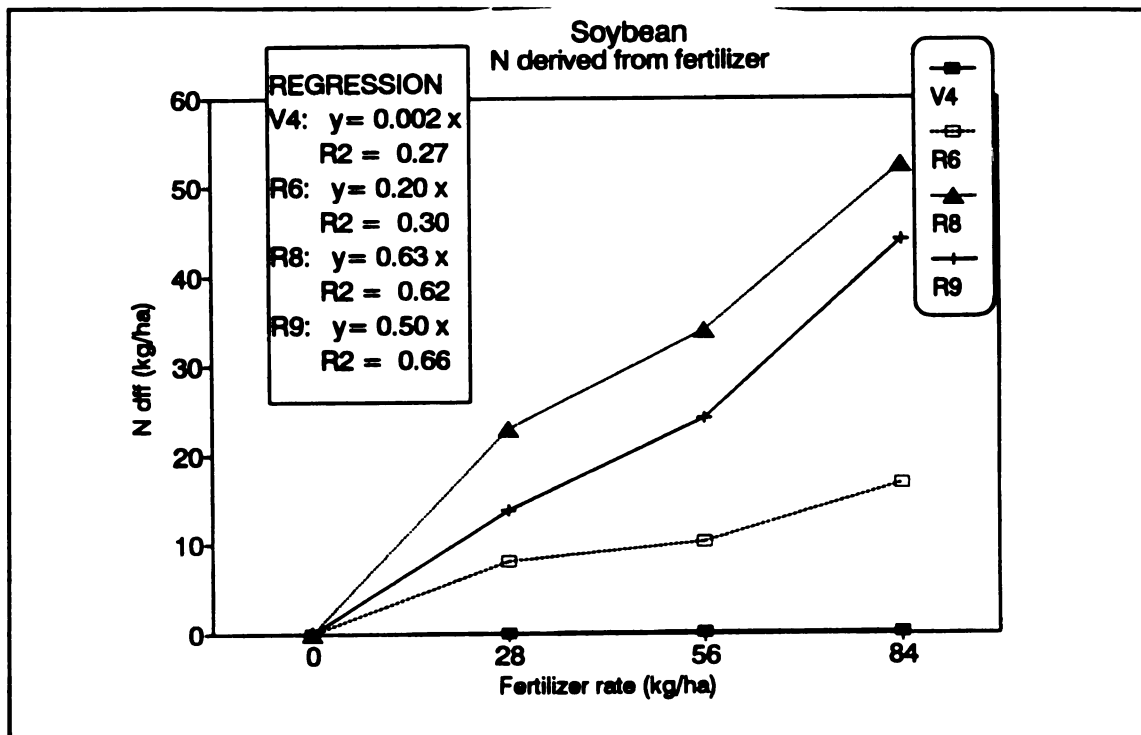


Figure B.19: Share of N derived from fertilizer by dry bean plant part at R8.

the old stem with a delay of a week or two. Along old stems, pods would develop and form seeds while flowers and trifoliates were still forming along new stems and feelers. The result was that growth in the dry bean was interspersed. Generally, leaves and stems formed before pods and pods before seeds. These observations match closely the nutrient accumulation curves for the dry bean as reported by Vitosh, Christenson and Knezek (1982.)

At R8 the share of N derived from fertilizer in soybean differed by plant fraction (Figure B.16). I believe that this difference is real, that it does not reflect any isotopic preferences for the ^{15}N label on the fertilizer. Instead it reflects the relative availability of fertilizer N at the time when that plant fraction was produced. Soybean leaves had the higher percentages of N dff than stems and stems were higher than pods. According to the time sequence discussed above, the fertilizer N must have become more available to the shoot as stems stopped growing. That availability was already declining as pods were forming. The surge of N fertilizer should be reflected in the amount of N dff in the shoot. Indeed, the N dff nearly triples in the soybean between R6 and R8 (Figure B.17).

During the same time, the N dff in the dry bean shoot increases by a factor of 5 (Figure B.18). Once again the share of N dff is significantly different between the plant parts (Figure B.19). This time, however, pods have the

highest share. Apparently fertilizer N is still available to the dry bean as the pods are forming. The dry bean fixes N while fertilizer N is readily available.

In the dry bean shoot the amount of N derived from the atmosphere increases by a factor of six (Figure B.11). Nevertheless, the amount of N in the shoot increases by a factor of sixteen (Figure B.13).

End of the season, R9: Between R8 and R9, dry weights of both the soybean and the dry bean decreased by more than the loss of leaves can account for (Figure B.12). The shoots were catabolized. Soybean N also decreased, but by less than the amount that had been in the leaves at R8, at leaf senescence (Figure B.13). The dry bean shoot N declined by more than the amount that had been in R8 leaves (Figure B.13)

Despite the decline in total shoot N, the amount of N derived from the atmosphere in the dry bean increased by 24 kg N ha⁻¹ (Figure B.11). The plant must have been cycling N. As some roots sent N dfa to the shoot, the shoot must have sent N richer in fertilizer N to other roots.

It is important to note that the amount of N dfa in the dry bean shoot more than doubled between R8 and R9. Nearly 60% of the fixed N appeared in the shoot during three weeks in which the plant had no leaves, stems were being catabolized for energy and nodules looked like dry, white stones. P.S. Cocks, as cited by Witty and Minchin (1988),

reported that Acetylene Reduction Activity in forage legumes peaked in Biological N₂ Fixation (BNF) in winter, but N accumulated most rapidly in spring.

Between R8 and R9, NO₃ concentrations rose in the top 30 cm of soil under the soybean and under the dry bean (Figures B.2 and B.9). The increase may represent continued mineralization of organic N, the a release of N from senesced roots.

Topic three: Treatment Effects on the plants.

Understanding the mechanism of N_2 fixation is only a first step toward improving fixation. None of the treatments significantly affected fixation. Other factors did respond to the treatments and, under different conditions, might affect fixation as well.

Effects on the N content of the soil: There were no significant tillage effects on soil inorganic NH_4 before the season starts (Figure C.1), even though NH_4 content under Conventional Tillage appears higher. At depths of 60 to 90 cm Conventional Tillage plots had significantly more NO_3 (Figure C.2). Other studies have suggested that No-Till plots have higher rates of N immobilization and denitrification (Gilliam and Hoyt, 1987). The decreased quantity of NO_3 below 60 cm under the Ridge Till as well as the No-Till may be due to increased denitrification (Gilliam and Hoyt, 1987).

By R6 the NO_3 content of the top 30 cm of soil under the RT and NT dry bean is significantly higher than that for CT (Figure C.3). Roots may not be able to penetrate as quickly against the higher penetration resistances of the RT and NT tillages (Figure A.10). Roots in the CT plots would

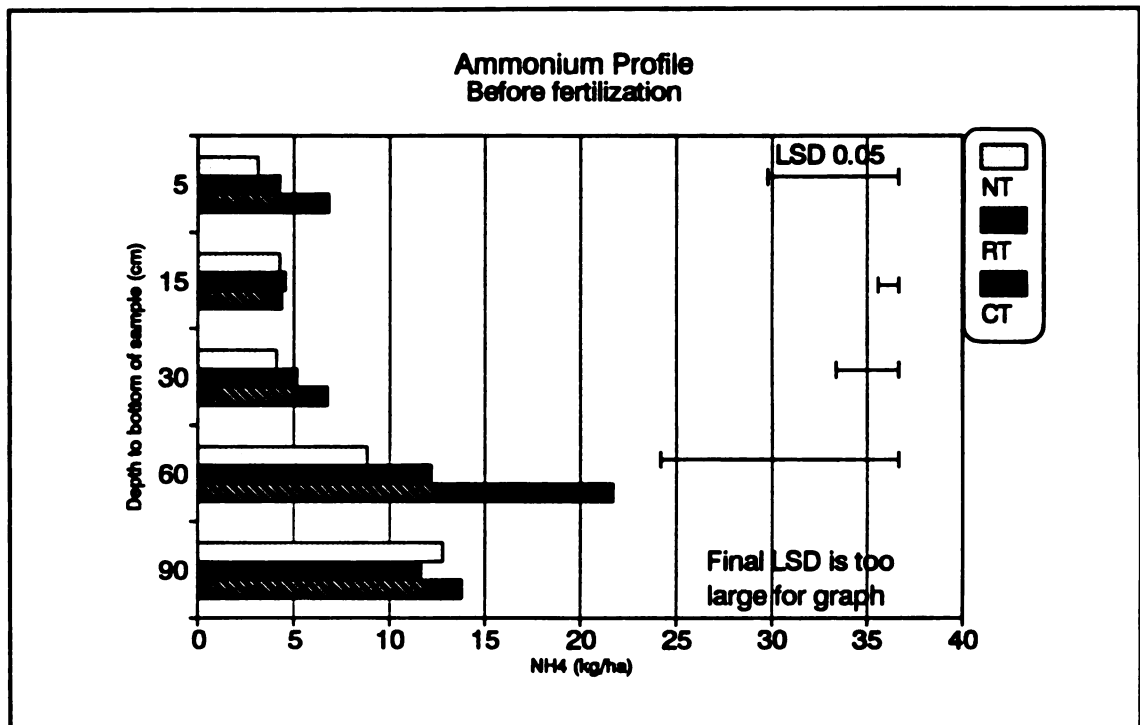


Figure C.1: Profile of the NH₄ content of the soil before fertilization.

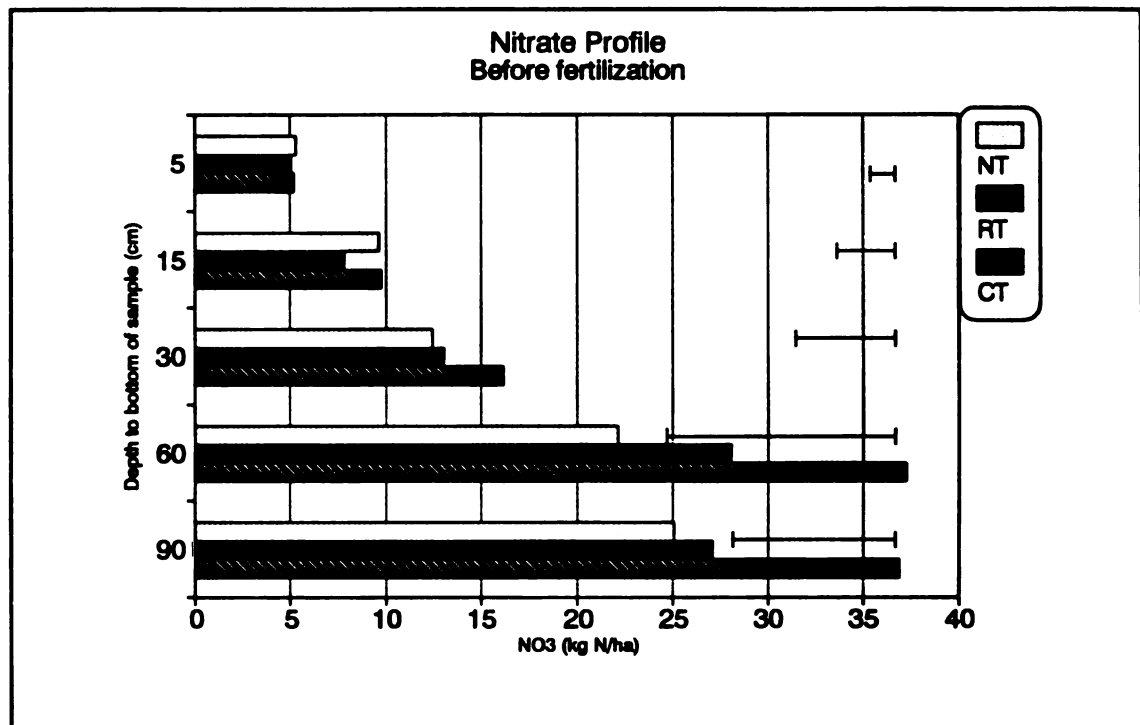


Figure C.2: Profile of the NO₃ content of the soil before fertilization.

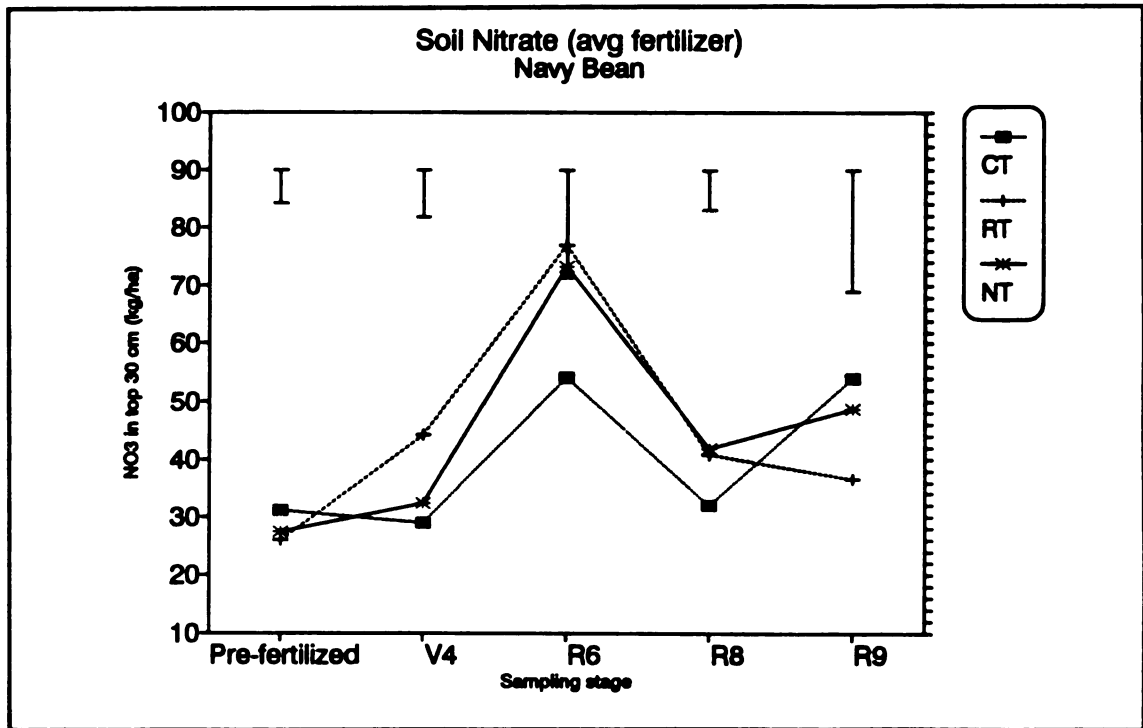


Figure C.3: Seasonal NO₃ content of the dry bean furrow slice, by tillage.

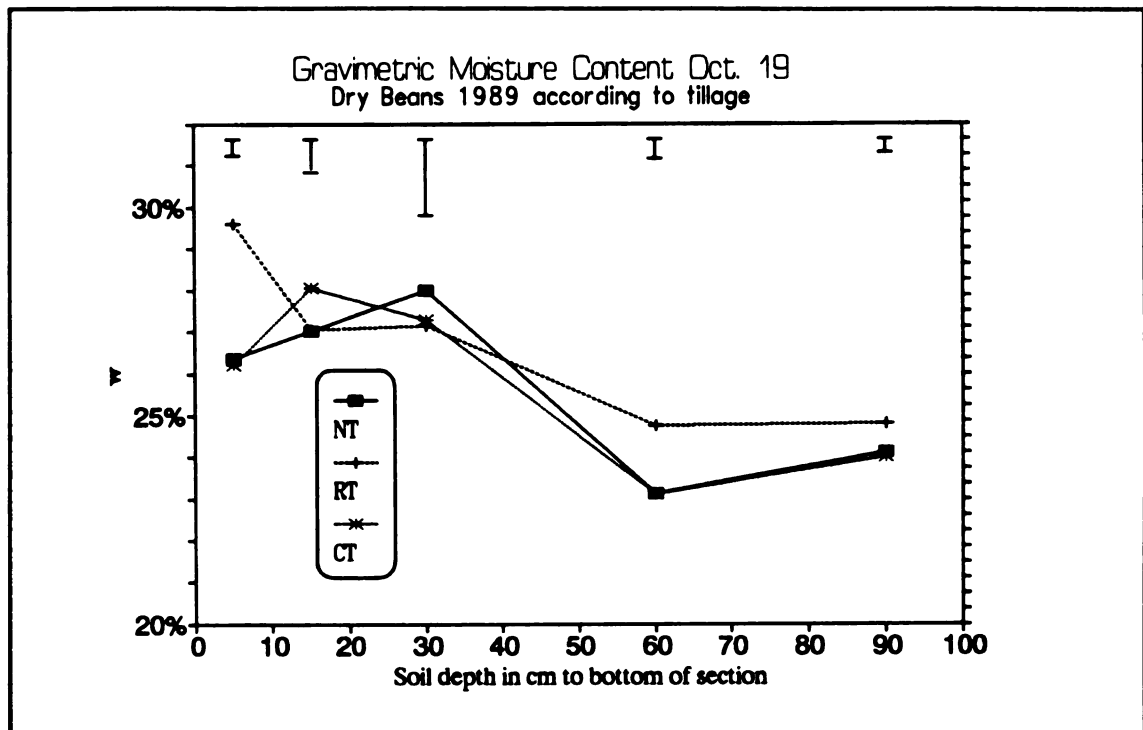


Figure C.4: Gravimetric wetness of a Misteguay soil under dry bean.

then be able to absorb more of the inorganic N than in the other plots. The NO_3 content under the soybean was not affected by tillage until R9 (Figure A.8) when CT was significantly higher than NT at 15 to 30 cm depth. At R8 Ridge Till NO_3 was consistently depleted in the top 30 cm and enriched below 30 cm (Figure A.8), although the difference is not statistically significant. This apparent movement of N may be related to the increased soil wetness under Ridge Till (Figure C.4). Soil NH_4 was not affected by tillage in either crop.

The fertilizer effect on the seasonal inorganic N content of the top 30 cm of soil was discussed in Topic two. The fertilizer effect on increasing NO_3 under the dry bean was significant at V4 through R8 (Figure B.2). The effect on dry bean NH_4 was significant only at V4 (Figure B.1). After V4 N cycling by dry bean roots and microflora apparently evened out all NH_4 traces of the fertilizer. Inorganic soil N indicated no treatment effects at all (Figures B.8 and B.9). The soybean took up whatever N became available.

Effects on N derived from fertilizer in the plant: The close dependancy of N dff on rate of fertilization has already been discussed (Figures A.4, A.5, B.3 and B.4). The quantity of N dff responded significantly to fertilizer at every sampling date, crop or plant fraction except dry beans at V4.

Tillage also affected N dff. As was discussed in Topic one, at harvest No-till stems and pods had a larger share of N dff than Conventional Tillage in both crops. This trend is foreshadowed at R8. The N dff in soybean pods and stems indicated a fertilizer-tillage interaction on N dff at R8 (Figure C.5). (The N dff in pods was highly variable and may not be revealing a significant effect when an effect does exist.) Although the treatments had the same average, No-till responded more to the fertilization rate. If roots stayed closer to the surface in NT plots the plant would have been more dependant on fertilizer than a plant in CT would.

Table c.1: Tillage effects on soybean pods at R8.

	<u>No Till</u>	<u>Conv.Till</u>	<u>LSD(0.05)</u>
R8 soybean pod dry wt. (kg ha ⁻¹)	323	391	26
R8 soybean pod N (kg N ha ⁻¹)	129	165	14

Other effects on soybean tissue at R8, leaf senescence:
The other significant effects on plant tissues all appeared in the soybean at R8. No-till treatment caused a significant decrease in the amount of N and the dry weight of the pods (Table c.1). Fertilization caused significant effects on N in stems and leaves (Figure C.6). As was stated in Topic two, the soybean may have scavenged all the available N at

R8, making the plant susceptible to influence by small changes in soil N.

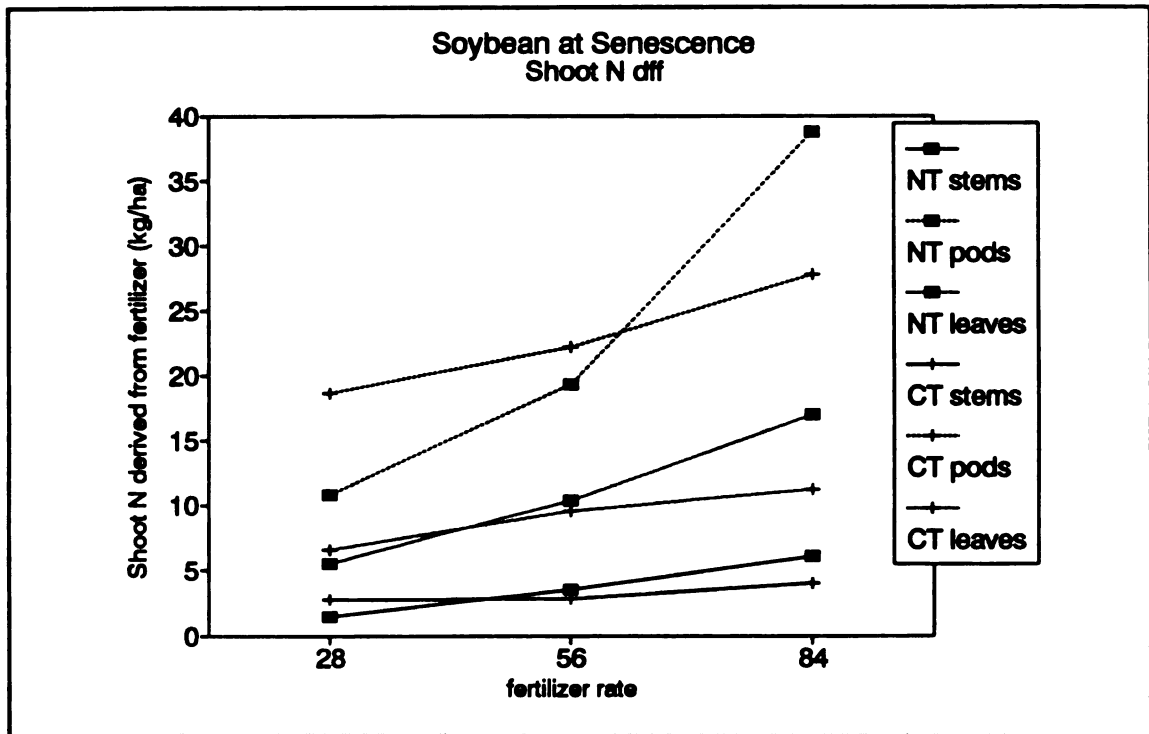


Figure C.5: Quantity of N derived from fertilizer in the soybean at R8.

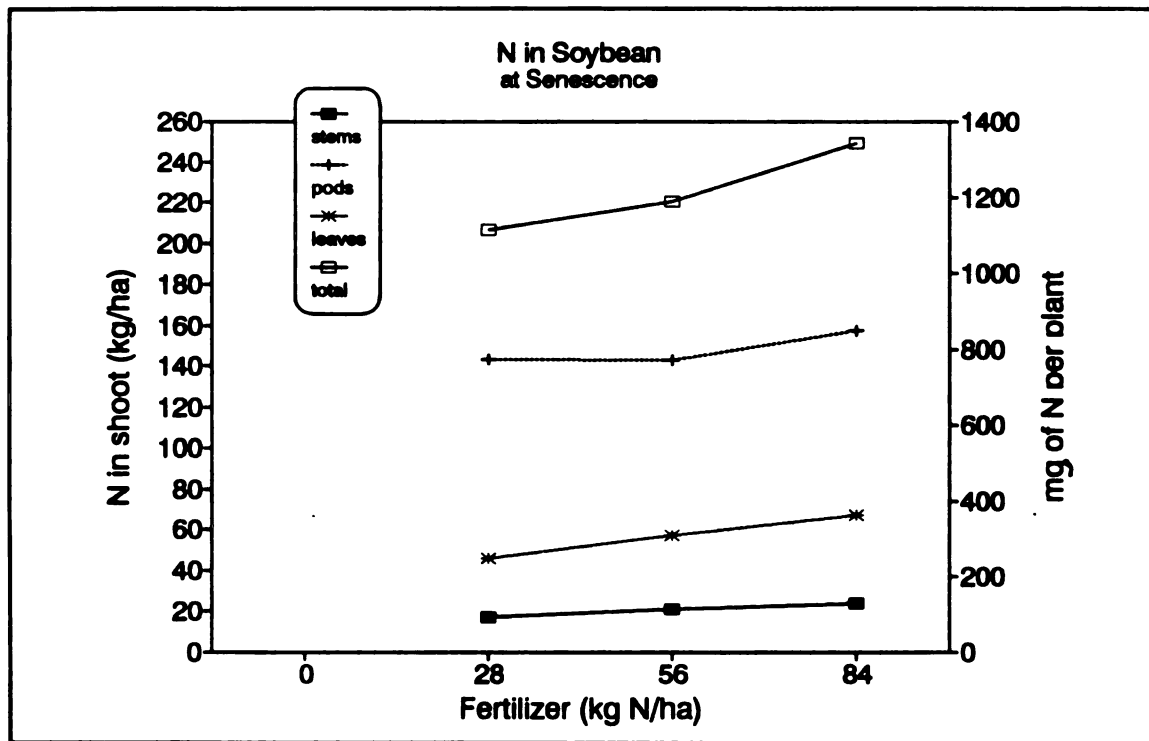


Figure C.6: Soybean N response to fertilization, at R8.

Conclusions

Neither tillage nor fertilizer treatments affected any measures of the yield in this experiment. Both treatments effectively modified soil N. Conventional Till apparently made more inorganic N available than Ridge Till or No Till. Even in treatments where N availability was lower, however, the dry bean and mutant soybean compensated with other N sources and were not affected.

On this soil, in this year the N fertilizer was unnecessary. Schild and Nuland (1988) reported that a dry bean needs 78 to 112 kg N ha⁻¹ from residual soil N, N₂ fixation and N fertilizer. In this experiment 195 kg N ha⁻¹ was contained in the shoot at harvest. The dry bean fixed 68 kg N ha⁻¹ and at least 165 kg N ha⁻¹ was mineralized during the season. Even in such relative abundance of N the dry bean fixed 35% of its own N. Given no stresses from drought, disease etc., 35% may be the minimum, inhibited fixation rate for this dry bean.

Fertilizer N was immobilized and nitrified soon after application. The fertilizer N did not become readily available to either crop until after flowering and this experiment suggests that N applied at planting may not act

as a "starter" fertilizer. The supply of soil N can influence the growth of a legume but evidence from the soybean suggests that the most likely stage for such an effect would be between R6 and R8, during pod-fill. According to Rennie and Kemp (1984) that is exactly the time of most rapid N_2 fixation and any fixing legume could be expected to mitigate the effects of a lack of fertilization.

Pod-fill was not the time of the most rapid accumulation of N derived from the atmosphere. The majority of fixed N accumulated in the shoot of the dry bean between R8 and R9. Wolyn et al. (1989) claims that N_2 fixation continues longer than is commonly believed, with the site of fixation moving to lateral root nodules as crown root nodules die. Wolyn et al. still report that fixation is most rapid near R4. It seems unlikely that the plant could have its peak fixation later than leaf senescence, after the plant has lost its best energy source to drive fixation.

Kahn, Kraus and Somerville (1985) offer a mechanism for legume/rhizobium interaction in which the legume uses N to get N. In an N rich field the dry bean might have kept N in the nodules as long as possible to be ready to respond to a need for more fixation. The fixed N may have stayed in the nodules until the bacteroids were completely dead, delaying the transfer of N dfa to the shoot.

A third explanation of the delay in transfer of N is that the dry bean may have fed the N dfa to microflora in

the root zone. In an N rich field the legume might have supported the growth of rhizosphere microbes with exudates rich in N dfa. The microbes might have dissolved other nutrients the dry bean needed, such as P. At the end of the season the dry bean stopped the exudates. Without a C source the microflora would die off, releasing the N dfa, P and other nutrients. The dry bean could then have reabsorbed the N dfa along with the released nutrients. This theory would explain (1) why there is a delay in transferring N dfa to the shoot, (2) why a legume would continue fixing N while ignoring fertilizer N and (3) what happened to the "lost" N. The dry bean wouldn't have been able to recover all the N exuded earlier. Some N from other sources would have been immobilized by soil microflora. At the end of the season this N could still be in the organic matter, creating a "hole" in the N budgets presented in Tables a.4 and a.5.

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CHAPTER 2

Introduction

There was concern in the field experiment as to whether the mutant soybean was the best possible non-fixing control to use with the Mayflower navy bean. At the time of the experiment a more adequate mutant legume was not available.

In 1988 Davis et al. announced the isolation of a mutant non-nodulating line of *Phaseolus vulgaris*. By Fall of 1989 seeds of the line were obtained. A greenhouse experiment was designed to assess the suitability of the soybean as a control by comparing plant parameters and N_2 fixation estimates to those of the mutant dry bean.

Materials and Methods

A greenhouse experiment was designed as a randomized block design with four replications. The experiment was grown on a Parkhill loam. Soil was passed through 7.7 mm stainless steel sieve and air dried. Seventy two plastic pots were covered with aluminum foil and lined with plastic bags. They were then filled with 7860 g each of the soil. The field capacity of this soil was determined according to the method by Jamison and Kroth (1958). Throughout the season the pots were watered once a day with distilled water to stay above half of field capacity. Note that no water was allowed to flow out of the pot. No N escaped as leachate.

Each pot was planted with five seeds of either mutant non-nodulating Chippewa soybeans, mutant NOD Rwandan dry beans or Mayflower navy dry beans. Seven days after emergence the pots were thinned to one plant per pot and N fertilizer was added. Pots were fertilized to 11, 22 or 56 kg N ha⁻¹ using (NH₄)₂SO₄.

At flowering, 34 days after emergence, half of the pots were sampled. At harvest, 67 days after emergence, the rest

of the pots were sampled. Shoots were processed as described in Chapter 1.

N₂ fixation was estimated using Isotope Dilution by Natural Abundance (Bergersen et al., 1990.) Natural Abundance estimates are based on the theory that isotopic fractionation causes soil N to be slightly higher in atom % ¹⁵N than atmospheric N. With a sufficiently precise mass spectrometer, enriching the fertilizer N is unnecessary.

Results and Discussion

At flowering there were no differences between the three cultivars in terms of dry weight (Table D.1) or N content (Table D.2). The dry weights at harvest of both

Table d.1: Dry weights of the navy bean and two controls by plant fraction.

Dry Weight		<u>navy bean</u>	<u>mutant soybean</u>	<u>mutant dry bean</u>	<u>LSD(.05)</u>
<u>STAGE</u>	<u>fraction</u>	in g plant ⁻¹			
R6	leaves	2.7	2.6	2.5	0.7
	stems	1.1	1.1	1.2	0.7
R9	leaves	2.8	3.7	4.4	0.5
	stems	1.8	3.0	3.6	0.5
	Pods	1.9	2.6	0.7	0.4
	seeds	4.9	2.7	0.2	1.0

controls were different from those of the navy bean (Table D.1), however the mutant soybean was more similar to the test crop than the mutant dry bean was. The N content of the mutant dry bean was different from the test crop in the stems, pods and seeds at harvest. The mutant soybean was different only in the pods and seeds.

The choice of control had no effect on measurements of N derived from the atmosphere. There was one control-

Table d.2: N content of the navy bean and two controls by plant fraction.

N content		<u>navy bean</u>	<u>mutant soybean</u>	<u>mutant dry bean</u>	<u>LSD(.05)</u>
<u>STAGE</u>	<u>fraction</u>	in mg N plant ⁻¹			
R6	leaves	98	91	95	18
	stems	18	19	15	5
R9	leaves	58	67	75	24
	stems	15	21	44	9
	Pods	10	22	16	5
	seeds	198	131	11	41

fertilizer interaction in the leaves at R6 (Figure D.1).

The lack of a fertilizer main effect suggests that the shape of the curve is caused by random variation in the navy bean. The mutant soybean apparently provides estimates that are more sensitive to the fluctuation in the navy bean than the mutant dry bean can.

Fertilization had no effect on dry weight or N content of any of the three cultivars. Fertilization did decrease the accumulation of N derived from the atmosphere in all plant fractions at harvest (Figure D.2).

At flowering the dry bean accumulated an average of 38.5 mg N dfa plant⁻¹. By harvest the average rose to 66.5 mg N dfa plant⁻¹.

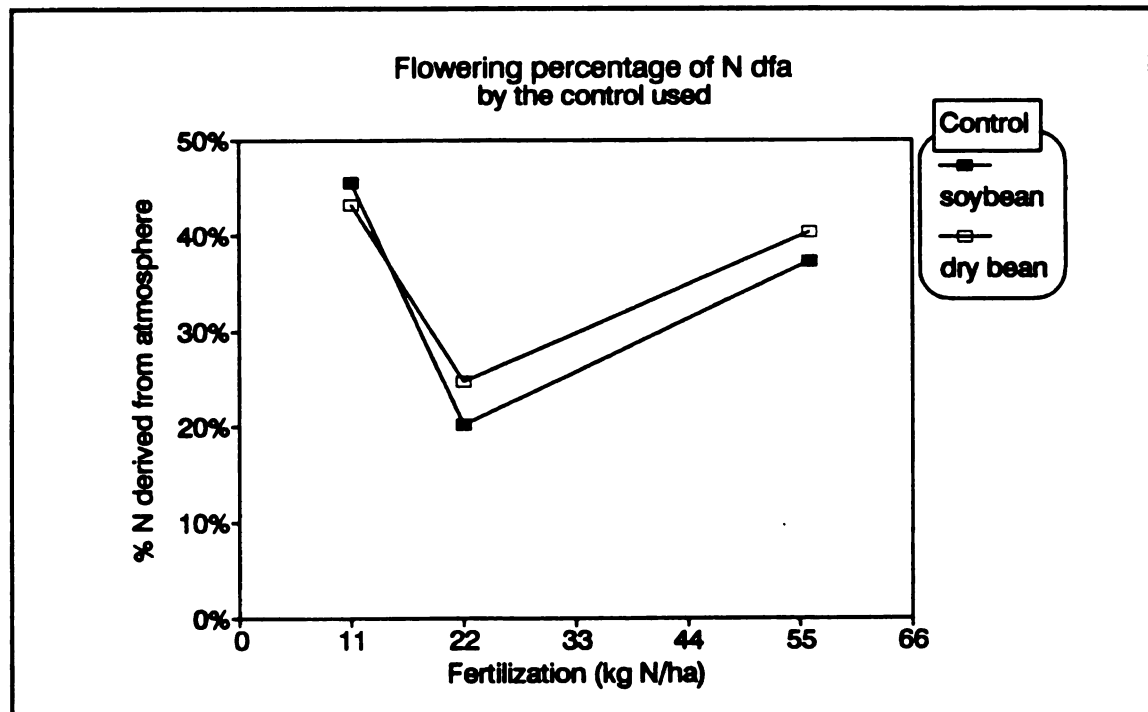


Figure D.1: Interactive effect of N derived from the atmosphere as measured by two controls on different fertilizer treatments.

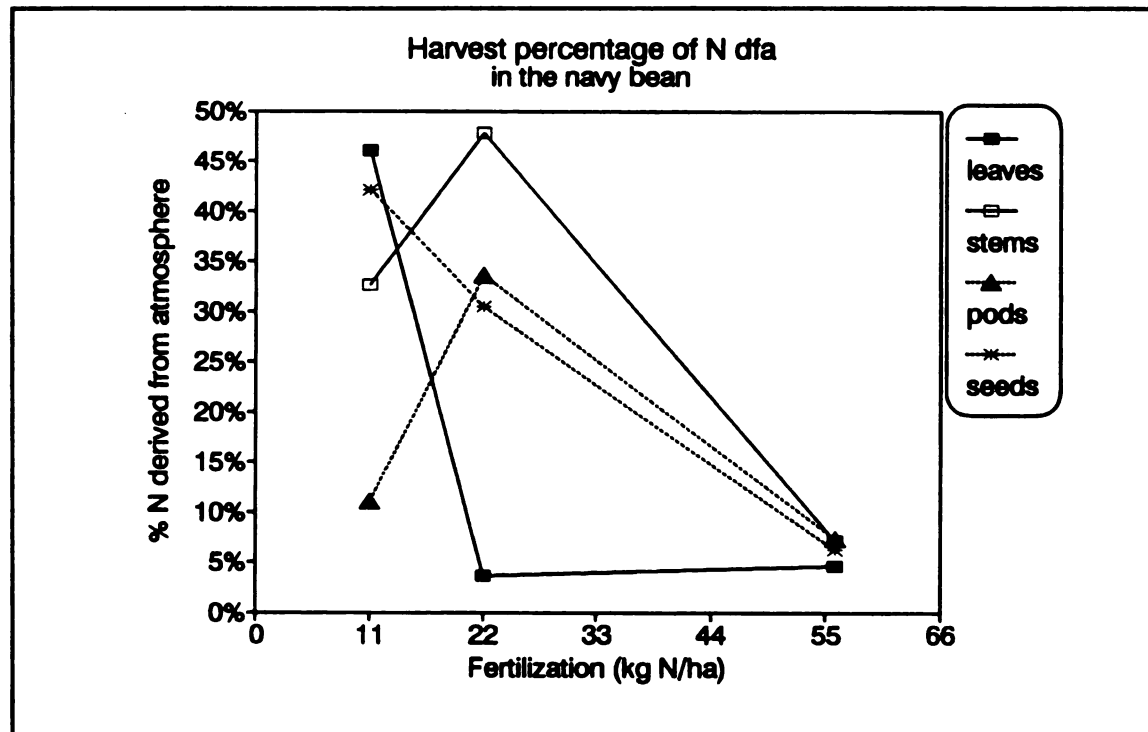


Figure D.2: Fertilizer effect on N derived from the atmosphere in the navy bean at harvest.

Conclusions

The mutant soybean was a closer approximation of the navy bean than the mutant dry bean was due to the similarity in dry weight accumulation and maturation to the navy bean. Until the mutant dry bean can be bred to have a similar maturation period to the navy bean it will not be the most suitable control. The difference in controls can lead to significant differences in measurements of N_2 fixation.

Fertilization did not improve the dry weight or N content of any of the three cultivars. There was no "starter" fertilizer on the navy bean and, in fact, fertilization inhibited N_2 fixation.

While the majority of N dfa accumulated before R6 in this experiment, a significant portion still accumulated during the reproductive stages of plant development.

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APPENDIX A

The weather during the season of 1989 was temperate (Table b.1.) Growing degree days were calculated by the formula used by Smucker et al. (1982):

$$\text{GDD} = \sum (\max T + \min T)/2 - 10^{\circ}\text{C}.$$

Table I: Weather data in Saginaw County, Michigan in 1989.

plant stage	Date	GDD since plant ing	avg daily temp. during period	rain during period (mm)	avg daily rain-fall (mm)
Planting Emergence V1	June 9 June 18	0 59	16°C	59.4	6.60
Vegetative V4	July 10	306	21°C	66.3	3.16
Flowering R6	Aug. 9	631	21°C	64.0	2.13
Senescence R8	Sept.10	949	20°C	137.7	4.30
Harvest R9	Sept.26	1032	14°C	11.9	0.75

Average daily temperatures were near those reported for a 30 year average by Smucker et al. (1982). Average daily rainfall is 0.3 mm above their report.

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