INHERITANCE OF PROTEIN AND OIL IN THE SEED OF EARLY MATURING SOYBEANS AND A CHARACTERIZATION OF THE PARENTAL CULTIVARS FOR NITROGEN ACCUMULATION AND REDISTRIBUTION

A Thesis
Submitted to the Faculty
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by

Anne Leslie McKendry
In Partial Fulfillment of the
Requirements for the Degree

of

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ABSTRACT

McKendry, Anne Leslie. M.Sc., The University of Manitoba. August 1984. Inheritance of Protein and Oil in the Seed of Early Maturing Soybeans and a Characterization of the Parental Cultivars for Nitrogen Accumulation and Redistribution. Major Professor; Dr. P.B.E. McVetty.

The inheritance of percent protein, percent oil and the sum of protein and oil as a percentage of the seed, was investigated in two crosses, Maple Presto/X446-2-1 and Maple Presto/Sioux, of early maturing soybeans. Spaced plants of the parental, F_1, F_2 and backcross generations were grown in 1981 while in 1982, selfed backcross and F_3 generations were added to the design. Plants were grown on the University of Manitoba's experimental land at Winnipeg (the point).

Generation means analyses indicated that gene action for percent protein was primarily additive, with partial dominance for low protein. Percent oil was also conditioned primarily by additive gene action. Partial dominance was not consistently detected over years and therefore was thought to be of minor importance. Additive X additive epistasic effects for percent oil were also detected but again, were not consistent with years. The sum of percent protein and oil was conditioned solely by additive gene action. Dominance and epistatic components were nonsignificant.

Variance analyses indicated a predominance of additive genetic variance for all three traits. Dominance variance was also detected for both percent protein and the sum of protein and oil. Broad sense heritability for the F_2 generation ranged from 76% to 83% for percent protein and from 68% to 86% for the sum of percent protein and oil, while narrow sense heritability ranged from 34% to 72% for percent protein and from 39% to 65% for the sum of protein and oil. Narrow sense heritability values equalled broad sense values for percent oil and ranged from 58% to 68%. Heritability was also estimated from standard unit parent-progeny regression analyses and values for each of the traits were found to be higher than the narrow sense estimates based on variance components.

The predominance of additive gene action suggested that recurrent selection or other conventional breeding methods aimed at accumulating favorable alleles would be effective in improving percent protein, percent oil or their sum.

Seasonal patterns of nitrogen accumulation and redistribution in the three parental cultivars, under spaced and plot density conditions, were also investigated in order to identify cultivar differences in these traits that were genetically related to percent protein in the seed.

Cultivar comparisons of the amount of nitrogen accumuluted in the vegetative tissues by the onset of pod development (R_2) and in the pod wall tissue by the onset of seed development (R_4), suggested that the high protein cultivars, X446-2-1 and Sioux, tended to accumulate more nitrogen per unit dry matter of these tissues than the low protein

cultivar, Maple Presto. Under plot conditions, X446-2-1 also remobilized more nitrogen to the developing seed than either Maple Presto or Sioux, while Sioux remobilized more nitrogen from the pod wall tissue to the developing seed than Maple Presto or X446-2-1. The rate of remobilization of nitrogen from pod wall tissue to the seed was also greater for Sioux than for either Maple Presto or X446-2-1.

Percent nitrogen in the vegetative tissue at R_2 and pod wall tissue at R_4, was also higher in X446-2-1 and Sioux than in Maple Presto. In the pod wall tissue of the high protein cultivars, this higher nitrogen cencentration was coupled to a greater net decline in nitrogen concentration during seed development.

A range of genetic variability was also noted for both harvest index (HI) and harvest nitrogen index (HNI) in the three cultivars. Independently, these traits were negatively related to percent protein in the seed, but the ratio of HNI/HI increased positively with percent protein in the seed.

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1. INTRODUCTION

The importance of grain legume crops is becoming ever more apparent as the world demand for food and feed protein increases. In both developed countries where environmentalists are voicing concerns over the effects of increased fertilizer use and in developing countries, where the rural farmer cannot afford fertilizers, there is an escalating demand for legumes which are both high in protein and capable of fixing atmospheric nitrogen. Although considered primarily as an oilseed crop, the soybean offers an economical source of these future protein needs of both man and animals.

It is well known that legume seed protein is generally deficient in the sulfur-containing amino acids (methionine and cystine) in contrast to cereal seed protein which is deficient in lysine. When comparing the legumes for sulfur-containing amino acid content as well as protein percentage, however, it can be seen that soybeans have relatively higher protein percentage and their protein contains relatively higher amounts of sulfur-containing amino acids than other leguminous species (Kaizuma 1979). As such, soybeans are one of the most promising plant protein sources known.

Historic and geographic evidence shows that the cultivated soybean Glycine max L. had its origin in Asia. It was introduced throughout the Far East in ancient times and to Europe and America in more recent times. The western world now contributes more than 80% of the world

soybean production, with the United States and Brazil being the major producers (Camacho 1981).

Early U.S. production of soybeans was concentrated in the eastern and southern states but by 1924 production had expanded into the Corn Belt area, which, by 1939, accounted for 84% of the acreage harvested and 91% of the total U.S. production (Probst and Judd 1973). The range of soybean production has also extended into Canada, where profitable production can be found in southern Ontario and more recently in southern Manitoba.

Varietal development and a clear understanding of photoperiodism in relation to varietal adaptation, have played a major role in extending the range of profitable soybean production from the southeastern United States north into Canada (Hartwig 1973). As a method of describing their responsiveness to daylength, ten maturity groups have been established for identifying the region of adaptation for soybean varieties in the U.S. and Canada. Groups 00, 0 and 1 are adapted to the longer days in the northern areas of adaptability while succeeding groups are adapted further south. An additional 000 group has been developed for areas of production above 50° latitude.

Photoperiod insensitivity has been reported in early maturing soybeans (Criswell and Hume 1972) and has been found to be controlled by a single recessive gene (Shanmugasundaram 1977). The inclusion of this gene into existing germplasm has helped to extend the range of the soybean yet further, through the production of widely adapted types.

The protein content of currently grown northern soybean cultivars, is 40-42% on a whole seed, zero moisture basis, which is considerably

below the maximum value of 53% reported in the northern soybean germplasm collection (Shannon et al. 1972). The main research objectives for genetic improvement in the protein of these lines are (1) further increase in protein percentage in the seed, (2) increase in the relative contents of sulfur-containing amino acids per unit weight protein and (3) genetic removal of anti-nutritional factors, eg. trypsin inhibitors. The emphasis however, has been on increasing percent protein while maintaining oil and yield levels (Brim 1973).

The extent of possible improvement in the precentage protein and oil in the seed by breeding, is determined by the amount of genetic variation in relation to environmental variation and by the nature and extent of their interaction. In turn, the breeding strategies are dependent on the type of action, interaction and linkage of the genes controlling these traits. The breeding strategy approaches for improvement of protein and oil percentages therefore, are determined by both a thorough knowledge of the extent of genetic and environmental variation and an understanding of the properties of the genes which control phenotypic expression.

The most significant improvement in soybeans to date has been in qualitative traits, which have relatively simple inheritance and therefore can be easily transferred from one line to another. Progress in selection for quantitative traits, which include the majority of commercially important characters, such as yield, protein and oil, has been achieved much more slowly, possibly as a result of the slower improvement in knowledge of breeding techniques for these more complexly inherited traits. With a better knowledge of the genetics

and gene action controlling these traits, it should be possible to make more rapid progress.

From a physiological viewpoint, the key to increasing percentage protein in the seed may be to increase the nitrogen accumulation in the seed. Yield has also been shown to be closely correlated to the accumulation of nitrogen throughout the life cycle of the plant (Lathwell and Evans 1951).

Increasing the quantity of nitrogen available for grain development in soybeans can be accomplished by increasing whole plant nitrogen accumulation and/or by increasing the proportion of vegetative nitrogen translocated to the developing grain. The application of nitrogen fertilizers has not proved an effective or economical method of increasing nitrogen accumulation in the seed of nodulated soybeans as these fertilizers inhibit the nitrogen fixation process (Weber 1966, Caviness and Hardy 1970). Therefore, maximizing the effectiveness and efficiency of the host symbiont interaction and increasing remobilization of nitrogen from vegetative structures to the grain appear to be the logical steps involved in increasing the supply of nitrogen to the grain.

Varietal differences in physiological strategies for accumulating nitrogen in the seed have been observed in some late maturing soybeans (Jeppson et al. 1978, Israel 1981). Should this variability exist in early maturing lines, it may be possible to increase the nitrogen accumulation in the seed and therefore the percentage protein and yield in the northern varieties.

The aim of this investigation was primarily to examine the inheritance of protein and oil in early maturing soybeans, in order to

gain a better understanding of gene action controlling these economically important traits. This information should enable the development of breeding strategies to further increase the percentage oil and protein in early maturing soybeans.

A secondary part of this study involved examination of the high and low protein lines included in the inheritance study for variable physiological strategies for accumulating nitrogen in the seed. This latter study was not intended to be an exhaustive one but rather a preliminary investigation of the parental material available, to determine if differing, complementary physiological strategies for nitrogen accumulation and remobilization, which could be employed in a soybean breeding program to increase accumulated nitrogen in the seed, were evident.

2. LITERATURE REVIEW

2.1 Quantitative Inheritance

The majority of economically important characters in crop plants are quantitative and usually display continuous variation. Whereas the kinds of qualitative characters which Mendel studied in his classic experiments could be easily classified without ambiguity into one of two or more classes, discrete classes can not be recognized in quantitative traits and therefore classical Mendelian methods of genetic analysis prove impractical. Also complicating the issue is the effect of the environment on these traits. The magnitude of differences between individuals with respect to qualitative characters, is so great that the effects of the environment for the purpose of genetical analysis can be effectively discounted. Quantitative traits, on the other hand, are controlled by many genes, inherited in a Mendelian fashion, but subject to environmental modification, to the extent that the environment is not infrequently a larger source of variation than the genotype itself (Breese 1971).

2.2 Genetic Variation for Protein and Oil

Variability has been observed in percentage protein and oil in many economically important crops. Experimental evidence that there is a significant genetic component to this variation dates back to the classical selection experiments for protein and oil content in corn,

conducted at the Illinois Agricultural Experiment Station. A steady slow response to selection was observed for both high and low protein and high oil content, throughout 50 generations of selection. Selection for low oil content, however, plateaued after 20 generations (Woodworth et al. 1952). In addition, evidence indicated that there was no marked increase or decrease in the variability of either the high or the low lines, even after 50 generations of selection. After 70 generations of selection, breeders have raised and lowered the protein percentage from 10.9 percent in the original sample to 23.5 percent and 4.0 percent respectively (Axtell 1981).

There is also ample evidence in other crops, as cited below, that demonstrates significant genetic variation for protein and oil concentration, which has enabled selection for these traits. In addition, although selection for increased protein concentration usually depressed yield, the examples cited in wheat and oats suggest that it is possible to combine high protein and acceptable yield levels.

Genetic investigations of the quantity of protein in the grain of wheat date back to 1926 (Clark 1926). Since then, many investigators have postulated the genetic control of protein in wheat to be multigenic and this is now generally accepted (Ausemus et al. 1946). Success in utilizing this genetic variability for protein without loss of yield has been reported for the cultivars Atlas 50 and Atlas 66 (Middleton et al. 1954). These cultivars have proved equally as productive as older cultivars and yet higher in percent protein in the grain. In breeding programs, Axtell (1981) reported that genes from Atlas 66 had been successfully transferred to Lancota, elevating its protein concentration by one to two percent without a reduction in yield.

Significant genetic variability for percent protein is also present in oats with the range of whole grain percent protein being from 9 to 16 percent (Frey and Watson 1950). As in wheat, high protein does not exclude acceptable yields as genes fron Avena sterilis L. have been discovered that increase yield without depressing protein concentration (Frey 1975).

In bean crops, a significant proportion of the variability in protein is genetic and thus can be selected for but the negative correlation between protein and yield persists.

The seed protein content of beans (Phaseolus vulgaris L.) has been reported to vary from 16 to 31 percent (Kelly and Bliss 1975a) and despite environmental factors known to affect protein levels (Kelly and Bliss 1975b), heritable differences for percentage protein and for protein quality have been reported (Leleji et al. 1972). Percentage protein was found to be negatively correlated with yield (Leleji et al. 1972, Kelly and Bliss 1975a), but the correlation was low.

Sesame seed is also rich in protein with relatively high proportions of the sulfur-containing amino acid methionine (Murty and Hashim 1973). A range of percent protein in the seed of 17 to 23 percent (Kinman and Stark 1954) indicated a high degree of variability in the expression of this trait. As this variability was observed for both varieties and locations, Kinman and Stark (1954) concluded that there were both genotypic and environmental components to the variation.

Soybeans, (Glycine max L.) also show significant variability for protein with the range for percent protein in early maturing lines extending from 40 to 53 percent. Evidence that this variability

consists of a significant genetic component is well established in the literature (Kwon and Torrie 1964, Smith and Weber 1968, Shannon et al. 1972, Brim and Burton 1979, Miller and Fehr 1979). Because of the importance of soybeans as a source of protein, breeders have intensified their efforts to increase protein productivity in the species, through selection of breeding lines for higher percent protein.

Breeding programs designed to increase protein content of soybeans have used crosses between locally adapted high yielding varieties and high protein exotic plant introductions in an effort to combine both high yield and high protein (Thorne and Fehr 1970). Three-way crosses ([adapted X exotic] X an adapted high protein variety) produced more superior lines for yield and protein than did two-way crosses (adapted X exotic) but in both cases, high protein content of the exotic strains was transmitted readily to the offspring and selection for high yielding, high protein strains was possible. Progress in developing strains with both high yield and high protein was slow, however this was generally thought to be due to the well documented negative correlation between protein and yield (Johnson et al. 1955a, Kwon and Torrie 1964, Hartwig and Hinson 1972, Shannon et al. 1972).

Less work has been reported on the nature of the variability of percent oil, but again, from the work on corn in Illinois reported earlier (Woodworth et al. 1952), it has been shown that there is a significant genetic component which allows selection to be practiced for either increased or decreased oil concentration. After 70 generations of selection, breeders have raised the percentage oil from 4.7 percent to 17 percent, but yield was adversely affected with very high oil

concentrations (Axtell 1981). Sprague and Brimhall (1950) have also shown that the oil content of corn can be modified by appropriate selection procedures, confirming the presence of a genetic component to the observed variability.

Traditionally, oats (Avena sp.) have not been used as a source of edible oil because the amount of oil is relatively low and the oil, once extracted, rapidly deteriorates in quality. However, new interest has been generated in developing oat cultivars with high oil content since a range of expression in oil of four percent to ten percent has been observed in the World Oat Collection (Brown and Craddock 1972) and studies have shown that a significant proportion of this variability is genetic (Baker and McKenzie 1972).

Other crops, important for their oil, also show significant genetic components to the observed variability in oil concentration.

In the sunflower (Helianthus annuus L.) the presence of significant genetic variability for percent oil has permitted Russian plant breeders to improve seed oil content from 30 percent in the 1920's to almost 50 percent in present day varieties (Alexander 1963).

Oil content of safflower has also responded well to selection indicating a significant genetic component to the observed variability. Knowles (1975) reported that oil content of the seed had been increased from 37 percent to 50 percent by breeding for reduced hull content.

Kinman and Stark (1954) reported a high degree of variability in the expression of oil in sesame, ranging from 45 to 63 percent for cultivars studied. Variability was found to exist both between environments and varieties indicating both an environmental and genetic component of the observed variability.

Variability for oil content in seeds of soybeans (Glycine max L.) is also high and results from genetic as well as environmental factors (Wilson et al. 1976). Smith and Weber (1968) effectively selected for high and low oil using mass selection techniques where low seed density was associated with high seed oil concentration and high seed density was associated with low oil concentration. Miller and Fehr (1979) reported that high or low oil could be selected for respectively, while Burton and Brim (1981) reported that oil percentage was increased significantly in soybean in three cycles of recurrent selection.

2.3 Components of Quantitative Genetic Variability

2.3.1 Types of Gene Action

The first attempt to partition the genetic component of continuous variation into its component parts was made by Fisher in 1918 (Allard 1960). He recognized three major components of genetic variance (1) additive (2) dominance and (3) epistatic gene action. In additive gene action, the substitution of one allele for another at a given locus, produces a plus or minus shift on the scale of measurement which is independent of other alleles present. On the other hand, alleles which interact with each other in the same gene pair, with one allele masking the effect of the other to varying degrees, produce dominance effects. Finally, phenotypic expression can also be caused by the interaction of genes at two or more loci. These inter-allelic interactions are called epistatic effects and are divisable into additive X additive, additive X dominance and dominance X dominance variation for two loci (Gardner

1963). Gardner (1963) also describes other components of the genetic variance which are useful to the plant breeder. These include: (1) the average degree of dominance which is the ratio of dominance variance to additive genetic variance, (2) genotype X environment interactions which are divisible into both additive gene effects X environment and non-additive gene effects X environment and (3) genotypic correlations among quantitative characters of importance for a particular crop.

Since Fisher's early work, many scientists have contributed over the years to the development of mating designs to allow these components to be estimated, most notably, R.E. Comstock, B.I. Hayman, K. Mather, and H.F. Robinson (Sprague 1965) but it is beyond the scope of this investigation to give a detailed review of these contributions.

2.3.2 Gene Action for Protein Content

The nature of the inheritance of protein content in corn has been studied for many years. Early work by East and Jones (1920) showed percent protein in the F_1 hybrid fell close to the low protein parent. Frey (1949) also found that low percentage protein was completely dominant. He suggested however that this interpretation of extreme dominance might be partially confounded by the increase in yield resulting from hybrid vigour. Genter et al. (1957) used correlations between similar characteristics in parents and progenies to eliminate confounding effects and found that very slight dominance for high protein percentage is evident in corn.

The earliest work in wheat (Clark 1926) indicated a degree of dominance for low percent protein. Later work by Chapman and McNeal

(1970) examined the inheritance of percent protein in each of five spring wheat crosses (Triticum aestivum L.). For all crosses, a good fit of observed and expected generation means was obtained and it was concluded that epistatic or non-allelic interactions, did not significantly contribute to the inheritance of grain protein. For all five crosses, a highly significant additive genetic effect was detected. In two of the five crosses, a significant dominance effect was observed, in the direction of the lower parent whereas the other three crosses positioned the F_1 between the midparent and the lower parent but not significantly different from the midparent. Conclusions reached from the experiment indicated that protein was primarily under additive control with some dominance for lower protein in the grain. Other work in the area indicated similar gene control of protein in wheat (Haunold et al. 1962, Stuber et al. 1962, Diehl et al. 1978). Diehl et al. (1978) also found that lysine expressed as percent of protein, appeared to be inherited primarily by additive gene action with significant genotype X environment interaction.

Early work on the inheritance of protein in oats (Frey et al. 1954) suggested dominance gene action for low protein. Campbell and Frey (1972) working on interspecific crosses with Avena sterilis L., important for its high protein content and acceptable yields, found that groat protein was primarily controlled by additive gene action with some epistatic gene action also influencing percent protein. Ohm and Patterson (1973), also working on interspecific crosses with A. sterilis L., similarly found groat protein to be controlled primarily by additive gene action but also found partial dominance for low protein. Sraon et al. (1975), working on interspecific crosses with A.

sterilis and A. sativa L. also found that groat protein percentage was primarily under additive genetic control with partial dominance for low protein. In summary, additive gene action would appear to be of primary importance in the expression of percent protein in oats with varying degrees of dominance or epistatis for low protein, the latter probably conditioned by the nature of the parental material used.

Culp (1959) found inheritance of protein in a sesame (Sesanum indicum L.) cross to be complex but with a large part of the genetic variance due to additive gene action. Little dominance gene action was reported. Later work (Murty and Hashim 1973) on ten inbred sesame lines also showed percentage protein to be governed by additive gene action. Murty and Hashim (1973) also reported that dominance for low protein played a major role in the expression of genes for protein.

Investigations in summer rape (Brassica napus L.) indicated that the expression of percentage protein is under nuclear control (Grami and Stefansson 1977a) and is governed by additive gene action (Grami and Stefansson 1977b) with a nonsignificant dominance component. Epistasis was found to be absent. Further investigations, (Grami et al. 1977) indicated that six to nine genes conditioned the difference between high and low protein.

Although beans (Phaseolus vulgaris L.) are an important source of protein, the literature relating to the inheritance of percent protein in the seed is meagre. Lelji et al. (1972) found the mean crude percentage protein in the F_1 to be slightly closer to the low protein parent indicating partial dominance of genes for low protein. Later

work (Kelly and Bliss 1975b) on both seed protein percentage and methionine as a percent of protein supported earlier work by suggesting partial dominance for low protein percentage and methionine percentage but also found these two traits to be primarily governed by additive gene action.

In soybeans (Glycine max L.) protein inheritance has also been studied but primarily in cultivars of maturity group II or later. Williams (1948) studied 15 crosses between wild and cultivated soybeans and found high protein content to be dominant. Brim and Cockerham (1961), after extensive investigations into quantitative traits in crosses involving group VI soybean varieties concluded that percent protein was conditioned primarily by additive gene action with some dominance towards the high protein parent. Their analysis also showed an additive X additive non-allelic component of the genetic variance which contributed significantly to the inheritance of protein in the crosses investigated. In a review of the literature, Johnson and Bernard (1963) found that the available estimates for the components of genetic variability were not in good agreement but the importance of additive genetic variance was well established. They felt, however, that the role of non-additive effects needed to be better established.

Singh and Hadley (1972), in examining cytoplasmic control of percent protein in soybeans found it to be also partially under maternal control in the F_1 and F_2. Percent protein was found to be significantly higher (four percent) in the population with cytoplasm from the higher protein parent. The authors concluded that this may have been an isolated occurence but felt that further investigation was warranted.

It would appear from the literature that a significant portion of the observed variability for percent protein is genetic and that additive gene action is the primary component of this variation. Partial dominance appears to be of secondary importance and is for lower percent protein in the crops reviewed, with the exception of corn and soybeans.

2.3.3 Gene Action for Oil Content

Numerous studies have provided information on the inheritance of oil content in corn. Early investigations (Brimhall and Sprague 1951) suggested that additive gene action was of major importance in the control of the expression of oil content. Later work (ElRouby and Penny 1966, Poneleit and Bauman 1970) supported these conclusions but ElRouby and Penny also suggested that slight positive dominance also contributed to oil content.

Investigations with cultivated oats (*Avena sativa* L.) (Brown *et al.* 1974) have suggested that percent groat oil was under polygenic control with primarily additive gene action controlling expression. Frey *et al.* (1975) determined the inheritance patterns of groat oil percentages in crosses between cultivated oats and *Avena sterilis* L., a weedy high oil content species, and found that inheritance seemed to follow the same pattern as that reported for *A. sativa* L. cultivars (Brown *et al.* 1974) with the exception that there appeared to be additional partial dominance for high oil percent.

Although little information is available on the inheritance of oil in sunflowers (*Helianthus annuus* L.), Russell (1953) suggested that

oil content was under polygenic control with partial dominance. Fisk (1975) showed that additive gene action was largely responsible for the expression of oil percentage with some dominance for high oil.

Yermanos et al. (1967) found similar inheritance patterns in seed-oil quantity in safflower (Carthamus tinctorius L.) crosses. Genes for oil content were found to be mainly additive with slight dominance for high oil.

Investigations by Culp (1959) on sesame (Sesanum indicum L.) indicated that the inheritance of oil content was complex. A large component of the variation was found to be nonheritable but of the heritable portion, the primary component was again additive gene action. Very little of the variance was attributed to dominance deviations. Murty and Hashim (1973) also concluded that oil content is governed primarily by additive gene action but also suggested that there is a significant dominance component for high oil in sesame.

Investigations into the inheritance of oil content in summer rape (Brassica napus L.) (Grami and Stefansson 1977b) indicated that oil was governed by additive gene effects. Dominance was a nonsignificant component of the genetic variance and epistasis was absent.

Finally, Brim and Cockerham (1961) found soybean oil content in the seed to be primarily under additive gene control with some additive X additive gene action. The latter component however was very small relative to the additive component of genetic variance. Gates et al. (1960) also found additive variance to be the principal component of genetic variance in soybean oil content, with little evidence of dominance variance. Singh and Hadley (1968) confirmed the importance

of additive genetic effects on the oil content of the seed in soybeans, but failed to detect any additive X additive epistatic interactions in the genotypes studied.

2.3.4 Gene Action for the Sum of Oil and Protein

It has been well established that protein and oil are negatively correlated in the seed of sesame (Culp 1959), rape (Brassica sp.) (Grami and Stefansson 1977b) and soybeans (Johnson et al. 1955a, Kwon and Torrie 1964, Shannon et al. 1972). Despite this negative correlation, positive advances for both protein and oil in rapeseed have been made simultaneously within the same program by selection for the sum of these two quality traits (Stefansson and Kondra 1975, Grami and Stefansson 1977b).

Grami and Stefansson (1977b), in investigations with two summer rape cultivars, Tower and Midas, found the sum of protein and oil to be governed by additive gene action. Dominance was found to be non-significant and epistasis was absent.

2.4 Heritability

Heritability is defined as "the portion of observed variability which is due to heredity, the remainder being due to environmental causes" (Allard 1960) and has value primarily as a method of quantifying the question of whether progress from selection for a plant character is relatively easy or difficult to make in a plant breeding program. It is used in both a broad and a narrow sense. For the broad sense, the genotype is considered as a unit in relation to the

environment and broad sense heritability is therefore defined as the "total genetic variability in relation to the phenotypic variability" (Hanson 1963). Heritability in the narrow sense expresses only the fraction of the phenotypic difference between parents which one expects to recover in the offspring. Hanson (1963) defines narrow sense heritability then, as "the additive portion of the genetic variability in relation to the phenotypic variability."

Among the simplest methods for estimating broad sense heritability is the replication of genotypes in a single micro-environment (Breese 1971). An analysis of variance then allows the total phenotypic variation to be partitioned into the genotypic component and the error component which is due to uncontrollable micro-environment fluctuations.

Narrow sense heritabilities are of more use to the plant breeder as they represent that proportion of the phenotypic variation that is fixable through selection. As such, a great deal of ingenuity has gone into developing mating plans which permit comprehensive breakdown of the genetic variance into component parts that allow the estimation of narrow sense heritability. These mating designs include analysis of variances from generations derived from the cross of two inbred lines, diallels, and North Carolina designs to name only a few (Mather and Jinks 1977). In a suitably designed experiment, then, it is possible to extract estimates of genetic variance and its components, environmental variance and genetic X environmental interactions.

Estimation of genetic variance and its components however suffers from statistical weaknesses. Simmonds (1979) in reviewing these weaknesses suggests variances may be unreliably estimated as they are

particularly sensitive to deviations from normality. He also indicated that variances tend to be less reliable than means as the variance of the mean is less than the variance of the variance.

An alternative approach to estimating narrow sense heritability is parent-progeny regression where close parent offspring similarity suggests a large genetic effect and a small environmental effect. The regression coefficient directly estimates narrow sense heritability empirically, without depending on genetic assumptions (Simmonds 1979). Although parent-progeny regressions are free of the troubles associated with the partition of variance, they may over-estimate narrow sense heritability when selfed progeny are regressed on their parents. This is because small amounts of dominance variance and dominance types of epistasis are usually contained in the heritability estimate (Casler 1982). In addition, if the parents and offspring are grown in the same environment, nonzero genotype X environment interaction covariances between parents and progeny will result in biased heritability estimates. However, when grown in separate environments, differential environmental expression on parents and progeny can also have an effect on the magnitude of heritability estimates (Frey and Horner 1957). Heritability estimates are therefore very much a function of the procedure used and the nature of the parental material studied. Each is unique and a property of a specific population in a specific experiment and therefore should be interpreted only in terms of general orders of magnitude.

In wheat, estimates of both broad and narrow sense heritabilities have been reported in the literature. Haunold et al. (1962) reported

from a study of crosses of the high protein soft winter wheat Atlas 66, with the hard winter wheat varieties, Comanche and Wichita, that broad sense heritability estimates ranged from 56 to 65 percent. Stuber et al. (1962) also studied the variability of grain protein content in a cross of Atlas 66 and Wichita. From an analysis of the variability in parental, F_1, F_2, and backcross populations, they found broad sense heritability estimates were higher and ranged from 68 to 83 percent. Finally, from an analysis of the genetic variance among hard red spring wheat cultivars, Baker et al. (1971) found broad sense heritability estimates of grain protein ranged from 61 to 89 percent. Narrow sense heritability estimates for crosses involving the winter wheat Atlas 66 have been reported by Davis et al. (1961). From an analysis of the genetic variance of parental, F_1 and derived selfed generations, they found that the narrow sense heritability of grain protein concentration ranged from 23 to 35 percent. Haunold et al. (1962) produced similar estimates from parent-progeny regression studies of winter wheat crosses with Atlas 66 as the high protein parent. They found narrow sense heritability ranged from 25 to 36 percent

Early studies of three oat crosses (Frey et al. 1955), showed broad sense heritability for grain protein content ranged from 88 to 90 percent. Narrow sense heritability was estimated from an analysis of the genetic variance found in interspecific oat crosses by Campbell and Frey (1972). They found that narrow sense heritability for groat-protein percentage, varied from 30 to 57 percent. Sraon et al. (1975) also studied the components of variation for grain protein percentage, using oat crosses and found that narrow sense heritability for percent protein in the grain was 41 percent.

Culp (1959) investigated the heritability of seed protein concentration in a cross of two sesame lines. From an analysis of the variability in the F_1 and F_2 generations, Culp found broad sense heritability was 61 percent. Narrow sense heritability was estimated from the variance of parental, F_1, F_2 and backcross generations and ranged from 48 to 58 percent (Culp 1959). Murty and Hashim (1973), also investigated the narrow sense heritability of percent protein in the seed of sesame from a number of crosses and found it was 30 percent.

The literature on the heritability of percent protein in the seed of rapeseed is meagre. An estimate of 26 percent for the broad sense heritability of percent protein was obtained however, from generations derived from the cross of two summer rape cultivars (Grami et al. 1977).

Broad sense heritability estimates in beans were calculated from F_1 and F_2 generations in crosses among five lines selected for their range of percent protein (Leleji et al. 1972). These estimates varied from 30 to 64 percent. Kelly and Bliss (1975a), reported broad sense heritability estimates for percent protein in crosses among four strains of beans that ranged from 32 to 71 percent. Narrow sense heritability estimates were also reported for percent protein by the above authors. Leleji et al. (1972) found from both an analysis of the variance in the parental, F_1, F_2 and backcross generations and from parent-progeny regression analyses that the narrow sense heritability of percent protein ranged from 5 to 20 percent. Using a similar type of analysis on different parental material, Kelly and Bliss (1975a)

found narrow sense heritability for percent protein varied from 32 to 63 percent.

Finally, a wide range of broad sense heritability for percent protein in the seed of soybeans, has been reported. Johnson et al. (1955b), analysed the genetic variance in the F_3, F_4 and F_5 generations of two soybean crosses and found the broad sense heritability of percent protein was 39 percent. Kwon and Torrie (1964) reported a broad sense heritability of 57 percent using a similar type of analysis but different parental material while Smith and Weber (1968) found that broad sense heritability for percent protein was 90 percent.

Heritability estimates for percent oil are equally variable, as illustrated by the literature on oats. Baker and McKenzie (1972), using crosses between cultivars with a range of oil concentrations, found that the broad sense heritability of oil percentage in the grain varied from 68 to 95 percent. Brown et al. (1974) evaluated crosses among eight Avena sativa varieties and found that broad sense heritability for oil percentage in the groat, ranged from 61 to 79 percent. Although narrow sense heritability estimates were not made, Brown et al. (1974) concluded that since the mode of gene action for oil content in oats was largely additive, the narrow sense heritability values would be very near the broad sense heritability values.

Culp (1959) investigated the heritability of seed oil concentration in a cross between 2 sesame lines. From an analysis of the F_1 and F_2 variability, Culp found broad sense heritability for oil percentage was 64 percent. From an analysis of the genetic variability in the parental, F_1, F_2 and backcross generations, narrow sense

heritability was found to vary from 33 to 82 percent (Culp 1959). Murty and Hashim (1973) also investigated the narrow sense heritability of percent oil in the seed of sesame and obtained an estimate of 23 percent.

The heritability of oil concentration in the seed of sunflowers was studied in parental, F_1, F_2 and backcross generations of two crosses (Fisk 1975). Broad sense heritability for percent oil in the seed was determined from the parental, F_1 and F_2 variances. An analysis of the F_2 and backcross variances, showed that narrow sense heritability for percent oil in the seed was 61 percent.

As with percent protein, the literature on the heritability of percent oil in the seed of rapeseed is also scarce. A broad sense heritability estimate for percent oil in the seed of 26 percent, was however reported from six generations derived from the cross of two summer rape cultivars (Grami et al. 1977).

A wide range of broad sense heritability estimates for percent oil in soybeans has been reported in the literature. Weber and Moorthy (1952), found broad sense heritability for percent oil ranged from 49 to 59 percent when estimated from an analysis of the genetic variance in the parental, F_1 and F_2 generations of three soybean crosses. Johnson et al. (1955b) found broad sense heritability for percent oil in soybean seed was 67.5 percent when derived from an analysis of the genetic variance of the F_3, F_4 and F_5 generations of two soybean crosses. Kwon and Torries (1964) found, from an analysis similar to that of Johnson et al. (1955b) but with different parental material, that the broad sense heritability for oil concentration in the seed of

soybeans was 51 percent. Finally, Hanson and Weber (1962) found that broad sense heritability for percent oil varied from 63 to 74 percent, when estimated from an analysis of the variation of the F_3 through F_7 generations derived from a single soybean cross.

Heritability for the sum of protein and oil was estimated from six generations derived from the cross of two summer rape cultivars (Grami et al. 1977). It was found that broad sense heritability for the sum of protein and oil was 33 percent.

2.5 Physiological Basis for Protein Concentration

Increase in protein concentration in seeds of crop species is an objective for many plant breeding programs. However, a sound physiological basis to account for genotypic differences in grain protein concentration is lacking. Various hypotheses have been proposed however, that could account for these genotypic differences.

Hoener and DeTurk (1938) investigated nitrogen utilization in high and low protein corn lines. Results showed high protein lines had a higher protein content in the vegetative parts of the plant than the low protein lines. In addition, they found that the high protein corn lines reduced nitrates to ammonia more readily than low protein corn lines. Deckard et al. (1973) also found that nitrate reductase activity was correlated to protein concentration in corn.

Croy and Hageman (1970) found nitrate reductase activity was correlated with grain protein concentration in wheat (Triticum aestivum L.). A similar conclusion was reached by Eilrich and Hageman

(1973) while Rao and Croy (1972) observed that a high protein wheat cultivar had higher protease activity in the leaves after flowering than did a low protein cultivar.

In rice, (Oriza sativa L.) high protease activity was also correlated with high protein (Perez et al. 1973) while Cruz et al. (1970) found that an increased concentration of soluble amino acids and an increased rate of amino acid incorporation in the grain were both associated with high protein cultivars.

Varietal differences in nitrogen translocation from vegetative tissue to the developing grain have been observed in both wheat and oats.

Johnson et al. (1967) found that a high protein wheat derived from Atlas 66 was more efficient in remobilization of nitrogen into the grains than was a comparable low protein cultivar. Mikesell and Paulsen (1971) have also shown that higher protein cultivars have a greater proportion of their shoot nitrogen in the grain at maturity than do low protein cultivars. However, McNeal et al. (1968) failed to find such a relationship in the study of seven spring wheat cultivars.

Peterson et al. (1975) also concluded from the ratio of total reduced nitrogen in the panicle to that in the entire shoot in oats, that the high protein cultivars were more efficient at remobilizing nitrogen from the vegetative tissue into the developing seed than were the low protein cultivars.

The protein content of soybean seed is one of the highest known (Thibodeau and Jaworski 1975) with a large range of genetic variability and yet little is known of the physiological basis for high or low protein concentrations in soybean seed.

Sinclair and deWit (1975) calculated the nitrogen required per gram of photosynthate for 24 crop species and found the soybean to be unique among the world's crops in both the composition of its seed and potential limitations to its productivity. They found that the soybean not only requires the greatest amount of nitrogen for seed production but is also one of the lowest producers of seed biomass per gram of photosynthate.

Hardy and Havelka (1975) pointed out the soybeans required four times as much nitrogen per unit yield as did cereals and illustrated this point by comparing soybeans and corn. A corn crop yielding 100 bushels per acre consumed 150 pounds of nitrogen while a soybean crop of the same yield consumed 600 pounds of nitrogen. They suggested that the development of a technology for increasing nitrogen input into the seed would be a key to increasing both the protein content and the yield of the soybean.

Increasing the quantity of nitrogen available for grain development in soybeans can be accomplished by increasing whole plant nitrogen accumulation and/or by increasing the proportion of vegetative nitrogen translocated to the developing grain (Jeppson et al. 1978).

The application of nitrogen fertilizers has not proved to be an economically feasible method for increasing total nitrogen accumulation in soybeans. Weber (1966) found nodule number, weight and size decreased with increasing nitrogen application. In addition, there was no yield response to nitrogen fertilization. This negative relationship between soil applied fertilizer nitrogen and symbiotically fixed nitrogen has been verified by a number of researchers (Caviness and Hardy 1970, Hanway and Weber 1971a and Ham et al. 1975).

The quantity of nitrogen available from symbiotic fixation appears to be regulated by photosynthate availability. Mague and Burris (1972) found marked diurnal variation in nitrogen fixation as measured by acetylene reduction techniques. They concluded that the process is quite sensitive to the supply of photosynthetic assimilates.

Lawn and Brun (1974) found that any factors that increased the photosynthate available to the nodules increased the rate of nitrogen fixation. Treatments designed to enhance the photosynthetic source/sink ratio such as supplemental light and depodding, resulted in an increase in the rate of nitrogen fixation. Lawn and Brun also found that the increase in nitrogen fixation with these manipulations was a product of three factors: (1) an increase in the number of nodules, (2) a delay in the loss of the exponential phase of nitrogen fixation and (3) a doubling in the nitrogen fixation acitivity/mass of nodules. They concluded that the observed decline in activity of the nodules during pod filling was the result of inadequate assimilate supply to the nodules.

Streeter (1974) doubled the shoot/root ratio in soybeans by grafting two stems together and then severing one root system after the stems had grown together. When the shoot/root ratio was doubled, he found that the weight of roots and nodules increased relative to the controls and that the rate of acetylene reduction was 60 to 70 percent greater than the controls. He concluded that nodulated soybean roots are capable of fixing nitrogen at rates greater than those which normally prevail and that the major limiting factor is photosynthate supply.

Hardy and Havelka (1975) also showed the photosynthate supply is a major factor limiting nitrogen fixation in soybeans. Carbon dioxide enrichment studies on soybeans showed dramatic increases in both nitrogen fixed per plant per day and the total amount of nitrogen fixed under carbon dioxide enriched conditions.

Growth type in soybeans may also limit photosynthate supply to the nodules and therefore decrease nitrogen fixing activity. Woodworth (1933) indicated that a determinate growth type was one in which the stem terminated in a raceme bearing several pods while the indeterminate type did not have a terminal inflorescence. Bernard (1972) has suggested that, since no anatomical evidence has been published in support of this definition, the difference between indeterminate and determinate soybeans is a result of the timing of the termination of stem growth and not the way in which it terminates. He defined a determinate type as one in which the stem growth terminated abruptly at the onset of flowering while in the indeterminate type, stem growth, node and leaf production continued for several weeks after flowering began. This results in competition between vegetative, reproductive and nodule tissue for photosynthate in indeterminate cultivars.

Egli and Leggett (1973) found that only 58 percent of the total dry weight of an indeterminate cultivar was present at initial flowering whereas 78 percent was present in a determinate cultivar. They concluded that partitioning of available photosynthate to component plant parts was different in determinate and indeterminate soybeans and suggested that less vegetative competition in determinate cultivars

during reproductive development could result in greater quantities of available photosynthate for nodule maintenance and nitrogen fixation.

Latimore et al. (1977) found that nodules on determinate cultivars import more photosynthate during late vegetative and early reproductive development than those of indeterminate cultivars. However, in the late reproductive stage of development, photosynthate import by nodules was similar to values for indeterminate cultivars.

Examination of seasonal nitrogen accumulation rates in the determinate cultivar Lee (Nelson and Weaver 1980) indicated that, during the period of most rapid nitrogen accumulation in the reproductive tissue, nitrogen fixation was sufficient to meet the reproductive tissue nitrogen demand. They concluded that competition between reproductive tissue and nodules for photosynthate does not appear to be as severe in determinate cultivars as in indeterminate cultivars.

Israel (1981) in a study of two determinate cultivars, found that a rapid rate of nitrogen fixation was sustained during most of the time that nitrogen was being accumulated in the reproductive tissue. This is contrary to findings for indeterminate cultivars (Lawn and Brun 1974, Thidobeau and Jaworski 1975) which show a marked decline in nitrogen fixation during early to mid podfill.

Nitrogen already accumulated in the vegetative tissues is also available to the developing grain. Studies examining the uptake and distribution of nitrogen by the soybean plant have shown that the loss of nitrogen from the vegetative tissue coincided with the accumulation of nitrogen in the seed (Borst and Thatcher 1931, Hanway and Weber 1971b) and occurred when the nitrogen assimilatory processes, nitrate

reduction and nitrogen fixation, were declining (Thibodeau and Jaworski 1975).

Hanway and Weber (1971b) reported that nitrogen levels in the leaves, petioles, stems and pods, decreased during reproductive development. They estimated that between 50 and 60 percent of the nitrogen in the seed comes from redistribution of nitrogen in the vegetative plant parts.

Further studies (Egli et al. 1978) on the effect of nitrogen stress and redistribution indicated that the proportion of redistributed nitrogen in the seed can vary from 20 to 60 percent and was proportional to the degree of nitrogen stress that the plant was under during reproductive development. Leaves and pod walls were found to be the major sources of redistributed nitrogen.

Jeppson et al. (1978) evaluated the nitrogen harvest index (the ratio of grain nitrogen to whole plant nitrogen) of a number of soybean cultivars. They concluded that there were cultivar differences in the efficiency with which nitrogen was remobilized to the developing seed.

Israel (1981) found that within the determinate cultivars, there were differential strategies for the accumulation of nitrogen in the seed. One cultivar studied showed a greater rate of fixation during reproductive development, coupled to a lower initial concentration of nitrogen in the vegetative tissue prior to reproductive development and a lower rate or remobilization of nitrogen out of the vegetative tissue to the developing seed. The second cultivar on the other hand, had a greater accumulation of nitrogen in the vegetative tissue prior to reproductive development coupled to a greater rate of remobilization

and a smaller rate of fixation during reproductive development. The final seed concentrations of nitrogen however, were remarkably similar.

Sinclair and deWit (1975) have suggested that the redistribution of nitrogen from the vegetative tissue to the seed as a result of the inability of the roots and nodules to meet the nitrogen demands of the developing seed reduced the physiological activity of the leaves ultimately causing senescence. They characterized the soybean plant as being self-destructive and concluded that nitrogen redistribution may limit yield by shortening the seed filling period.

3. MATERIALS AND METHODS

3.1 Description of Cultivars

Three soybean genotypes, Maple Presto, X446-2-1, and Sioux were selected for this study. The origins and pedigrees of these lines are given in Table 1.

Maple Presto was selected because it was the adapted, recommended cultivar for Manitoba areas with a minimum of 2200 corn heat units. It has a relatively low percentage of protein in the seed but has a high oil percentage in the seed.

The selection of the high protein genotypes was based primarily on two criteria. First, the genotype had to be early maturing in order to ripen under the short, cool growing conditions of Manitoba. In addition, the protein and oil concentrations in the seed had to be such that the differences in protein and oil percentages between the "high" and "low" parents, were maximized.

X446-2-1 was selected as an early maturing experimental line that had a high protein percentage and low oil percentage in the seed while Sioux was one of the earliest maturing named U.S. varieties and is a common source of high seed protein concentration (Hartwig 1973).

To simplify the nomenclature with regards to the three genotypes used in this study, they are consistently referred to as cultivars.

Yield and seed quality data for parental cultivar plots grown at Winnipeg in 1981 are presented in Table 2.

TABLE 1. Origin and pedigree of cultivars.

Cultivar	Origin	Pedigree[1]
Maple Presto	Agric. Canada Ottawa Canada	Amsoy/Portage//840-7-3
X446-2-1	Agric. Canada Ottawa Canada	Portage/P1153.302//Morsoy/3/ Merit/P1153.293/4/Morsoy G. ussuriensis//Acme
Sioux	Hokkaido Japan	Selection from a landrace

[1]Pedigree is written according to Purdy et al. (1968)

TABLE 2. Performance of cultivars grown at Winnipeg in 1981.

Cultivar	Maturity group	Growth type	Yield kg/ha	Protein %	Oil %
Maple Presto	000	Indeterminate	3300±143	41.3±.3	20.5±.2
X446-2-1	00	Indeterminate	2467±130	55.9±.2	12.2±.1
Sioux	00	Determinate	2900±107	50.9±.1	14.7±.2

3.2 Inheritance Study

3.2.1 Crosses

Two crosses were made between the adapted, low protein cultivar and the high protein cultivars with Maple Presto being the common female parent for both crosses. Because of the difficulty in generating crossed material in soybeans, reciprocal crosses were not attempted in this study.

F_1 material was generated in the greenhouse in the fall of 1980. Crosses attempted, generated 40 F_1 seeds of each of the crosses. 20 F_1 seeds were grown out in the greenhouse during the winter of 1981. Plants were selfed to produce F_2 seed and simultaneously backcrossed to the paternal and maternal parents to produce the backcross progeny (B_1 and B_2 respectively). F_2 and backcrossed material, selfed in the field experiments of 1981, generated F_3 and selfed backcross material for the 1982 summer field experiments. Additional F_1 seeds from each cross, which were required for the 1982 field experiments, were generated in the greenhouse in the fall of 1981.

3.2.2 Field and Laboratory Methods

Six generations of each of the two crosses Maple Presto/Sioux and Maple Presto/X446-2-1, were planted on the University of Manitoba's experimental land at Winnipeg (the point) during the summer of 1981. The generations and number of seeds in each generation planted, are listed in Table 3.

TABLE 3. Generations and numbers of plants per generation, planted in the 1981 and 1982 field experiments.

Generation	Number of Plants	
	1981	1982
P_1	20	40
P_2	20	40
F_1	20	40
F_2	232	400
F_3	-	160
B_1	30	-
B_2	30	-
$B_{1(s)}$[1]	-	160
$B_{2(s)}$	-	160

[1] $B_{1(s)}$ and $B_{2(s)}$ represent selfed backcrosses.

The number of plants in each generation represents the numbers required to equalize the genetic variance among generations. The proportions were based on the assumption that additive genetic variance equals dominance genetic variance and equals four times the environmental variance. Due to the difficulty encountered in generating backcrossed material, the number of plants in the B_1 and B_2 generations was lower than optimal.

In 1982, seven generations (Table 3) of each of the two crosses were spaced planted in a completely randomized design. The backcrossed generations were replaced by selfed backcrosses and F_3 plants from selfed F_2 plants of the 1981 study, were added as the seventh generation.

Within each cross, for both years, entries were completely randomized in order to equalize environmental variance due to soil heterogeneity. Seeds were planted on one metre centres in order to eliminate the effects of interplant competition on the estimation of genetic parameters (Hamblin and Rosielle 1978). Seeds were hand planted and inoculated with soil implant, granular Rhizobium japonicum obtained from the Nitragin Co. Ltd., 3101 W. Custer Avenue, Milwaukee, Wisconsin, U.S.A.

During the growing season, bacterial blight was controlled by weekly spraying with the bactericide Kocide 101 obtained from the Kocide Chemical Corporation, 12701 Almeda Road, Houston, Texas, U.S.A. The rate of application used was 2 g/L with all leaves completely covered.

At maturity, plants were cut and mechanically threshed. Each plant was handled separately at all times and only those plants that survived to maturity were harvested.

A 15 gram sub-sample of the seed from each plant was finely ground and analyzed for percent protein, oil and moisture using near-infrared reflectance spectroscopy. These analyses were carried out at the Central Canada Agricultural Farm in Ottawa, Ontario, Canada, under the supervision of Dr. H. Voldeng using a Technicon InfraAlyzer, Model 500R obtained from Technicon Industrial Systems, Tarrytown, New York U.S.A.

3.2.3 Statistical Methods

Percent protein and percent oil data from each generation, were tested against a normal distribution, with the mean and variance equal to the sample mean and variance, using the Kolomogorov-Smirnov D statistic (Steel and Torrie 1980). The mean, standard deviation, standard error of the mean, variance and coefficient of variation for each of percent protein, percent oil and the sum of percent protein and percent oil ("sum") were calculated for each generation according to Steel and Torrie (1980). Variances of the means for each generation were tested for homogeneity using a Bartlett's test (Steel and Torrie 1980).

The means of the six generations in 1981 were used to estimate the magnitudes of gene action and the conformity of the genetic system governing the expression of the three traits investigated, to a simple additive-dominance model. The methodology used was a joint scaling test proposed by Cavalli (1952) and outlined by Mather and Jinks (1977), and involved a simple weighted least squares analysis. The weights used were reciprocals of the standard errors of the generation means. A chi-square analysis of observed and expected generation means

was used to test the fit of the genetic model to the data and a lack of fit implied the existence of non-additive (epistatic) gene effects other than dominance. The epistatic variation was then separated from additive and dominance variation in the means of the six generations, by increasing the complexity of the simple additive-dominance model (m, d, and h) to include additive X additive, additive X dominance and dominance X dominance interactions (i, j, and l respectively) as outlined by Hayman (1958). The coefficients used for the components of means are given in Appendix Table 1.

A similar type of analysis was performed on the means of the seven generations in 1982. The model, however, was adjusted to incorporate the F_3 and selfed backcross generations. In addition, the analysis differed from that performed on the 1981 data, in that, the least square analysis was unweighted because generation variances were homogeneous.

An analysis using second degree statistics was also performed on both the 1981 and 1982 data. The variation observed in the parental and F_1 generations, was used to estimate the non-heritable variation while the overall variance in the F_2, F_3 and backcross derived generations, was partitioned into additive, dominance and environmental components as outlined by Mather and Jinks (1977). The model was estimated using an unweighted least squares approach. For each trait, nonsignificant parameters were dropped and the analysis was re-run to give a better estimate of the significant components and to allow for a test of the fit of the model to the data. The coefficients used for the components of variance are given in Appendix Table 2.

It has been demonstrated from a symbolical analysis of variance (Mather and Jinks 1977) that the genetic variance of an F_2 population is equal to 1/2D + 1/4H, where D represents that portion of the variance attributable to the additive gene effects and H represents that portion due to deviations from additivity or the dominance gene effects. Heritabilities for the F_2 generation, were calculated from these theoretical proportions and the values for each component estimated in the analysis of variance. Broad sense heritability was calculated as the total genetic variance in the F_2 (1/2D + 1/4H) divided by phenotypic variance in the F_2 (1/2D + 1/4H + E), while narrow sense heritability was calculated as the additive variance in the F_2 (1/2D) divided by the phenotypic variance in the F_2.

3.3 Parent-Progeny Regression

3.3.1 Field and Laboratory Methods

30 F_2 plants from each cross of the 1981 inheritance study were selected to form parental material for parent-progeny regression analysis. F_2 plants were selected to maximize the range of percent protein in the seed.

Five seeds from each F_2 plant were randomly selected to form the F_3 families. Within each cross, the 150 F_3 seeds were hand planted on three foot centres and again inoculated with soil implant, granular Rhizobium japonicum.

At harvest maturity, plants were cut and mechanically threshed. The seed was stored in envelopes for subsequent chemical analyses. Plants were handled individually throughout the harvest procedure.

A 15 gram sub-sample of seed was taken from each F_3 plant and sent to the Central Canada Agricultural Farm in Ottawa, Canada for chemical analyses, as outlined earlier.

All protein and oil values were converted to zero moisture and all subsequent statistical analysis were performed on these converted data. The sum of protein and oil calculated as the simple algebraic sum of percent protein and percent oil at zero moisture.

3.3.2 Statistical Methods

The mean, standard deviation, standard error of the mean and variance for each of percent protein, percent oil and their "sum", was calculated for each F_3 family, according to Steel and Torrie (1980). F_3 family means then were regressed on F_2 parental values (Simmonds 1979). Three linear regressions were performed for each cross, one for each of the traits listed above. The slope of the regression line was taken as an estimate of the narrow sense heritability of the trait concerned (Simmonds 1979).

Heritability estimates were also calculated in standard units as outlined by Frey and Horner (1957). F_2 and F_3 data were coded in terms of the phenotypic standard deviation and standard unit heritabilities were obtained from the slope of the regressions of coded F_3 means on coded F_2 parental values. This regression coefficient (b') is identical to the correlation coefficient on the original data.

3.4 Nitrogen Accumulation and Redistribution

3.4.1 Field and Laboratory Methods

The three parents used in the inheritance study and described in section 3.1 formed the three entries for this study. These cultivars were grown on the University of Manitoba's experimental land at Winnipeg (the point) during the summers of 1981 and 1982. Soil tests showed mean soil nitrogen levels to be 20 kg/ha in 1981 and 16.5 kg/ha in 1982. No additional nitrogen was added as the seed was inoculated with soil implant, granular *Rhizobium japonicum* at the time of planting.

Two planting densities were used for this experiment. Each cultivar was planted in a 4 row, 5 metre plot with rows 30 cm apart at a density of 400 seeds per plot. Adjacent to the plot of each cultivar, 20 plants of the entry were hand planted on one metre centres. One plot and the adjacent 20 spaced plants of a cultivar formed one replicate of that entry. Each entry was replicated 4 times in 1981 and 6 times in 1982 in a randomized complete block design.

Plant development was assessed throughout the growing season using the scale of Fehr *et al.*(1971) as outlined in Table 4. This permitted an accurate comparison of data from the 1981 and 1982 growing seasons.

At growth stage V_3, 15 spaced plants and 30 plants, from the inner rows of the adjacent plot for each replicate, were tagged, with the assumption that if they were at the same growth stage they were the same age. Plants sampled throughout the season were randomly chosen from among the tagged plants to ensure plants sampled later in the season, when age differences were more difficult to detect, were of a comparable physiological age.

TABLE 4. Stage of development descriptions for soybeans (Fehr et al. 1971).

Stage of development	Description
Vegetative	
V_1	Completely unrolled leaf at the unifoliate stage
V_2	Completely unrolled leaf at the first node above the unifoliate node.
V_3	Three nodes on the main stem beginning with the unifoliate node.
V_8	Eight nodes on the main stem beginning with the unifoliate node.
V_9	Nine nodes on the main stem beginning with the unifoliate node.
Reproductive	
R_1	One flower at any node
R_2	Flower at the node immediately below the uppermost node with a completely unrolled leaf.
R_3	Pod 0.5 cm long at one of the four uppermost nodes with a completely unrolled leaf.
R_4	Pod 2.0 cm long at one of the four uppermost nodes with a completely unrolled leaf.
R_5	Beans beginning to develop at one of the four uppermost nodes with a completely unrolled leaf.
R_6	Pod containing full size green beans at one of the four uppermost nodes with a completely unrolled leaf.
R_7	Pods yellowing; 50% of leaves yellow Physiological maturity.
R_8	95% of the pods brown. Harvest maturity.

Individual plants were sampled at weekly intervals commencing with a late vegetative phase (V_8) and ending with harvest maturity (R_8). One spaced plant and one plant from the adjacent plot, were sampled, from each replicate for each of the three cultivars, over the course of the season. Sample dates, expressed in days after planting, and the corresponding stage of development are given in Table 5.

Each plant sampled was cut at ground level. No attempt was made to collect senesced leaves. Each sample was then individually tagged, bagged and oven dried at 70°C for 48 hours. All data collected were expressed on a per-plant basis at zero percent moisture.

Plants were weighed and separated into vegetative and reproductive components. Stems, leaves and petioles were bulked into the vegetative component while the reproductive component was separated into pod wall and seed. Because of the differential age of pods along the stem in the indeterminate cultivars, a sub-sample of the 10 oldest pods (ssp) was taken from each sample plant and used to track the accumulation and redistribution of nitrogen in the reproductive tissue. The age of the pod was determined by the width of the pod after Bravo et al. (1980), and by the position of the pod on the plant. This latter criterion was applied to the indeterminate cultivars in particular, where lower pods were more mature. After being weighed, plant parts were ground with a Wiley mill through a two millimeter screen and were analysed for percent nitrogen using the standard macro-Kjeldahl technique.

TABLE 5. Sampling date and the corresponding stage of development.

Sample date[1]		Stage of development[2]	
1981	1982	1981	1982
43	48	V_9	V_8
-	55	R_1	V_9
63	62	R_2	R_1
70	70		R_2
76	76	R_4	R_4
85	83	R_5	
91	91	$R_{6.5}$	$R_{5.5}$
-	98		R_6
108	105	R_8	R_7
-	112		R_8

[1]Expressed in days after planting.
[2]After Fehr et al. (1971)

Total accumulated nitrogen in each of the vegetative, pod wall and seed components was calculated from the percent nitrogen data and the dry weight of the component parts. The percentages of redistributed nitrogen were estimated from the changes in total nitrogen (grams) in each plant part between the harvest at growth stage R_4 and the harvest at physiological maturity at R_7. Thus, the nitrogen redistributed from the vegetative portion was represented by the total nitrogen at R_4 minus the total nitrogen in the leaves (less the abscised leaves) at R_7. R_7 was selected as the final growth stage for this comparison because no attempt was made to collect senesced leaves and at this stage, most of the leaves still adhered to the plant. In addition, this was the point of maximum dry matter, and therefore, nitrogen accumulation in the seed. Similar calculations were made to determine the amount of nitrogen redistributed from the pod wall. It was assumed that nitrogen lost from the vegetative and pod wall tissue was redistributed to the seed. The estimated amount of redistributed nitrogen for the vegetative and pod wall components, was assumed to give the total amount of redistributed nitrogen and the contribution of each component was calculated as a percent of the total.

The total nitrogen (grams) in the seed at R_4 was subtracted from the total nitrogen in the seed at R_7 to give the net gain in seed nitrogen. Remobilized nitrogen from the vegetative tissues and pod walls was then calculated as a percentage of this net gain in seed nitrogen to determine the proportion of total seed nitrogen that came from redistribution (RN/SN).

Harvest index, the ratio of seed yield to biological yield, was calculated for each sampling date from R_4 through to R_8. For the harvest sample, apparent harvest index, the ratio of seed yield to mature plant weight (Schapaugh and Wilcox 1980), was calculated. Similarly, harvest nitrogen index, the ratio of seed nitrogen yield to total biological nitrogen yield was calculated for each sampling date from R_4 through to R_8. The harvest value was also an apparent harvest nitrogen index as no attempt was made to collect senesced leaves.

3.4.2 Statistical Methods

Pairwise comparisons of percent nitrogen, total accumulated nitrogen and total accumulated dry matter in both the vegetative and pod tissue were made at R_2 and R_4 respectively. All calculations were carried out with four decimal places and then rounded to fewer decimals for presentation purposes.

The means of the 4 replicates in 1981 and the 6 replicates in 1982 were compared for high versus low protein cultivars, determinate versus indeterminate cultivars and spaced versus plot plants. These pairwise comparisons of the means were made for percent nitrogen, total accumulated nitrogen and total accumulated dry matter, in both the vegetative tissue at the onset of pod development (R_2) and the pod tissue at the onset of seed development (R_4). Student's t-statistic was used to determine the significance of the differences in the means. The level of significance used for all comparisons was $P = .05$.

The rate of redistribution of nitrogen was measured by the change in nitrogen concentration in the vegetative and pod tissue from R_2

and R_4 respectively, to physiological maturity (R_7). For the purposes of this study, physiological maturity was measured visually using Fehr's scale (Fehr et al. 1971), and quantitatively, as the point of maximum dry matter accumulation in the seed.

Individual sample data for percent nitrogen in vegetative and pod tissue were regressed on days after planting. The slope of the regression line was taken as the rate of change in nitrogen concentration with time. It was assumed that a decrease in nitrogen concentration reflected a decrease in nitrogen content and that nitrogen lost from the vegetative plant parts and the pod walls was redistributed to the seed. The slopes of the regression lines were used to compare high versus low protein cultivars, determinate versus indeterminate high protein cultivars, and spaced versus plot density plants. Slopes were compared through the construction of confidence limits (Neter and Wasserman 1974). A significant difference in two slopes was interpreted as a significant difference in the rate of remobilization of nitrogen in the cultivars compared.

4. RESULTS AND DISCUSSION

4.1 Preliminary Analyses

The frequency distributions of each generation for percent protein and percent oil, were tested against a normal distribution, with mean and variance equal to the sample mean and variance, using the Kolomogorov Smirnov D-statistic. The analysis indicated that the data from each generation approximated the normal distribution and consequently no transformation of the data was required. The original percent data were therefore used for all subsequent analyses.

The means, variances and coefficients of variability for percent protein and oil in each of the generations of the cross Maple Presto/Sioux are presented in Tables 6 and 7. The 1981 data are summarized in Table 6 while the 1982 data are summarized in Table 7. The same descriptive statistics for the cross Maple Presto/X446-2-1 are presented in Tables 8 and 9. The 1981 data are summarized in Table 8, while the 1982 data are summarized in Table 9.

4.1.1 Percent Protein

The difference in percent protein between the two parents in the cross Maple Presto/Sioux, was 9.4 percent in 1981 (Table 6) and 11.3 percent in 1982 (Table 7). Over the two experimental years, there appeared to be a greater environmental influence on the expression of genes for percent protein in Maple Presto than on Sioux. In both years

TABLE 6. Summary of preliminary 1981 data for percent protein and percent oil in the seed for the cross Maple Presto/Sioux.

Generation	No. of plants	Protein			Oil		
		Mean[1]	Variance	C.V.	Mean[2]	Variance	C.V.
P_1	18	50.9±.2	.843	1.80	14.7±.1	.314	3.81
P_2	19	41.5±.3	.656	1.95	20.5±.2	.189	2.12
F_1	20	45.4±.4	.976	2.18	17.1±.2	.435	3.86
F_2	100	46.0±.1	2.389	3.37	17.2±.1	.744	5.01
B_1	23	47.0±.4	1.113	2.24	16.4±.2	.470	4.18
B_2	14	43.6±.3	1.321	2.64	18.7±.2	.442	3.56

[1] χ^2 homogeneity for the variance of the mean = 52.2
[2] χ^2 homogeneity for the variance of the mean = 60.1
P (.05) = 11.1

TABLE 7. Summary of preliminary 1982 data for percent protein and percent oil in the seed for the cross Maple Presto/Sioux.

Generation	No. of plants	Protein			Oil		
		Mean[1]	Variance	C.V.	Mean[2]	Variance	C.V.
P_1	33	49.4±.2	.912	1.93	13.4±.1	.389	4.67
P_2	27	38.1±.2	.779	2.32	19.2±.1	.317	2.93
F_1	28	41.6±.2	.831	2.19	16.5±.1	.331	3.50
F_2	140	43.6±.2	3.687	4.40	16.1±.1	.946	6.04
$B_1(s)$	107	44.8±.2	2.657	3.64	15.2±.1	.970	6.48
$B_2(s)$	106	41.2±.2	2.847	4.09	17.2±.1	.825	5.29
F_3	104	44.1±.2	3.850	4.45	15.9±.1	1.198	6.88

[1] χ^2 homogeneity for the variance of the mean = 7.42
[2] χ^2 homogeneity for the variance of the mean = 11.11
P (.05) = 12.6

TABLE 8. Summary of preliminary 1981 data for percent protein and percent oil in the seed for the cross Maple Presto/X446-2-1.

Generation	No. of plants	Protein			Oil		
		Mean[1]	Variance	C.V.	Mean[2]	Variance	C.V.
P_1	19	56.0±.2	.556	1.33	12.2±.1	.331	4.72
P_2	19	41.2±.2	.617	1.91	20.7±.2	.229	2.31
F_1	16	47.4±.2	.597	1.63	17.2±.2	.472	3.99
F_2	100	48.5±.1	3.442	3.83	16.5±.1	1.089	6.32
B_1	24	51.3±.3	1.980	2.74	14.8±.2	.772	5.94
B_2	20	45.4±.4	2.434	3.44	18.6±.3	.861	4.99

[1] χ^2 homogeneity for the variance of the mean = 28.35
[2] χ^2 homogeneity for the variance of the mean = 55.16
P (.05) = 11.1

TABLE 9. Summary of preliminary 1982 data for percent protein and percent oil in the seed for the cross Maple Presto/X446-2-1.

Generation	No. of plants	Protein			Oil		
		Mean[1]	Variance	C.V.	Mean[2]	Variance	C.V.
P_1	22	55.6±.2	.697	1.50	11.6±.1	.356	5.14
P_2	30	38.8±.2	.896	2.44	18.7±.1	.241	2.63
F_1	33	46.0±.2	.806	1.95	15.7±.1	.313	3.56
F_2	110	45.8±.2	3.457	4.06	15.9±.1	.708	5.29
B_1(s)	85	49.9±.2	2.720	3.31	14.2±.1	.721	5.98
B_2(s)	80	42.9±.2	2.638	3.79	17.6±.1	.679	4.68
F_3	106	46.4±.2	3.604	4.09	15.7±.1	.900	6.04

[1] χ^2 homogeneity for the variance of the mean = 9.69
[2] χ^2 homogeneity for the variance of the mean = 12.22
P (.05) = 12.6

however, the difference in percent protein between the parental cultivars was significant.

The mean F_1 percent protein in 1981 (Table 6) was lower than the mid-parent value of 46.2 percent, indicating a partial dominance for low percent protein. The F_2 mean value was close to the mid-parent value, and suggested that most of the gene action governing the expression of genes for percent protein, was additive. The 1982 data (Table 7) also indicated a partial dominance for low protein as the F_1 value was lower than the mid-parent value of 43.8. The F_2 value was close to the mid-parent, again suggesting that the gene action for percent protein was additive.

In considering the variance data for percent protein, it was clear from the data of both experimental years, that the variances of the segregating generations were large in comparison to the variances for the non-segregating parental and F_1 generations. The mean variance of the parents and F_1 was taken as an estimate of the environmental component of the variability observed for percent protein, and therefore it was concluded that there was a large genetic component of the observed variability for percent protein.

The variance in the backcross generations in 1981, was low compared to the value for the F_2. This may have been due to a limited range of expression within the excessively small sample size of the backcross generations. This small sample size was due to the difficulty experienced generating material. As expected however, selfed backcross variances were much greater than the backcross values of 1981 due to the more extreme range of values expected if the variance contained a genetic component.

In the cross Maple Presto/X446-2-1, the difference in percent protein between the two parents was 14.8 percent in 1981 (Table 8) and 16.8 percent in 1982 (Table 9). Again, Maple Presto varied more in the two experimental years with respect to percent protein, than did X446-2-1. In both years, the difference was significant and acceptable for genetic analysis.

The mean F_1 value for percent protein in 1981 (Table 8) was again lower than the mid-parent value of 48.6, which suggested a partial dominance for low protein in this cross. The F_2 mean approximated the mid-parent value and therefore suggested a predominance of additive gene action governing the expression of percent protein. The 1982 data for this cross (Table 9), again showed similar trends, with the F_1 value being lower than the mid-parent value of 47.2 percent. The mean of the F_2 data was lower than the mid-parent, but with selfing, tended towards the mid-parent, indicating additive gene action.

Again, with this cross, an examination of the variances for each generation (Tables 8 and 9) indicated a large genetic component to the variability for percent protein in the seed, as the variances for the segregating generations were larger than the mean of the parental and F_1 variances.

4.1.2 Percent Oil

The difference in percent oil in the seed of the two parents in the cross Maple Presto/Sioux was 5.8 percent in both 1981 and 1982 (Tables 6 and 7 respectively). This was a significant difference and adequate for genetic analysis. There appeared to be no genotype by

year interaction for percent oil as the magnitude of the difference between the two parents remained the same over the two years but a frost at R_6 resulted in lower values for percent oil for both parents in 1982. This was expected, as a frost at R_6, or earlier, has a more pronounced effect on oil than on protein (Saliba et al. 1982).

The mean F_1 value for percent oil in 1981 (Table 6) was slightly lower than the mid-parent value of 17.6 percent which suggested that there was some dominance for low percent oil. The proximity of the F_2 mean to the mean F_1 value and the mid-parent, indicated the prevalence of additive gene action. The F_1 data for 1982 suggested a partial dominance for high oil percentage as the F_1 mean was higher than the midparent value of 16.3 percent (Table 7). The F_2 mean value however, indicated predominantly additive gene action. The preliminary analysis for percent oil in the cross Maple Presto/Sioux did not conclusively show a significant partial dominance but did however, suggest a significant additive component to the genetic variation. The extent of partial dominance for percent oil was determined in the subsequent genetic analysis.

The variance data for percent oil for this cross clearly showed a significant genetic component of variance as the segregating generations in both 1981 and 1982 had a larger variance than the mean of the nonsegregating generations. Again, the selfed backcross generations showed a larger variance than the backcross generations, as would be expected from the more extreme values segregating, if the variation was genetic.

For the cross Maple Presto/X446-2-1, the difference in percent oil in the seed of the parents was 8.5 percent in 1981 (Table 8) and

7.1 percent in 1982 (Table 9). The difference was significant in both years and sufficient for genetic analysis. The frost that occurred at R_6 however, resulted in a lower percent oil in both parents in 1982. In addition to the lower percentage oil in both parents, there appeared to be a genotype by year interaction for percent oil in this cross, as the magnitude of the difference between the two parents changed over the two experimental years.

The mean F_1 value for 1981 (Table 8) was higher than the mid-parent value of 16.5 percent, suggesting, as did the 1982 results of the Maple Presto/Sioux cross, a partial dominance for high percent oil. The F_2 value fell right at the midpoint and therefore, suggested a predominance of additive gene action. The mean F_1 value for percent oil in 1982 (Table 9) also was higher than the mid-parent, again indicating partial dominance for high percent oil. The mean F_2 and F_3 values, although not right at the mid-parent, suggested much of the gene action for percent oil was additive.

Again, as with percent oil in the cross Maple Presto/Sioux, the variances of the generations in both experimental years clearly showed a large genetic component to the observed variability for percent oil. In both 1981 and 1982 (Tables 8 and 9 respectively), the variances for the segregating generations were two to three times larger than those for the nonsegregating parental and F_1 generations, indicating a large genetic component.

4.1.3 Sum of Percent Protein and Percent Oil

The means, variances and coefficients of variability for the sum of percent protein and percent oil, for each of the generations of the

cross Maple Presto/Sioux, are presented in Table 10 and Table 11. The data for 1981 are summarized in Table 10 while the data for 1982 are summarized in Table 11.

The difference in the sum of percent protein and percent oil between the two parents was 3.6 percent in 1981 (Table 10) and 5.4 percent in 1982 (Table 11). The difference was significant in each year. The larger observed difference in 1982 resulted from the apparent genotype by year interactions noted earlier and the influence of the frost at R_6 on the expression of percent oil, also noted earlier.

In both 1981 and 1982, the mean F_1 values fell below the mid-parent values of 62.8 and 59.1 respectively (Tables 10 and 11 respectively), which suggested a partial dominance for a lower sum of percent protein and percent oil ("sum"). As with percent protein and percent oil independently, the F_2 value in 1981 and the F_2 and F_3 values in 1982, clearly showed a large additive component to the observed variability.

Examination of the F_2 variance data for 1981 suggested a genetic component to the variance but backcross values were lower than expected, again, due in part to the very small sample size used. Data from the segregating and nonsegregating generations in 1982 (Table 11), were more consistent with a large genetic component to the variation.

The coefficients of variability for the sum of percent protein and percent oil were notably smaller than for either percent protein or percent oil independently. This was not unexpected, as the well documented negative correlation that exists between protein and oil (Johnson et al. 1955a, Kwon and Torrie 1964, Shannon et al. 1972)

TABLE 10. Summary of preliminary 1981 data for the sum of percent protein and percent oil in the seed for the cross Maple Presto/Sioux.

Generation	No. of plants	Mean[1]	Variance	C.V.
P_1	18	64.6±.2	.500	1.09
P_2	19	61.0±.2	.722	1.39
F_1	20	61.5±.3	.924	1.56
F_2	100	62.3±.1	1.761	2.13
B_1	23	62.6±.3	.797	1.43
B_2	14	61.2±.2	.695	1.36

[1] χ^2 homogeneity for the variance of the mean = 119.5
P (.05) = 11.1.

TABLE 11. Summary of preliminary 1982 data for the sum of percent protein and percent oil in the seed for the cross Maple Presto/Sioux.

Generation	No. of plants	Mean[1]	Variance	C.V.
P_1	33	61.8±.2	.704	1.36
P_2	27	56.4±.2	.694	1.48
F_1	28	57.4±.1	.477	1.20
F_2	140	58.9 .1	2.045	2.43
B_1(s)	107	59.1±.2	1.535	2.10
B_2(s)	106	57.5±.1	1.835	2.36
F_3	104	59.1±.2	2.239	2.53

[1] χ^2 homogeneity for the variance of the mean = 11.32
P (.05) = 12.6.

would suggest that the sum of these two traits would be less variable than either trait independently.

The means, variances and coefficients of variability for the sum of percent protein and percent oil for the cross Maple Presto/X446-2-1, are presented in Tables 12 and 13 respectively. The 1981 data are summarized in Table 12 while the 1982 data are summarized in Table 13.

The mean F_1 values for 1981 and 1982 were lower than the mid-parent values of 64.1 and 61.4 (Tables 12 and 13 respectively) which indicated a partial dominance for a lower "sum", as was found in the cross Maple Presto/Sioux. The F_2 data in 1981 and the F_2 and F_3 data in 1982 however, suggested that most of the gene action governing the sum of percent protein and percent oil, was additive.

As found earlier, the variance data clearly showed a large genetic component to the variability.

Finally, the coefficients of variability were found to be much lower than for percent protein or percent oil independently. This again was thought to be due to the relative stability of the sum of protein and oil because of the negative correlation between these two traits.

4.2 Genetic Analyses

Barlett's test for homogeneity was used to determine whether the genetic information in the mean of each generation was estimated with the same precision.

In 1981, the variance of the means for the six generations of the cross, Maple Presto/Sioux (Tables 6 and 10), and the cross,

TABLE 12. Summary of preliminary 1981 data for the sum of percent protein and percent oil in the seed for the cross Maple Presto/X446-2-1.

Generation	No. of plants	Mean[1]	Variance	C.V.
P_1	19	67.3±.2	.434	.98
P_2	19	60.9±.2	.429	1.08
F_1	16	63.7±.1	.210	.72
F_2	100	63.8±.1	1.494	1.92
B_1	24	65.3±.2	.655	1.24
B_2	20	63.2±.2	.662	1.29

[1] χ^2 homogeneity for the variance of the mean = 55.24
χ^2_P (.05) = 11.1

TABLE 13. Summary of preliminary 1982 data for the sum of percent protein and percent oil in the seed for the cross Maple Presto/X446-2-1.

Generation	No. of plants	Mean[1]	Variance	C.V.
P_1	22	66.3±.2	.603	1.17
P_2	30	56.5±.1	.462	1.20
F_1	33	60.8±.1	.351	.97
F_2	110	60.7 .1	1.581	2.07
B_1(s)	80	63.2±.1	1.369	1.85
B_2(s)	85	59.6±.1	1.119	1.77
F_3	106	61.2±.2	1.789	2.19

[1] χ^2 homogeneity for the variance of the mean = 9.34
χ^2_P (.05) = 12.6

Maple Presto/X446-2-1 (Tables 8 and 12), were found to be significantly heterogeneous for the three traits under investigation. This was largely due to the excessively small sample size in the backcross generations. This, as noted earlier, was due to the difficulty in generating adequate material, and to the subsequent loss of some plants in the field. Because the genetic information in the means was not estimated with the same precision for all generations, a weighted least squares approach was used for the genetic analyses in 1981. The weights used were the reciprocals of the squared standard errors of the generation means.

In 1982, larger backcross generations were achieved by using the selfed backcross generations and adjusting the genetic model appropriately. Bartlett's test for homogeneity showed, for both the Maple Presto/Sioux cross (Tables 7 and 11) and the Maple Presto/X446-2-1 cross (Tables 9 and 13), that the variances of the means were homogeneous for each of percent protein, percent oil and their "sum". Consequently, an unweighted least squares approach was used for all genetic analyses of the 1982 data.

4.2.1 Means Analyses

The means for percent protein, percent oil and their "sum", were analyzed using the joint scaling test outlined by Mather and Jinks (1977). Initially, a three parameter, nonepistatic model was applied to the generation means of each trait. This model included the mean (m) which was the mid-parent point in the absence of any interaction, an additive genetic component (d) and a dominance genetic component

(h). A chi-square test was used to compare expected and observed means in order to test the goodness-of-fit of the model to the data. The additive/dominance model however, assumes that the genes involved are independent of each other in producing their effects and therefore, where the three parameter model did not provide a satisfactory explanation of the observed means at P=.05, the model was extended to a six parameter model. This model included additive X additive (i), additive X dominance (j) and dominance X dominance (l) nonallelic interactions. The significance of each parameter was tested using a z-statistic. Where parameters were nonsignificant, they were dropped and the analysis was re-run to give a better estimate of the significant parameters. The simplest model that adequately fit the data was reported for each trait in 1981. For the 1982 data however, where the simplest model did not minimize the chi-square value, both the simplest model and a second model which minimized the chi-square value for goodness-of-fit, were reported.

4.2.1.1 Percent Protein. The results of the 1981 means analysis for the cross, Maple Presto/Sioux, are presented in Table 14. A highly significant additive genetic effect was detected for percent protein. Dominance gene action for low protein percentage was also significant, although the magnitude of the contribution of this component was small in comparison to the additive genetic component. The simple additive/dominance model did not adequately describe percent protein for this cross and therefore, the model was extended to the six parameter epistatic model. Of the three interaction components, the

TABLE 14. Estimates of genetic parameters for percent protein, percent oil and their "sum" for the cross Maple Presto/Sioux (1981).

Parameter	Protein	Oil	Sum
m	46.2 ± 0.2	17.5 ± 0.1	62.3 ± 0.2
[d]	4.7 ± 0.2	-2.8 ± 0.1	1.7 ± 0.3
[h]	-0.8 ± 0.3	-0.4 ± 0.2	-
[j]	-2.0 ± 1.0	-	-
χ^2[1]	5.53 (2df)	7.20 (3df)	6.21 (4df)
P(.05)	5.99	7.81	9.49

[1]Test of the fit of the model to the data.

only significant component was the additive X dominance component (j). The inclusion of the (j) nonallelic interaction term resulted in an adequate fit of the model to the data at P=.05.

The results of the 1982 means analysis for percent protein for this cross are presented in Table 15. In this case, the simplest model did not minimize the chi-square value for the test of goodness-of-fit and therefore, both the simplest model and the model that generated the best fit to the data, are presented. For both models, a highly significant, additive genetic component was again detected. As well, a less significant dominance component was again evident for low percent protein. Although a simple additive/dominance model fit the data, the inclusion of the additive X dominance interaction component to the model, generated a lower chi-square value and therefore, a better fit of the model to the data.

From the data over the two experimental years, it would appear that gene action for percent protein in the cross Maple Presto/Sioux is governed predominantly by additive gene action, with some dominance gene action for low percent protein and a significant additive X dominance nonallelic interaction component.

The results of the 1981 means analysis for percent protein in the cross, Maple Presto/X446-2-1, are presented in Table 16. The analysis showed that additive gene action was again, the major component contributing to the inheritance of percent protein. In addition, there was some dominance for low percent protein and a significant non-allelic additive X dominance component.

TABLE 15. Estimates of genetic parameters for percent protein, percent oil and their "sum" for the cross Maple Presto/Sioux (1982).

Parameter	Protein Model		Oil Model		Sum
	Simplest	Best fit	Simplest	Best fit	
m	43.9 ± 0.5	43.9 ± 0.5	16.2 ± 0.1	16.2 ± 0.1	58.6 ± 0.3
[d]	5.3 ± 0.6	5.7 ± 0.5	-2.7 ± 0.2	-2.9 ± 0.1	2.5 ± 0.5
[h]	-1.9 ± 1.0	-1.9 ± 0.8	-	-	-
[i]	-	-	-	3.9 ± 1.3	-
[j]	-	-8.3 ± 4.3	-	-	-
χ^2[1]	3.1 (4df)	1.4 (3df)	0.6 (5df)	0.2 (3df)	2.8 (5df)
P(.05)	9.49	7.80	11.10	7.80	11.10

[1]Test of the fit of the model to the data.

TABLE 16. Estimates of genetic parameters for percent protein, percent oil and their "sum" for the cross Maple Presto/X446-2-1 (1981).

Parameter	Protein	Oil	Sum
m	48.7 ± 0.1	16.4 ± 0.1	64.0 ± 0.2
[d]	7.4 ± 0.2	-4.2 ± 0.1	3.0 ± 0.3
[h]	-1.1 ± 0.3	0.5 ± 0.2	-
[j]	-2.8 ± 1.0	-	-
χ^2[1]	1.90 (2df)	6.40 (3df)	4.30 (4df)
P(.05)	5.99	7.81	9.49

[1]Test of the fit of the model to the data.

The results of the 1982 analysis are presented in Table 17. Again, as with the 1982 results for protein in the cross Maple Presto/ Sioux, the simplest model did not provide the best fit to the data. Although the chi-square value was nonsignificant for the three parameter model, the inclusion of the additive X dominance (j) component provided a better fit to the data, as evidenced by the lower chi-square value. The 1982 data clearly showed a highly significant additive genetic component with some dominance for low percent protein. Again, there was also a significant additive X dominance non-allelic component contributing to the inheritance of percent protein.

The results from the two experimental years, for the two crosses under investigation, clearly showed that percent protein in the seed of these early maturing lines, was predominantly governed by additive gene action. There was also a smaller, but significant, contribution of dominance gene action for lower percent protein. As well, when the model providing the best fit to the data was considered, there was a significant additive X dominance epistatic component contributing to the inheritance of percent protein.

Percent protein in other crops has also been found to be governed primarily by additive gene action, with some dominance for low percent protein. This is illustrated by work in wheat (Chapman and McNeal 1970), oats (Ohm and Patterson 1973, Sraon et al. 1975), sesame (Murty and Hashim 1973) and beans (Lelji et al. 1972, Kelly and Bliss 1975b). Work in soybeans in cultivars of maturity group II or later has shown percent protein to be primarily conditioned by additive gene action but, contrary to the results presented here for early maturing

TABLE 17. Estimates of genetic parameters for percent protein, percent oil and their "sum" for the cross Maple Presto/X446-2-1 (1982).

Parameter	Protein Model		Oil Model		Sum
	Simplest	Best fit	Simplest	Best fit	
m	46.9 ± 0.3	46.9 ± 0.3	15.6 ± 0.1	15.6 ± 0.1	61.2 ± 0.2
[d]	8.2 ± 0.4	8.4 ± 0.3	-3.5 ± 0.2	-3.5 ± 0.1	4.6 ± 0.3
[h]	-1.3 ± 0.7	-1.3 ± 0.5	-	-	-
[i]	-	-	-	0.6 ± 0.2	-
[j]	-	-5.7 ± 2.9	-	-	-
χ^2 [1]	1.5 (4df)	0.6 (3df)	0.7 (5df)	0.1 (4df)	1.2 (5df)
P(.05)	9.49	7.80	11.10	9.49	11.10

[1]Test of the fit of the model to the data.

soybeans, dominance was for high percent protein in the later lines (Williams 1948, Brim and Cockerham 1961). There is limited evidence of non-allelic gene interaction contributing significantly to the inheritance of percent protein. Brim and Cockerham (1961), did however conclude that an additive X additive epistatic component significantly contributed to the expression of percent protein. This again differs from the significant additive X dominance component detected for the genetic material under investigation in this study.

In summary then, it is clear that additive gene action predominantly governs the expression of genes for percent protein in soybeans, but there appears to be a difference in the direction of dominance with maturity group. Percent protein in later maturing lines appears to be partially controlled by dominance for high percent protein, while percent protein in the early maturing lines appears to be partially governed by a dominance for low percent protein.

4.2.1.2 Percent Oil. The results of the analysis for percent oil for the cross Maple Presto/Sioux, are presented in Table 14. A highly significant additive genetic component was detected for this trait. The sign of the additive component for oil was different than that for protein or for the sum of protein and oil, because of the use of the high protein, low oil parent as P_1 and the low protein, high oil parent as P_2 consistently for all three analyses. Therefore, the difference between P_1 and P_2 was positive for both percent protein and the "sum" and was negative for percent oil. Percent oil also appeared to be conditioned by a significant dominance component for low oil

percentage, although the standard error associated with the estimate was large. Significant epistasis was not detected by the analysis of percent oil.

Significant additive genetic effects were also obtained for percent oil in 1982 (Table 15). As with percent protein, the simplest model, although fitting the data adequately, did not minimize the chi-square value and therefore, both the simplest model and the model providing the best fit are presented. For both models, there was no significant dominance component for percent oil. The inclusion of an additive X additive (i) epistatic component did however, provide a better model to explain the data.

The results of the 1981 genetic analysis for percent oil for the cross Maple Presto/X446-2-1 are presented in Table 16. Again, as was found for the cross Maple Presto/Sioux, there was a highly significant additive genetic component governing the expression or percent oil. In addition, there was some dominance, but in this cross, it was for high oil percentage in the seed. No epistasis was detected.

Data from the 1982 genetic analysis for percent oil in this cross are presented in Table 17 and, as was the case with percent oil for the Maple Presto/Sioux cross, the simplest model adequately fit the data but did not provide the best fit. Both models indicated a highly significant additive genetic component and no dominance component contributing to the inheritance of percent oil. There was also evidence of an additive X additive epistatic component conditioning percent oil.

The results of the genetic analyses for percent oil in the early maturing lines investigated clearly showed the importance of additive

gene action in the inheritance of this trait. Of lesser importance was dominance gene action, but it was not possible to generalize about the direction of dominance. In the cross Maple Presto/Sioux, dominance gene action for low oil percentage was significant in 1981 and non-significant in 1982. For the cross Maple Presto/X446-2-1, dominance gene action for high oil percentage was significant in 1981 and non-significant in 1982. For both crosses, the magnitude of the dominance relative to the mean, suggested that the role of dominance gene action was minor. For both crosses, additive X additive epistasis may have contributed to the inheritance, but again, the inconsistency of this finding suggested that it played a minor role.

The importance of additive gene action and partial dominance for high oil percentage has been well documented in corn (Brimhall and Sprague 1951, ElRouby and Penny 1966, Poneleit and Bauman 1970), oats (Frey et al. 1975), sunflowers (Fisk 1975), safflower (Yermanos et al. 1967) and sesame (Murty and Hashim 1973). Investigations into the inheritance of oil percentage in summer rape also indicated the predominance of additive gene action. Dominance gene action however, was nonsignificant (Grami and Stefansson 1977b).

The importance of additive genetic effects in late maturing soybeans has also been well established (Brim and Cockerham 1961, Gates et al. 1960 and Singh and Hadley 1968), but there has been little evidence of dominance gene action for either high or low percentage oil. The presence of an additive X additive component for percent oil has also been noted in the literature on soybeans (Brim and Cockerham 1961). The results for percent oil in early maturing soybeans then, were consistent with the published results from other maturity groups in soybeans.

4.2.1.3 The Sum of Percent Protein and Percent Oil. The results for the 1981 and 1982 genetic analyses of the sum of percent protein and percent oil, for the cross Maple Presto/Sioux, are presented in Tables 14 and 15 respectively. In both years, a simple additive genetic model adequately explained the data. Additive gene action was highly significant, while dominance gene action and non-allelic interactions were nonsignificant.

The 1981 and 1982 results of the means analyses for the sum of protein and oil, for the cross Maple Presto/X446-2-1 are presented in Tables 16 and 17 respectively. Again, the only genetic component contributing to the inheritance of this "sum" was an additive genetic component. For both experimental years, dominance and epistatic effects were absent.

The literature on gene action controlling the sum of protein and oil is meagre but these results in early maturing soybeans are in agreement with those in summer rape (Grami and Stefansson 1977b), where this "sum" was conditioned entirely by additive gene action.

4.2.2 Variance Analyses

The variances of the generations used in the means analyses were used to estimate genetic components of variance. The variation in each of the true breeding parental generations (P_1 and P_2) provided estimates of the nonheritable variance or, the proportion of the observed variability that was due to the environment (E). Similarly, the F_1 population of the two true-breeding parents, although heterozygous, is genetically homogeneous and therefore, any variation observed is

environmental. Given genetic differences between the parents, the F_2 generation includes this nonheritable component and a heritable component. The heritable component may consist of additive genetic variance (D) and/or dominance genetic variance (H). The (d) and (h) components of the means analysis may be influenced by cancellation effects as a result of the distribution of alleles for the trait between the two parents. By contrast the D and H values will remain uninfluenced by the distribution of alleles. This is because they represent the contribution of (d) and (h) to the sum of squares of deviation from the mid-parent. D and H however, are estimated less precisely than (d) and (h) as was clear from the experimental results.

The analyses of the variances of the experimental generations as outlined by Mather and Jinks (1977) provided estimates of D, H, and E as well as a fourth component (F) which arose from the partitioning of the variation in the backcross generations. The F component was nonsignificantly different from zero in all of the analyses for each cross and therefore, only the D, H and E components are reported.

In 1981 of the six generations used to estimate the four components above, three were used to estimate the environmental component. This permitted only an exact solution for the four parameters. Dropping the nonsignificant components, provided a better estimate of the remaining components and also provided degrees of freedom to test the goodness of fit of the model to the data. In 1982, the seven experimental generations provided extra degrees of freedom, again, to test the fit of the model to the data.

4.2.2.1 Percent Protein. The results of the 1981 and 1982 analyses of the variances of the experimental generations for the cross Maple Presto/Sioux, are presented in Tables 18 and 19. For both years, there was clearly a large genetic component of the observed variance comprised of both an additive and a dominance component. In 1981, the dominance component was small compared to the additive component whereas in 1982, the dominance component was large. In both years however, the standard error associated with the dominance component was large relative to that for the additive component. A significant environmental component was also detected, as expected.

Similarly, for the 1981 and 1982 analyses for the cross Maple Presto/X446-2-1 (Tables 20 and 21), there were significant additive and dominance components observed but again, the precision of the estimates was poor, as the standard errors were large. A significant environmental component was also present.

The sample size of the segregating generations for both crosses was not adequate to provide good estimates of the heritable components of variance as the standard errors were very high when compared to the standard error for the estimate of the environmental component. This was particularly true for the estimate of the dominance component where standard errors were as high as 50 percent. Clearly the F_2 and backcross generations, which provide estimates of 1/4 H each (Appendix Table 2), should be larger in order to increase the precision of the estimate.

TABLE 18. Estimates of the components of variance for percent protein, percent oil and their "sum", for the cross Maple Presto/Sioux (1981).

Component	Protein	Oil	Sum
D	3.033 ± .331	0.816 ± .203	4.062 ± 1.039
H	0.751 ± .214	-	3.939 ± 1.548
E	0.801 ± .083	0.301 ± .051	0.715 ± 0.122
χ^2[1]	0.069 (1 df)	0.034 (2 df)	0.090 (1 df)
P(.05)	3.84	5.99	3.84

[1]Test of the fit of the model to the data.

TABLE 19. Estimates of the components of variance for percent protein, percent oil and their "sum", for the cross Maple Presto/Sioux (1982).

Component	Protein	Oil	Sum
D	2.419 ± .972	1.136 ± .071	1.495 ± .527
H	5.486 ± 2.744	-	2.303 ± 1.290
E	0.808 ± .166	0.346 ± .031	0.613 ± .090
χ^2[1]	0.334 (2 df)	0.015 (3 df)	0.074 (2 df)
P(.05)	5.99	7.81	5.99

[1]Test of the fit of the model to the data.

TABLE 20. Estimates of the components of variance for percent protein, percent oil and their "sum", for the cross Maple Presto/X446-2-1 (1981).

Component	Protein	Oil	Sum
D	4.940 ± .916	1.570 ± .234	3.342 ± .627
H	1.528 ± .636	-	2.139 ± .934
E	0.590 ± .108	0.364 ± .059	0.358 ± .074
2^1	0.105 (1 df)	0.046 (2 df)	0.033 (1 df)
P(.05)	3.84	5.99	3.84

[1]Test of the fit of the model to the data.

TABLE 21. Estimates of the components of variance for percent protein, percent oil and their "sum", for the cross Maple Presto/X446-2-1 (1982).

Component	Protein	Oil	Sum
D	2.226 ± .741	0.797 ± .051	1.333 ± .578
H	5.385 ± 2.093	-	1.314 ± .802
E	0.775 ± .127	0.304 ± .022	0.458 ± .099
2^1	0.193 (2 df)	0.080 (3 df)	0.089 (2 df)
P(.05)	5.99	7.81	5.99

[1]Test of the fit of the model to the data.

4.2.2.2 Percent Oil. The results of the analyses of the variances of the experimental generations for percent oil in the cross Maple Presto/Sioux are presented in Tables 18 and 19. For both of the experimental years, the heritable variance was entirely additive and therefore the trait would respond well to selection. The dominance variance was nonsignificant. In addition to the additive component there was a significant environmental component to the observed variation.

For the cross, Maple Presto/X446-2-1, the results of the two experimental years are presented in Tables 20 and 21. Once again, the genetic variance was entirely additive as the dominance component was nonsignificant. For this cross, as with the Maple Presto/Sioux cross, a significant portion of observed variation was environmental.

The estimates of the additive component for percent oil are much smaller than those for percent protein. This, of course, reflects the smaller difference between the parental cultivars for the trait. Consequently the segregates that would result from a cross of these parents would be less variable.

4.2.2.3 Sum of Percent Protein and Percent Oil. The results of the analyses of variance for the sum of percent protein and percent oil for the cross Maple Presto/Sioux, are presented in Tables 18 and 19. A significant genetic component was detected for both experimental years, which consisted of both an additive and dominance component. As with percent protein, the precision of the estimates was poor, as evidenced by the large standard errors associated with the estimates, and in particular, with the dominance component.

Similarly, for the cross Maple Presto/X446-2-1 (Tables 20 and 21), the variance of the experimental generations was partitioned into significant additive and dominance genetic components and significant environmental component. As was the case with the Maple Presto/Sioux cross, the standard errors for D and H were very large.

In order to estimate D and H with the same precision as the environmental component, it would be necessary to increase the size of the experimental generations from which the estimates of the genetic components of variance are made (Appendix Table 2). This should decrease the standard error and therefore increase the precision of the estimates.

4.2.3 Heritability

The extent to which selection for a particular trait is likely to be effective, is determined by the heritability of that trait or, the proportion of the total phenotypic variation for the trait that is genetic or heritable. This is termed heritability in the broad sense. It is the ratio of the heritable variance, both the additive and non-additive components, to the overall phenotypic variance. Of particular interest to the plant breeder, is the narrow sense heritability because it represents that portion of the genetic variance that will respond to selection or is fixable. It is defined as the ratio of the additive genetic variance to the total phenotypic variance.

Techniques for estimating the degree of heritability are varied and include the use of variance components from an analysis of generation variances and, parent-offspring regressions. Both of these techniques were used in this study.

4.2.3.1 Heritability from Components of Variance. The heritabilities of the investigated traits in the F_2 generation, were calculated using the estimates of the components of variance determined earlier. The phenotypic variance of the F_2 was equal to 1/2D + 1/4H + E, as outlined by Mather and Jinks (1977). Narrow sense heritability was taken as the ratio of the additive variance component of the F_2 to the total phenotypic variance, while broad sense heritability for the trait was taken as the ratio of the genetic variance (both additive and non-additive) in the F_2 to the overall phenotypic variance. Results of these calculations for both crosses in the 1981 and 1982 experimental years are presented in Tables 22 and 23 respectively.

Heritabilities in the broad sense for percent protein, ranged from .68 to .83 in 1981 but were identical at .76 in 1982. In 1981, the narrow sense heritability for percent protein was close to the broad sense value for both crosses, which indicated that most of the variance was additive and therefore could be selected for. In 1982 however, heritability in the narrow sense was .34 for both of the crosses, which indicated that only half of the genetic variance was fixable. Broad sense values over the two years were very closely related and high. The difference in narrow sense values over the two experimental years was more marked and due in part to the large dominance estimates. The precision of the estimates however, was poor and consequently, the narrow sense heritabilities may have been underestimated.

The range of the broad sense heritability estimates for percent protein reflects the range found in the literature (Kwon and Torrie

TABLE 22. Estimates of broad and narrow sense heritabilities for percent protein, percent oil and their "sum" from estimates of the components of variance (1981).

Cross	Heritability[1]	Protein	Oil	Sum
Maple Presto/Sioux	Broad sense	.68	.58	.81
	Narrow sense	.61	.58	.54
Maple Presto/X446-2-1	Broad sense	.83	.68	.86
	Narrow sense	.72	.68	.65

[1]Estimates calculated according to Warner (1952).

TABLE 23. Estimates of broad and narrow sense heritabilities for percent protein, percent oil and their "sum" from estimates of the components of variance (1982).

Cross	Heritability[1]	Protein	Oil	Sum
Maple Presto/Sioux	Broad sense	.76	.62	.68
	Narrow sense	.34	.62	.39
Maple Presto/X446-2-1	Broad sense	.76	.57	.68
	Narrow sense	.34	.57	.46

[1]Estimates calculated according to Warner (1952).

1964, Smith and Weber 1968) and very much reflects the range in material and methods used for the estimations.

Broad sense heritabilities for percent oil were moderately high and ranged from .57 to .68 for the two crosses over the two experimental years. Narrow sense values were the same as broad sense values, which indicated that all of the genetic variation was additive for percent oil and therefore, could be selected for. The close correlation between years reflected the precision of the estimates of the components of variance as evidenced by the lower standard errors for the components noted earlier.

These heritabilities for percent oil were in close agreement with other values in the soybean literature from similar methods of estimation (Weber and Moorthy 1952, Johnson et al. 1955b, Kwon and Torrie 1964).

4.2.4 Parent-Progeny Regression

Parent-progeny regression involves the regression of the mean value of a trait in the progeny on the value of that trait in the parent. In practice, this involves regressing the data obtained from the progeny in one year on the parental data obtained from the previous year. Consequently, any environmental variation that tends to reduce or increase the range of variability in one year over the next could affect the heritability estimate.

Examination of the parental and mean progeny data for the three traits in the two crosses of this study (Appendix Tables 3 and 4), clearly shows that the range of values in the F_2 and F_3 differed for each trait. It differed most significantly for percent protein and the sum of protein and oil in the cross Maple Presto/X446-2-1. In

considering the nature of the calculation of the conventional regression coefficient, a range in the F_3 means that exceeds the range in the F_2 values, could result in a narrow sense heritability estimate that is greater than one. A value of that magnitude is meaningless in a practical sense and therefore, standard unit heritabilities were calculated in addition to the conventional estimates.

The heritability estimates for the three traits in the two crosses under investigation, are presented in Table 24. Both conventional (b) and standard unit (b') heritabilities are given.

For percent protein, the narrow sense heritabilities for the F_2, calculated by the conventional regression method, ranged from .78 to 1.22. The standard unit heritabilities more accurately reflected the F_2/F_3 correlation and indicated, by the lower values, that the range of values in the F_3 was greater than that in the F_2. The standard unit heritability estimates were higher than the narrow sense values calculated for the F_2 by the components of variance method. This however was expected, as the F_2 contains some dominance variance which biases the F_2/F_3 regression estimate (Hanson and Weber 1961).

Conventional heritability estimates for percent oil were again higher than the standard unit estimates, reflecting the slightly larger range of values in the F_3 over the F_2. The narrow sense heritability estimate for the cross Maple Presto/X446-2-1 was higher than that calculated from the components of variance. This again may have reflected a dominance variance bias.

TABLE 24. Heritability estimates for percent protein, percent oil and their "sum", based on regression analysis (b) and standard unit regression analysis (b') (Frey and Horner 1957) of F_3 family means on F_2 parental values.

Cross	Heritability	Protein	Oil	Sum
Maple Presto/Sioux	b	.78	.49	.66
	b'	.76	.43	.74
Maple Presto/X446-2-1	b	1.22	.80	1.01
	b'	.81	.70	.71

The comparison of the conventional and standard unit heritabilities for the sum of percent protein and oil again indicated that the range of values in the F_2 and F_3 differed. For the cross Maple Presto/Sioux, the range of values in the F_2 was greater than that in the F_3 as evidenced by the increase in the heritability estimate from .66 to .74 when the standard unit regression analysis was used. For the cross, Maple Presto/X446-2-1, the opposite was true, as the conventional estimate was 1.01 while the standard unit estimate was .71. Standard unit values were higher than the narrow sense estimates calculated from the components of variance, again reflecting a potential bias from dominance variance in the early generations.

The range of values observed for the traits, was consistent with the range of heritabilities reported in the literature. The magnitude of the value was very much a function of the genetic material and the method of estimation used. For each of the three traits investigated, the narrow sense value was moderately high and therefore, each of the traits could be successfully selected for in early maturing soybeans.

4.3 Physiology

4.3.2 Nitrogen Accumulation and Redistribution

Increasing the quantity of nitrogen available for grain development in early maturing soybeans can be accomplished by increasing the whole plant nitrogen accumulation and/or by increasing the proportion of vegetative nitrogen translocated to the developing grain. If genetic variability exists for these physiological processes, it should be possible to select for them in order to increase protein percentage in the grain.

A comparison of the patterns of nitrogen accumulation for the three parental cultivars of the inheritance study was made. Cultivars were compared for accumulated nitrogen in the whole plant, and the vegetative and seed components of the plant. The results from plants grown under spaced conditions in 1981 and 1982 are presented in Figures 1 and 2 respectively.

Under spaced conditions, the two indeterminate cultivars continued to assimilate nitrogen throughout reproductive development, as evidenced by a sustained accumulation of nitrogen in the whole plant throughout the growing season (Figures 1 and 2). Sioux, the only determinate cultivar, showed a marked decline in accumulated nitrogen after 76 days, which corresponded with the onset of seed growth. This was due in part to the cessation of vegetative growth of the determinate cultivar at the onset of reproductive development but may have been due as well to genetic variability for the duration of N_2-fixation and assimilation.

Examination of accumulated nitrogen in the vegetative tissue suggested that the high protein cultivar X446-2-1 accumulated more nitrogen in the vegetative tissue than the low protein cultivar Maple Presto. The determinate high protein cultivar Sioux, however, did not consistently accumulate more nitrogen than the low protein cultivar Maple Presto. In 1981 (Figure 1), Sioux accumulated more nitrogen in the vegetative tissue than Maple Presto, but in 1982 (Figure 2), the reverse was true.

For all three cultivars, a decline in the vegetative tissue nitrogen coincided with an increased accumulation of nitrogen in the seed,

Figure 1. Total nitrogen content of the whole plant and the vegetative and seed components for Maple Presto (●), X446-2-1 (■) and Sioux (▲) under spaced conditions. (1981)

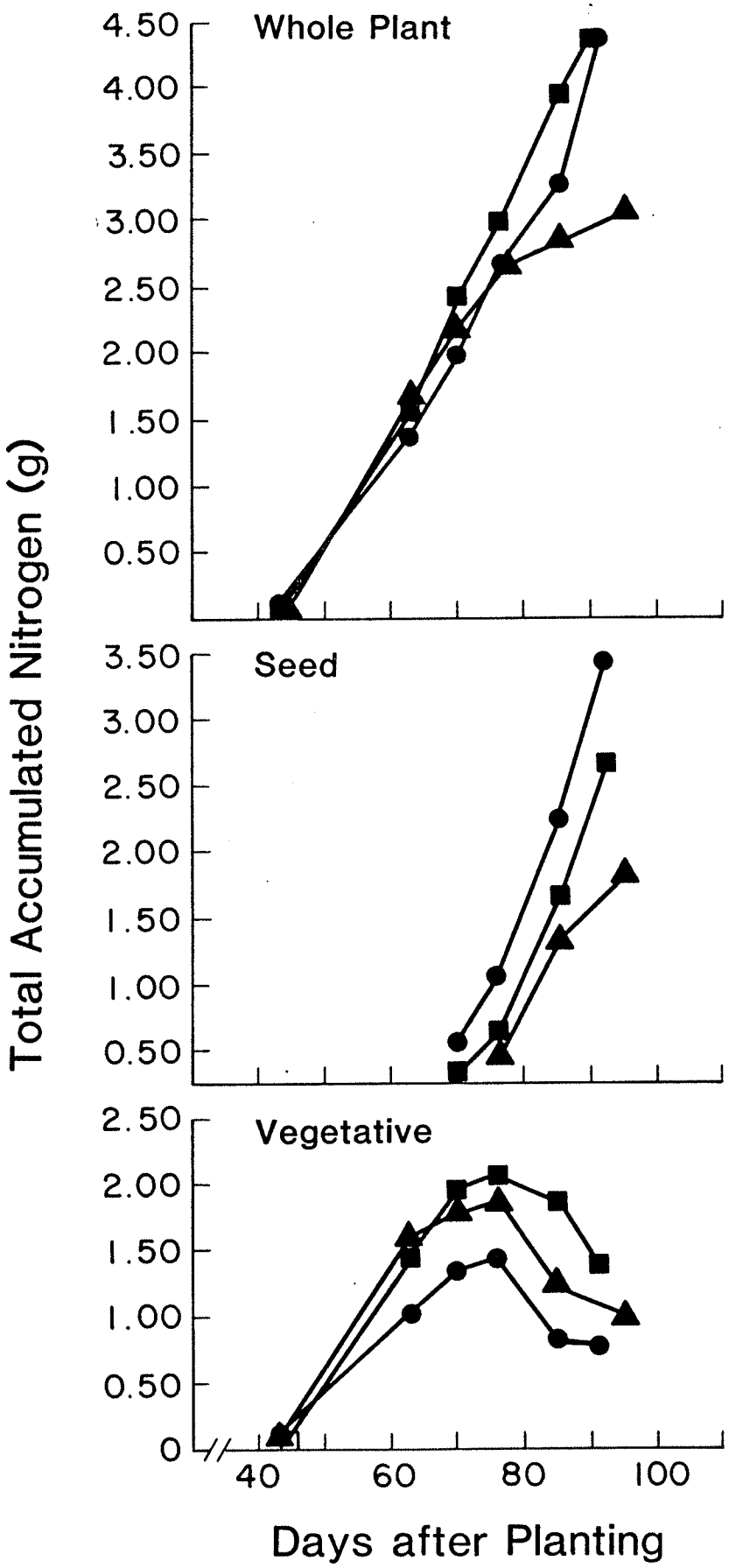
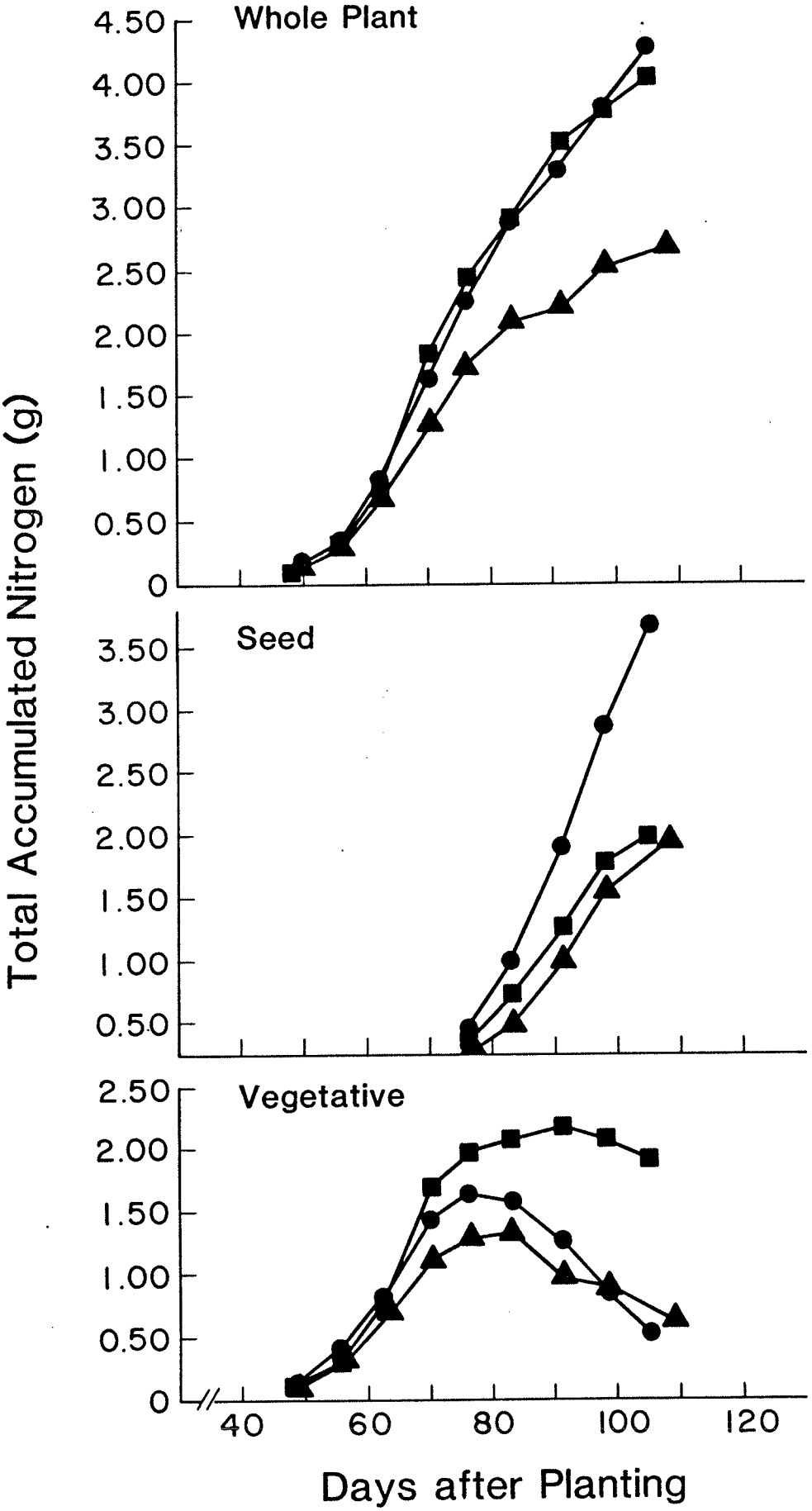

Whole Plant
Seed
Vegetative
Total Accumulated Nitrogen (g)
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0
40
60
80
100
Days after Planting

Figure 2. Total nitrogen content of the whole plant and the vegetative and seed components for Maple Presto (●), X446-2-1 (■) and Sioux (▲) under spaced conditions. (1982)


Whole Plant
Seed
Vegetative
Total Accumulated Nitrogen (g)
Days after Planting
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0
40
60
80
100
120

as nitrogen was remobilized from the vegetative tissue to the developing seed. The gain in seed nitrogen for all three cultivars, however, was greater than the net loss from the vegetative tissue. This suggested that nitrogen continued to be assimilated by the plant during reproductive development. During the initial stages of seed development, the nitrogen assimilated was adequate to meet the seed demand but during the later stages both assimilated and remobilized nitrogen were required. In 1982, the nitrogen assimilated by the X446-2-1 cultivar appeared to be almost adequate for seed development as there was a marked decrease in the amount of nitrogen remobilized from the vegetative tissues (Figure 2).

Examination of the rate of accumulation of nitrogen in the seed again indicated that nitrogen lost from the vegetative tissue was remobilized to the seed, as the rate of accumulation of nitrogen in the seed exceeded the rate of accumulation in the whole plant. This was consistent for all three cultivars in 1981, and Maple Presto and Sioux in 1982. The X446-2-1 cultivar showed a lower rate of accumulation of nitrogen in the seed in 1982, which reflected, at least to some extent, the decrease in the amount of nitrogen remobilized from the vegetative tissues.

The comparisons of accumulated nitrogen in the three cultivars under plot conditions for 1981 and 1982, are presented in Figures 3 and 4 respectively. Nitrogen was again assimilated throughout much of the growing season as indicated by the steady increase in whole plant nitrogen in the three cultivars in both experimental years. A marked decline, however, was noted 85 days after planting in 1981 and at 90

Figure 3. Total nitrogen content of the whole plant and the vegetative and seed components for Maple Presto (●), X446-2-1 (■) and Sioux (▲) under plot conditions. (1981)

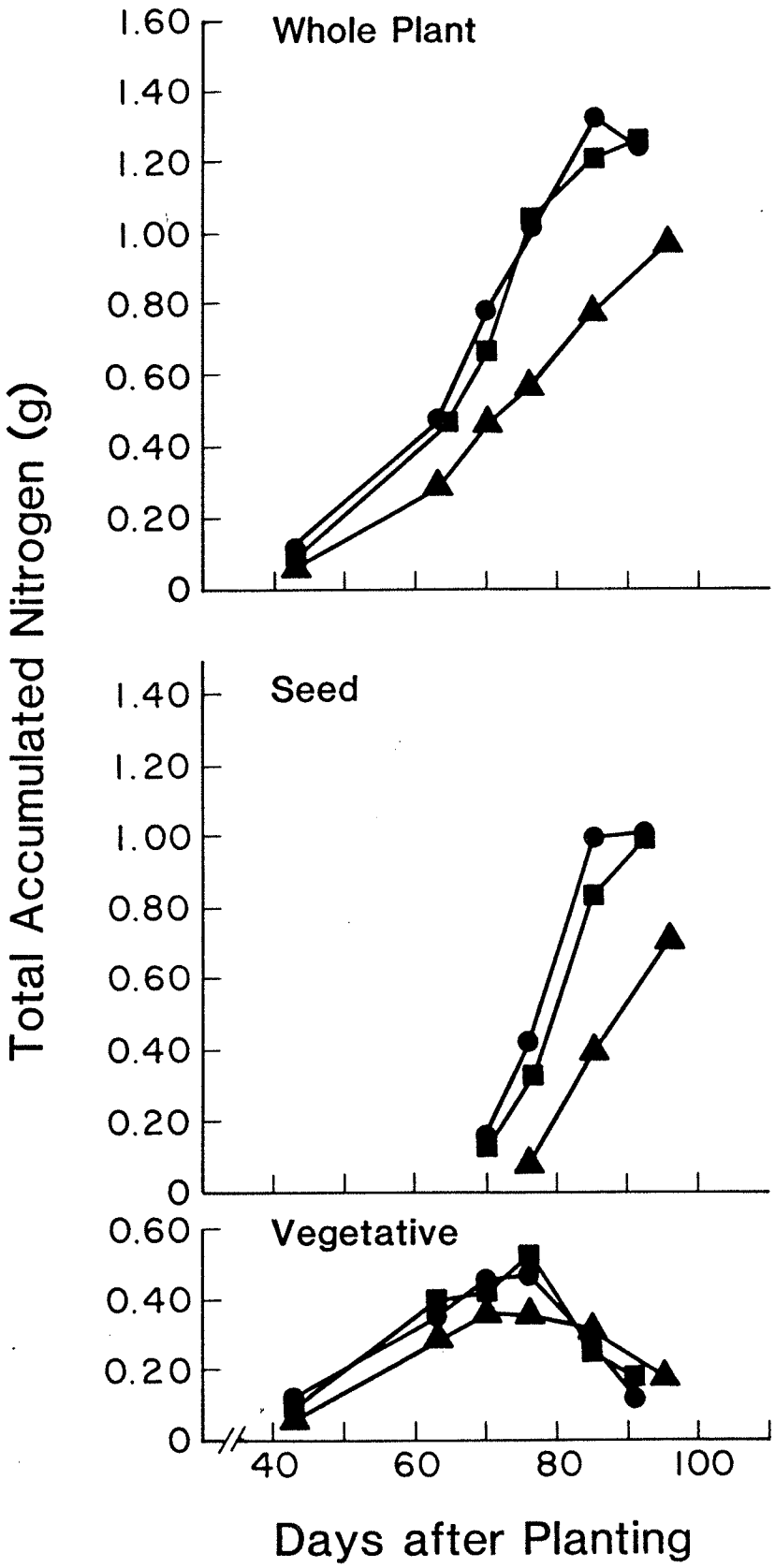
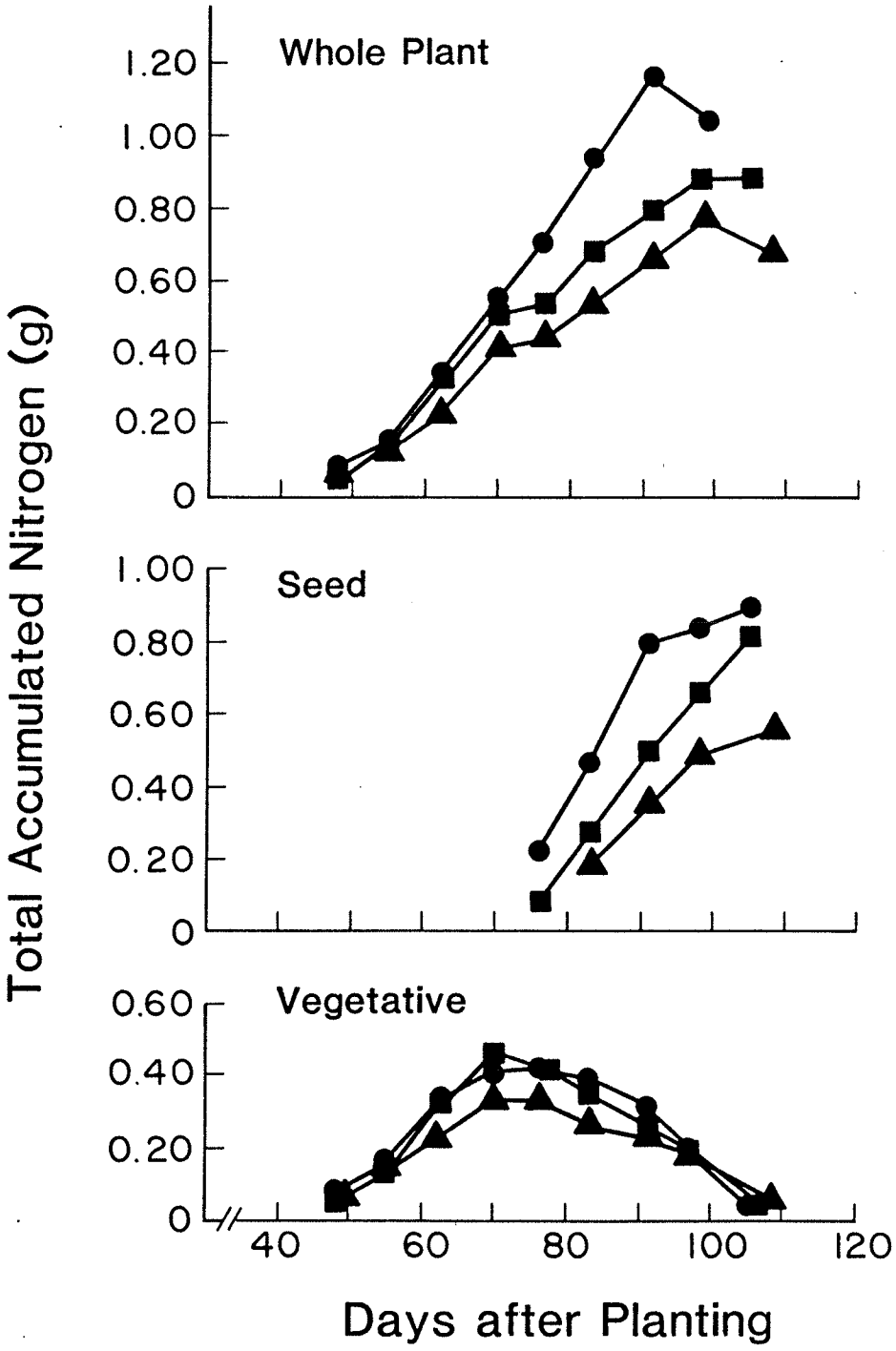

Whole Plant
Seed
Vegetative
Total Accumulated Nitrogen (g)
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0
40
60
80
100
Days after Planting

Figure 4. Total nitrogen content of the whole plant and the vegetative and seed components for Maple Presto (●), X446-2-1 (■) and Sioux (▲) under plot conditions. (1982)


Whole Plant
Seed
Vegetative
Total Accumulated Nitrogen (g)
1.20
1.00
0.80
0.60
0.40
0.20
0
40
60
80
100
120
Days after Planting

days after planting in 1982 for the indeterminate cultivars. This corresponded to approximately the R_5 stage of development. The determinate cultivar Sioux, under plot conditions, showed no decline in total plant nitrogen in 1981 but a marked decline at R_6 in 1982.

This decline in whole plant nitrogen accumulation during mid to late podfill, observed under plot conditions, may have been due to the well documented relationship between nitrogen fixation and photosynthate supply (Mague and Burris 1972, Lawn and Brun 1974, Streeter 1974). Under plot conditions, the increased competition for light and nutrient supply, could have resulted in photosynthate limitation, which would result in a decrease in the total amount of nitrogen fixed and a decline in the rate of nitrogen accumulation in the whole plant.

Examination of the levels of accumulated nitrogen in the vegetative tissue, under plot conditions, indicated that the indeterminate cultivars appeared to accumulate more nitrogen than the determinate cultivar, Sioux. This was directly related to the larger vegetative dry matter of Maple Presto and X446-2-1 and the nature of the calculation of accumulated nitrogen. Of the two indeterminate cultivars, the high protein cultivar X446-2-1, appeared to have a higher nitrogen content at the onset of reproductive development than did the low protein cultivar, Maple Presto.

Again, as with the spaced plants, nitrogen was remobilized from the vegetative tissue to the developing seed, as indicated by the coincidental decrease in vegetative nitrogen and increase in seed nitrogen. The much greater rate of increase in seed nitrogen over the rate of increase in whole plant nitrogen, suggested that remobilization

contributed more to seed nitrogen under plot conditions than under spaced conditions. Under plot conditions, a decline in vegetative nitrogen began at the onset of seed development whereas under spaced conditions, N_2-fixation was initially adequate to meet the seed demand. This again suggested the importance of remobilization of accumulated vegetative nitrogen to the accumulation of nitrogen in the seed, under plot conditions. Results were consistent over both experimental years.

The relative importance of the vegetative and pod wall components of the three cultivars, as a source of redistributed nitrogen under both spaced and plot conditions, was determined from changes in the levels of accumulated nitrogen in the component parts over the course of the season. This was calculated as the amount of redistributed nitrogen contributed by each component as a percent of the total amount redistributed. In addition, the contribution of this redistributed nitrogen to the total seed nitrogen content (RN/SN) was determined. The results of these calculations for the 1981 and 1982 seasons are presented in Tables 25 and 26 respectively.

As was expected, the vegetative tissue, including the stems, petioles and leaves, was the major source of redistributed nitrogen. The range of contribution from the vegetative tissue of the spaced plants was from 71 to 85 percent in 1981 and from 78 to 86 percent in 1982. For plants grown under plot conditions, the vegetative tissue contributed 71 to 82 percent of the redistributed nitrogen in 1981 and 79 to 90 percent of the redistributed nitrogen in 1982.

TABLE 25. Nitrogen redistribution for the three cultivars under spaced (S) and plot (P) conditions (1981).

Cultivar	Planting density	Source of redistributed N[1]		RN/SN[2]		
		Vegetative	Pod	Vegetative	Pod	Total
		%				
Maple Presto	S	85	15	57	3	60
	P	82	18	63	7	70
X446-2-1	S	80	20	38	5	43
	P	72	28	54	9	63
Sioux	S	71	29	60	10	70
	P	71	29	70	11	81

[1]Amount of redistributed nitrogen contributed by each plant part, as a percent of the total amount redistributed.
[2]Proportion of the total seed N that came from redistribution.

TABLE 26. Nitrogen redistribution for the three cultivars under spaced (S) and plot (P) conditions (1982).

Cultivar	Planting density	Source of redistributed N[1]		RN/SN[2]		
		Vegetative	Pod	Vegetative	Pod	Total
		%				
Maple Presto	S	86	14	40	7	47
	P	90	10	54	6	60
X446-2-1	S	81	19	20	6	26
	P	88	12	52	5	57
Sioux	S	78	22	47	12	59
	P	79	21	73	11	84

[1]Amount of redistributed nitrogen contributed by each plant part, as a percent of the total amount redistributed.
[2]Proportion of the total seed N that came from redistribution.

The importance of nitrogen derived from the vegetative tissues to the total seed nitrogen varied with planting density and with cultivar. When taken as a percent of total seed nitrogen (RN/SN), the contribution of vegetative tissue nitrogen to the developing seed, under spaced conditions, ranged from 38 percent to 60 percent of the seed nitrogen in 1981 and from 20 percent to 47 percent of the seed nitrogen in 1982. Under plot conditions, the range of the contribution of the vegetative tissues to seed nitrogen was from 54 percent to 70 percent of the seed nitrogen in 1981 and from 52 percent to 73 percent of the seed nitrogen in 1982.

In general, nitrogen remobilized from the vegetative tissues formed a greater proportion of the seed nitrogen in plants grown under plot conditions than in plants grown under spaced conditions. This could have resulted from a decreased capacity to assimilate nitrogen under the competitive conditions of plot density planting. Of the three cultivars studied under plot conditions, the highest proportion of seed nitrogen derived from vegetative nitrogen was found in the high protein determinate cultivar, Sioux. The high protein indeterminate cultivar, X446-2-1, relied the least on remobilized nitrogen and therefore must have been more efficient in assimilating nitrogen throughout the reproductive period in order to maintain its high protein seed quality.

The pod tissue also contributed to the total seed nitrogen. However, this was a less important source of seed nitrogen for the indeterminate cultivars, Maple Presto and X446-2-1, than for the determinate high protein cultivar, Sioux. Under spaced conditions in 1981,

the percentage of seed nitrogen derived from pod tissue ranged from 3 percent to 5 percent for Maple Presto and X446-2-1 while for Sioux it was 10 percent. Similarly, in 1982, under spaced conditions, the pod tissue contributed 7 percent and 6 percent of the seed nitrogen in Maple Presto and X446-2-1 respectively while it was the source of 12 percent of the seed nitrogen in the cultivar, Sioux.

Under plot conditions, a similar trend was in evidence, in that, the pod tissue was a relatively more important source of seed nitrogen in Sioux than it was in either Maple Presto or X446-2-1. In 1981, 7 percent and 9 percent of the nitrogen in the seed of Maple/Presto and X446-2-1 respectively, was derived from the pod tissue, whereas 11 percent of the seed nitrogen in Sioux was derived from the pod tissue. Similarly, in 1982, 6 percent and 5 percent of the seed nitrogen in Maple Presto and X446-2-1 respectively, had been remobilized from pod tissue, whereas in Sioux, 11 percent of the seed nitrogen was derived from this source.

From the data of the two experimental years, it was apparent that the pods of the determinate cultivar Sioux, were a more important source of seed nitrogen than were the pods of either Maple Presto or X446-2-1. This suggested a strategy for accumulating nitrogen in the seed of the high protein, determinate cultivar that was different from that of either the high or the low protein indeterminate cultivars.

The ability of the high protein, determinate cultivar, Sioux, to efficiently remobilize pod nitrogen to the developing seed, was also detected through an analysis of the rate of change of percent nitrogen in the pod tissue of the three cultivars.

Percent nitrogen for each of the cultivars was regressed on days after planting. Significant differences in the slopes of the regression lines were then used to detect cultivar differences in the rate of decline of percent nitrogen in the pod tissues. Although this decline in percent nitrogen also reflected the change of other cell components in addition to nitrogen, it was thought to indicate, at least in part, a greater rate of remobilization of nitrogen from the pod to the developing seed. The results of the analyses for the two experimental years are presented in Figures 5 and 6.

Under spaced conditions, in 1981 (Figure 5a), the rate of decline in percent nitrogen in the pod tissues of Sioux (b = -.09) was significantly greater than that for either Maple Presto (b = -.03) or X446-2-1 (b = -.06). Similarly, in 1982 (Figure 5b), the rate of decline in percent nitrogen was significantly greater for Sioux (b = -.09) than it was for either Maple Presto (b = -.05) or X446-2-1 (b = -.04). Differences detected between the high and low protein, indeterminate cultivars, X446-2-1 and Maple Presto, were not as conclusive as the slopes were significantly different in 1981 but not significantly different in 1982.

Under plot conditions in 1981 (Figure 6a) Sioux again had a significantly greater rate of decline in percent nitrogen in the pod tissue during seed development (b = -.08) than did either Maple Presto (b = -.04) or X446-2-1 (b = -.05). The same trend was apparent in 1982 (Figure 6b) as percent nitrogen in the pod tissue decreased at a significantly greater rate in Sioux (b = -.08) than it did in either Maple Presto (b = -.04) or X446-2-1 (b = -.04). Under plot conditions, no significant differences were detected in the rate of decline in percent nitrogen in the pod tissue of either Maple Presto or X446-2-1.

Figure 5. Change in nitrogen concentration in the pod tissue during reproductive development for Maple Presto (●), X446-2-1 (■) and Sioux (▲) under spaced conditions for 1981 (a) and 1982(b).

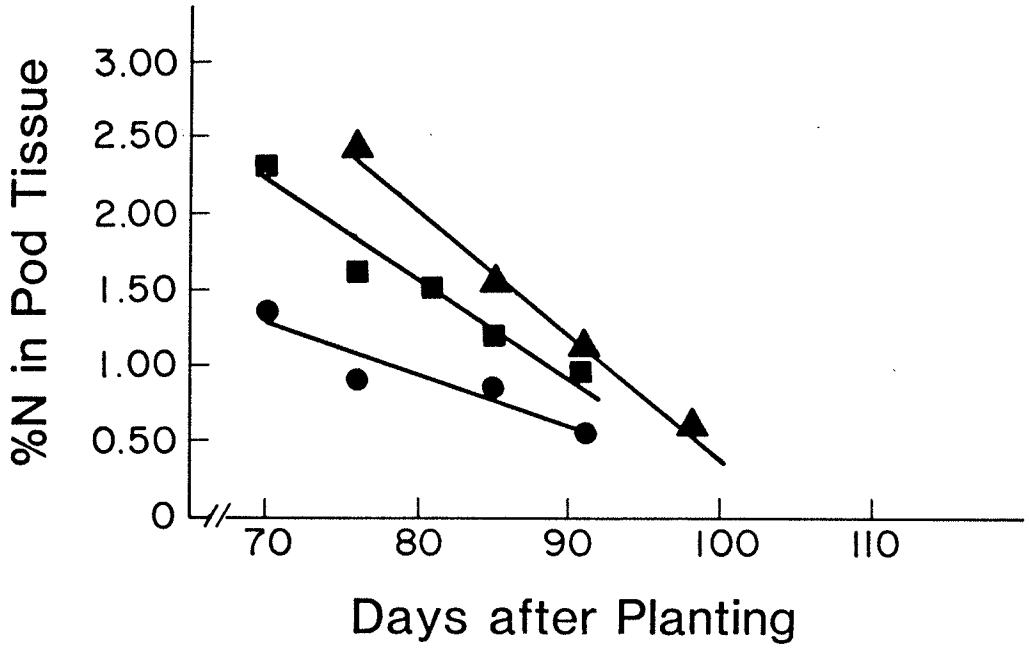


Figure 5a

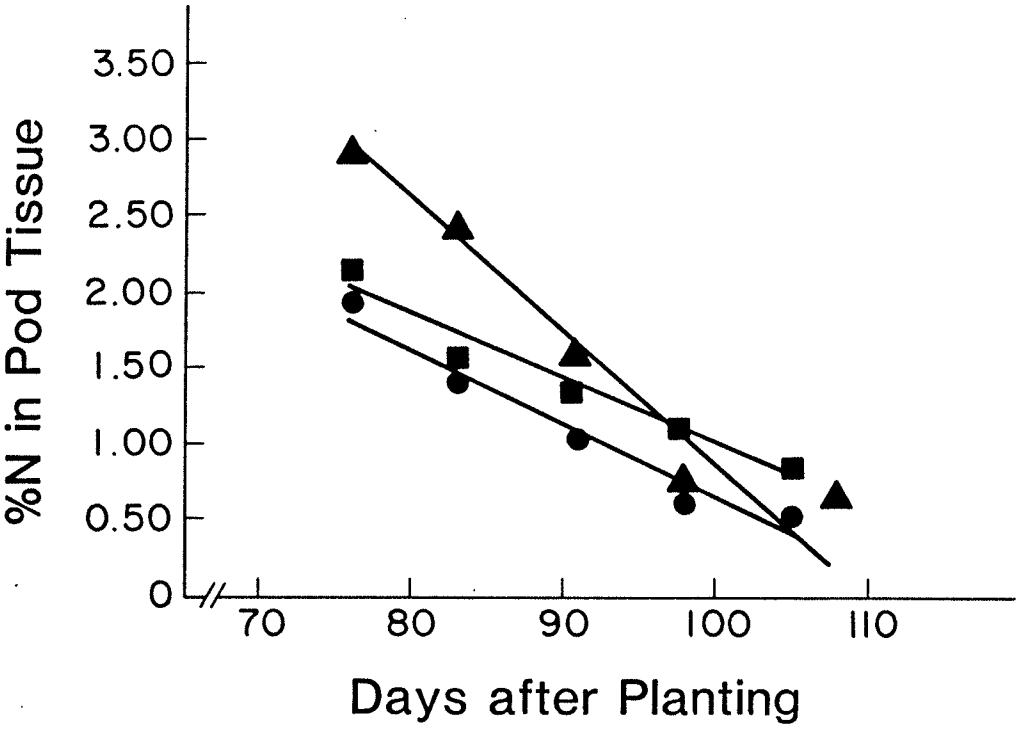


Figure 5b

Figure 6. Change in nitrogen concentration in the pod tissue during reproductive development for Maple Presto (●), X446-2-1 (■) and Sioux (▲) under plot conditions for 1981 (a) and 1982(b).

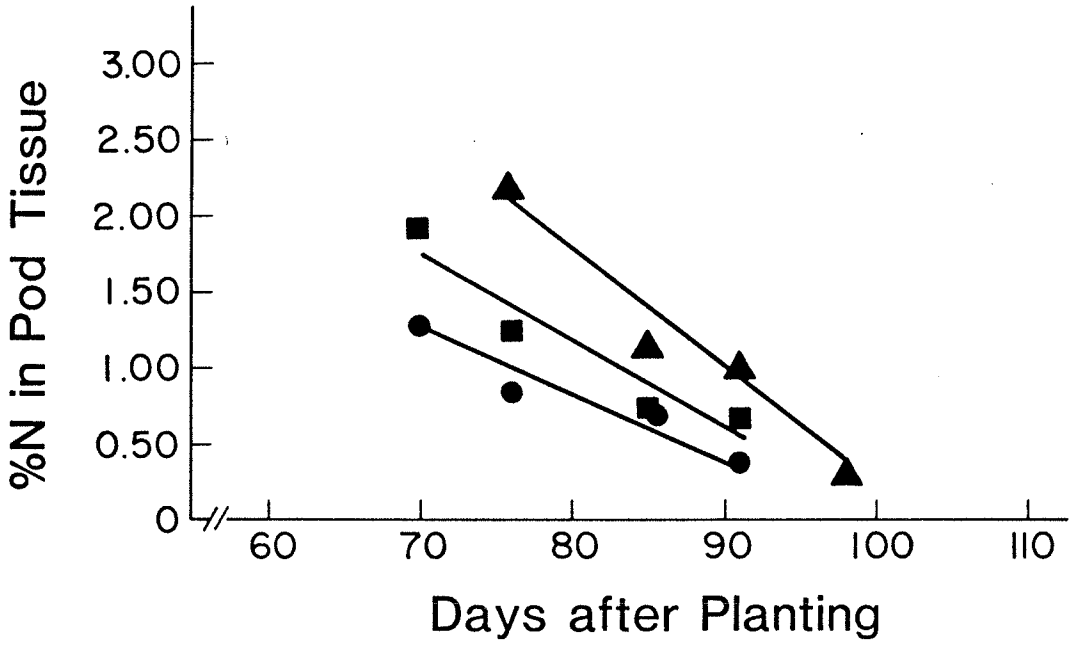


Figure 6a

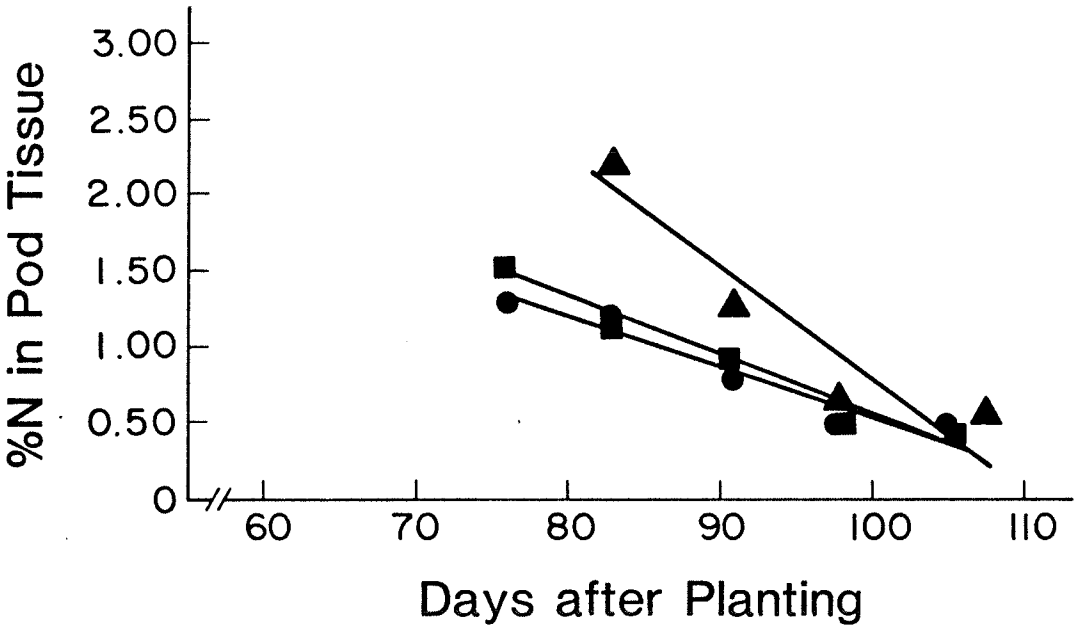


Figure 6b

In summary, the data on the remobilization of pod tissue nitrogen suggested that genetic variability exists for the rate of remobilization from the pod tissue and the extent of the contribution of pod nitrogen to the seed. Therefore, the two traits could be selected for as a means of increasing the accumulation of nitrogen in the seed. Similar variability for the decrease in percent nitrogen in the vegetative tissue was not apparent.

Comparisons among the three cultivars of the amount of nitrogen accumulated in the vegetative tissue by R_2, the onset of pod development, were made. The choice of the R_2 stage of development resulted from the assumption that nitrogen, accumulated to this point, was available for subsequent remobilization to the developing reproductive tissue. Similarly, comparisons were made among the three cultivars of the amount of nitrogen accumulated in the pod tissue by R_4, the onset of seed development. Again, the choice of this stage of development resulted from the assumption that the nitrogen accumulated to this point, was available for subsquent remobilization to the developing seed.

The results of the comparison of high and low protein cultivars for accumulated nitrogen and accumulated dry matter at the onset of pod development (R_2) are presented in Table 27. Maple Presto, a low protein indeterminate cultivar was compared initially to X446-2-1 which had an indeterminate growth type but had high percent protein in the seed. The second comparison involved Maple Presto and Sioux, a determinate, high protein cultivar.

TABLE 27. Pairwise comparisons of accumulated nitrogen and dry matter in the vegetative tissue at R_2, of high (H) and low (L) protein cultivars under spaced (S) and plot (P) conditions.

Cultivar	Protein	Planting density	1981[1]		1982[2]	
			Nitrogen	Dry matter	Nitrogen	Dry matter
			g			
Maple Presto	L	S	1.10±.09	33.5±4.3	1.44±.07	40.6±1.6
X446-2-1	H	S	1.51±.06*	37.0±2.6	1.98±.09*	41.1±1.8
Maple Presto	L	P	0.35±.03	12.1±0.6	0.41±.09	13.2±0.9
X446-2-1	H	P	0.43±.05	13.4±1.5	0.45±.03	12.7±0.8
Maple Presto	L	S	1.10±.09	33.5±4.3	1.44±.07*	40.6±1.6*
Sioux	H	S	1.60±.13*	40.7±4.7*	1.12±.08	27.2±1.7
Maple Presto	L	P	0.35±.03	12.1±0.6*	0.41±.03	13.2±0.9*
Sioux	H	P	0.38±.01	10.5±0.2	0.33±.02	9.2±0.6

[1]Mean based on 4 replicates
[2]Mean based on 6 replicates
*Means significantly different at P=.05.

For the comparison of Maple Presto and X446-2-1, the mean vegetative dry weight data indicated that there were no significant differences in dry weight between the two cultivars for either spaced or plot density samples. However, a comparison of the accumulated nitrogen in the vegetative tissue showed significant differences between the high and low protein cultivars. The comparisons of cultivars grown under spaced conditions showed the high protein cultivar, X446-2-1, accumulated significantly more nitrogen in the vegetative tissue than the low protein cultivar, Maple Presto. Under plot conditions, the difference in the means was not significant but the trend suggested that the high protein cultivar accumulated more nitrogen per unit dry matter than the low protein cultivar. Calculations based on the data presented in Table 27 indicated that X446-2-1 accumulated .08 g/sample or 23 percent more nitrogen in 1981 while accumulating only 11 percent more dry matter. In 1982, Maple Presto accumulated more dry matter and yet, X446-2-1 still accumulated .04 g/sample or 10 percent more nitrogen than Maple Presto. Some environmental variation with years was observed but the trend was consistent over both experimental years.

The comparison of the determinate, high protein cultivar Sioux with Maple Presto, was not as conclusive as it was difficult to eliminate the effect of growth habit on the results. Both the 1981 and the 1982 results for spaced plants indicated significant differences, between the two cultivars, in the amount of dry matter accumulated by R_2. In 1981, Sioux accumulated significantly more nitrogen and dry matter than Maple Presto while in 1982, the opposite was true.

Calculations based on the data presented in Table 27 however showed that, while Sioux accumulated 7.2 g/plant or 21 percent more dry matter than Maple Presto in 1981, it accumulated .50 g/plant or 45 percent more nitrogen. In 1982, while Maple Presto accumulated 13.4 g/plant or 40 percent more dry matter than Sioux, it accumulated only .32 g/plant or 29 percent more nitrogen. Although not conclusive, these results suggested that, again, on a per unit dry matter basis, the high protein cultivar had accumulated more nitrogen by R_2 than the low protein cultivar.

Under plot conditions, the results from both experimental years showed that plot samples of the two cultivars differed significantly in dry matter. Calculations based on the means presented however, showed that in 1981, Maple Presto accumulated 1.6 g/sample or 15 percent more dry matter than Sioux, while Sioux accumulated .03 g/sample or 9 percent more nitrogen than Maple Presto. Similarly, in 1982, Maple Presto accumulated 4 g/sample or 43 percent more dry matter while accumulating only .08 g/sample or 24 percent more nitrogen than Sioux. These data suggested therefore that under plot conditions, the high protein cultivar accumulated more nitrogen per unit dry matter than the low protein cultivar.

The conclusions drawn from the comparison of accumulated nitrogen in the vegetative tissue by R_2 support those of Israel (1981) who showed that cultivars differ in the amount of nitrogen accumulated in the vegetative tissue prior to reproductive development. The results of this study, from comparisons of Maple Presto, X446-2-1 and Sioux, suggested that the levels of accumulated nitrogen per unit dry matter were positively correlated with percent protein in the seed at maturity.

Comparisons were also made, among the three cultivars, of the amount of nitrogen retained in the vegetative tissues at physiological maturity (R_7) under both spaced and plot conditions. The results of these comparisons are presented in Table 28.

Large standard errors confounded the interpretation of data taken at this growth stage. The magnitude of the standard errors of the means resulted from severe storm damage in both experimental years, which caused the loss of dry matter and therefore, because of the nature of the calculations, resulted in increased variability in the data for accumulated nitrogen.

Under spaced conditions, X446-2-1 tended to retain green leaves through to physiological maturity and therefore the normal yellowing of the leaves observed in soybeans was not evident. This trait has been reported in the literature (Mondal et al. 1978) where it was related to sink limitation but in this experiment the cause was not clearly evident. This "evergreen" quality of the leaves was reflected in the amount of nitrogen retained in the vegetative tissues at R_7. X446-2-1 had significantly greater amounts of nitrogen in the vegetative tissues at this stage in both experimental years, than Maple Presto, while dry matter differences between the two cultivars were nonsignificantly different.

Under plot conditions no significant differences were detected in dry matter or retained nitrogen between Maple Presto and X446-2-1 although the data suggested that more nitrogen per unit dry matter was retained by the high protein cultivar X446-2-1. From calculations based on mean values in Tables 27 and 28, under plot conditions, the

TABLE 28. Pairwise comparisons of accumulated nitrogen and dry matter in the vegetative tissue at R_7, of high (H) and low (L) protein cultivars, under spaced (S) and plot (P) conditions.

Cultivar	Protein	Planting density	1981[1]		1982[2]	
			Nitrogen	Dry matter	Nitrogen	Dry matter
			g			
Maple Presto	L	S	0.73±.14	41.5±5.7	0.58±.10	51.4±5.3
X446-2-1	H	S	1.38±.09*	59.7±3.0	1.92±.11*	65.3±2.4
Maple Presto	L	P	0.12±.06	6.8±1.4	0.03±.01	6.5±0.9
X446-2-1	H	P	0.19±.09	6.3±1.5	0.04±.01	5.7±1.1
Maple Presto	L	S	0.73±.14	41.5±5.7	0.57±.10	51.4±5.3
Sioux	H	S	1.03±.15	35.5±2.1	0.67±.04	26.3±1.5
Maple Presto	L	P	0.12±.06	6.8±1.4	0.03±.01	6.5±0.9
Sioux	H	P	0.16±.04	9.0±1.1	0.06±.02	5.2±0.8

[1]Mean based on 4 replicates
[2]Mean based on 6 replicates
*Means significantly different at P=.05.

high protein cultivar X446-2-1 tended to accumulate more nitrogen in the vegetative tissues prior to reproductive development (R_2) and remobilize more nitrogen to the developing seed than Maple Presto.

The comparison of Maple Presto and Sioux at R_7 was again confounded by large standard errors of the means due to storm damage in both 1981 and 1982. Under both spaced and plot conditions, no significant differences were detected for either accumulated dry matter or the amount of nitrogen retained by the vegetative tissues. The tendency however was for the high protein cultivar to retain more nitrogen per unit dry matter in the vegetative tissue at R_7 than the low protein cultivar. Calculations based on the data presented in Tables 27 and 28 showed the net losses of nitrogen from the vegetative tissues of Sioux, during reproductive development, to be smaller than those for Maple Presto. The greater amount of nitrogen accumulated in the vegetative tissues of Sioux by R_2 therefore, although assumed to have been available for remobilization, was not reflected in the amount of nitrogen redistributed.

A similar analysis of the amount of accumulated nitrogen in a sub-sample of the pod wall tissue (ssp) at the onset of seed development (R_4) was done and the results are presented in Table 29.

The comparison of Maple Presto and X446-2-1 did not conclusively show significant differences between these two cultivars in the amount of nitrogen accumulated in the pod wall tissue by R_4. In 1981, under spaced conditions, the means for accumulated dry matter were not significantly different yet X446-2-1 had accumulated significantly more nitrogen in the pod wall tissue than Maple Presto. Although the

TABLE 29. Pairwise comparisons of accumulated nitrogen and dry matter in the pod wall tissue at R_4, of high (H) and low (L) protein cultivars, under spaced (S) and plot (P) conditions.

Cultivar	Protein	Planting density	1981[1]		1982[2]	
			Nitrogen	Dry matter	Nitrogen	Dry matter
			g			
Maple Presto	L	S	.030±.001	2.28±.11	.037±.003	1.95±.13
X446-2-1	H	S	.145±.003*	1.95±.09	.038±.002	1.78±.11
Maple Presto	L	P	.028±.005	2.10±.22*	.023±.004*	1.27±.15*
X446-2-1	H	P	.028±.003	1.43±.15	.018±.002	0.85±.08
Maple Presto	L	S	.030±.001	2.28±.11	.037±.003	1.95±.13
Sioux	H	S	.063±.003*	2.53±.13	.065±.004*	2.62±.15*
Maple Presto	L	P	.028±.002	2.10±.22	.023±.004	1.27±.15
Sioux	H	P	.038±.001*	1.63±.18	.030±.001*	1.42±.09

[1]Mean based on 4 replicates
[2]Mean based on 6 replicates
*Means significantly different at P=.05.

results for 1982 indicated that neither accumulated nitrogen nor accumulated dry matter differed significantly for the two cultivars, Maple Presto accumulated .17 g/ssp or 10 percent more dry matter and therefore suggested that X446-2-1 accumulated more nitrogen per unit dry matter of pod wall tissue, than Maple Presto.

Under plot conditions in 1981, no significant differences were detected between Maple Presto and X446-2-1 but the high protein cultivar again tended to accumulate more nitrogen per unit dry matter than the low protein cultivar. Calculations based on Table 29 showed that in 1981, Maple Presto accumulated .67 g/ssp or 47 percent more dry weight than X446-2-1 while the values for accumulated nitrogen were equal. In 1982, Maple Presto accumulated significantly more nitrogen than X446-2-1 but the results were confounded by significant dry matter differences in the sub-sample. Calculations similar to those for 1981 showed that, although Maple Presto accumulated .42 g/ssp or 49 percent more dry matter, it accumulated only .005 g/ssp or 28 percent more nitrogen.

The comparison of Maple Presto and Sioux showed more conclusively that the high protein cultivar accumulated significantly more nitrogen in the pod wall tissue than the low protein cultivar. Under spaced conditions in 1981, the means of the two cultivars for accumulated dry matter were not significantly different yet Sioux accumulated significantly more nitrogen than Maple Presto. In 1982, the results were confounded by significant dry matter differences but still suggested that the high protein cultivar accumulated more nitrogen per unit dry matter of pod wall tissue than the low protein cultivar as Sioux

accumulated .67 g/ssp or 34 percent more dry matter than Maple Presto while at the same time accumulating .028 g/ssp or 76 percent more nitrogen.

Under plot conditions, there were no significant cultivar differences in the means for accumulated dry matter in the pod wall tissue yet in both experimental years, Sioux accumulated significantly more nitrogen in this tissue. These data strongly indicated that the high protein cultivar accumulated more nitrogen in the pod wall tissue than the low protein cultivar.

Differences were influenced by the environment over years, but the consistency of the results on a per unit dry matter basis over years and planting densities, suggested that there was a significant genetic component of the variability in accumulated nitrogen in the pod wall tissues that could possibly be selected for.

Comparisons of the amount of nitrogen and dry matter retained in the pod wall tissue at physiological maturity (R_7) were made and the results are presented in Table 30.

No significant dry matter differences were noted in the sub-sample of pod wall tissue for any of the comparisons and therefore, accumulated nitrogen for each pair could be compared without dry matter bias. For the comparison of Maple Presto and X446-2-1 under spaced conditions, the data indicated that X446-2-1 retained significantly more nitrogen in the pod wall tissue at R_7 than Maple Presto. This was consistent with earlier results for vegetative tissue nitrogen in the cultivar X446-2-1 and may reflect the "evergreen" nature of this cultivar when grown under spaced conditions.

TABLE 30. Pairwise comparisons of accumulated nitrogen and dry matter in the pod wall tissue at R_7, of high (H) and low (L) protein cultivars, under spaced (S) and plot (P) conditions.

Cultivar	Protein	Planting density	1981[1]		1982[2]	
			Nitrogen	Dry matter	Nitrogen	Dry matter
			g			
Maple Presto	L	S	.023±.003	3.35±.36	.013±.001	2.37±.08
X446-2-1	H	S	.039±.004*	3.08±.24	.027±.002*	2.43±.10
Maple Presto	L	P	.013±.001	2.48±.25	.009±.001	1.85+.02
X446-2-1	H	P	.014±.002	2.05±.13	.009±.001	1.65±.09
Maple Presto	L	S	.023±.003	3.35±.36	.013±.001	2.37+.08
Sioux	H	S	.031±.001	2.95±.10	.018±.001*	2.60±.05
Maple Presto	L	P	.013±.001	2.48±.25	.009±.001	1.85±.02
Sioux	H	P	.021±.004	1.88+.13	.010+.001	1.68±.07

[1]Mean based on 4 replicates
[2]Mean based on 6 replicates
*Means significantly different at P=.05.

Under plot conditions, although the tendency was for X446-2-1 to retain more nitrogen in the pod wall tissue than Maple Presto, no significant differences in the amount of nitrogen retained were detected. From a comparison of the levels of nitrogen in this tissue at R_4 and R_7 (Tables 29 and 30) it was noted that the net loss of nitrogen from the pod wall tissue during seed development was approximately equal for the two cultivars in 1981 but was greater for the low protein cultivar in 1982.

The comparison of Maple Presto and Sioux under spaced conditions was not consistent over experimental years but suggested that Sioux retained more nitrogen per unit dry matter of pod wall tissue than Maple Presto. In 1981, although the difference was not significant, the mean data for dry matter and accumulated nitrogen, when taken together, showed Maple Presto to have .4 g/ssp or 14 percent more dry matter while Sioux had .008 g/ssp or 35 percent more nitrogen. In 1982, Sioux retained a significantly greater amount of nitrogen in the pod wall tissue than Maple Presto.

Under plot conditions, no significant differences were detected in the amount of nitrogen remaining in the pod wall tissue of the two cultivars. Calculations from the data presented in Table 30 however, suggested again that the high protein cultivar Sioux retained more nitrogen in this tissue at R_7 than the low protein cultivar Maple Presto. In 1981, the mean data showed Maple Presto had .6 g/ssp or 32 percent more dry matter than Sioux, while Sioux had .008 g/ssp or 62 percent more nitrogen than Maple Presto. Similarly, in 1982, Maple Presto had .17 g/ssp or 10 percent more dry matter than Sioux while Sioux retained .001 g/ssp or 11 percent more nitrogen.

Assuming that nitrogen lost from the pod wall during seed development (R_4 through to R_7) was remobilized to the seed, the importance of this source of nitrogen to the developing seed in the determinate cultivar is evident from the combined data of Tables 29 and 30. Although the high protein determinate cultivar, Sioux retained more nitrogen per unit dry matter of pod wall tissue at R_7 than the low protein indeterminate cultivar Maple Presto, the net loss of nitrogen from this tissue between R_4 and R_7, was greater for Sioux than for Maple Presto.

In summary, Sioux accumulated more nitrogen in the pod wall tissue than Maple Presto and subsequently remobilized more nitrogen from this source to the developing seed. Since this result was consistent over years and planting densities, it reinforced the conclusion drawn earlier that the pods are a more important source of seed nitrogen in Sioux than in Maple Presto. This consistency also suggested that the variability that exists is, at least in part, genetic and therefore could possibly be selected for.

4.3.2 Percent Nitrogen

The results for accumulated nitrogen in both the vegetative and pod wall tissues were confounded by significant dry matter differences between the means being compared. This variability in dry matter and therefore, nitrogen accumulation, resulted from sampling that was based on an individual plant basis. In addition, dry matter variability for the R_7 samples in particular, resulted partially from storm damage during both experimental years. Mean percent nitrogen values

were therefore compared for high versus low protein cultivars, under spaced and plot conditions. This enabled a direct comparison of the cultivars on a per unit dry matter basis. Comparisons, similar to those for accumulated nitrogen and dry matter, were made.

The results of the comparisons for percent nitrogen in the vegetative tissue at R_2, are presented in Table 31. The comparison of the low protein cultivar, Maple Presto, with the high protein cultivar, X446-2-1, clearly showed that X446-2-1 had a higher percentage of nitrogen in the vegetative tissue at the onset of pod development. This difference was consistent over years and planting densities.

For the comparison of Maple Presto with the determinate high protein cultivar Sioux, it was again clear that the high protein cultivar had a greater percentage of nitrogen in the vegetative tissue at R_2 than the low protein cultivar.

The data for comparisons of Maple Presto with both of the high protein cultivars, reinforced earlier conclusions that the high protein cultivars accumulated more nitrogen on a per unit dry matter basis by R_2, than the low protein cultivars

The data from the comparison of percent nitrogen in the vegetative tissue at R_7 are presented in Table 32.

Under spaced conditions, the comparison of Maple Presto and X446-2-1 reflected the "evergreen" nature of X446-2-1 when grown under conditions of zero competition, in that there was a significantly greater nitrogen concentration, in the vegetative tissue of X446-2-1 at R_7 than in Maple Presto. Under plot conditions, the means of these two cultivars were not significantly different although

TABLE 31. Pairwise comparisons of percent nitrogen in the vegetative tissue at R_2, of high (H) and low (L) protein cultivars, under spaced (S) and plot (P) conditions.

Cultivar	Protein	Planting density	Nitrogen (1981)[1]	Nitrogen (1982)[2]
			%	
Maple Presto	L	S	3.42±.13	3.57±.14
X446-2-1	H	S	3.97±.05*	4.27±.11*
Maple Presto	L	P	2.88±.02	3.07±.12
X446-2-1	H	P	3.23±.10*	3.60±.11*
Maple Presto	L	S	3.42±.13	3.57±.14
Sioux	H	S	3.94±.06*	4.13±.14*
Maple Presto	L	P	2.88±.02	3.07±.12
Sioux	H	P	3.33±.05*	3.71±.07*

[1]Mean based on 4 replicates
[2]Mean based on 6 replicates
*Means significantly different at P=0.5.

TABLE 32. Pairwise comparisons of percent nitrogen in the vegetative tissue at R_7, of high (H) and low (L) protein cultivars, under spaced (S) and plot (P) conditions.

Cultivar	Protein	Planting density	Nitrogen (1981)[1]	Nitrogen (1982)[2]
			%	
Maple Presto	L	S	1.83±.12	1.27±.10
X446-2-1	H	S	2.59±.11*	2.95±.19*
Maple Presto	L	P	1.06±.11	0.41±.04
X446-2-1	H	P	1.66±.17	0.57±.09
Maple Presto	L	S	1.83±.12	1.27±.10
Sioux	H	S	2.53±.05*	2.43±.07*
Maple Presto	L	P	1.06±.11	0.41±.04
Sioux	H	P	2.09±.07*	1.00±.13*

[1]Mean based on 4 replicates
[2]Mean based on 6 replicates
*Means significantly different at P=.05.

the high protein cultivar tended to have a higher mean nitrogen concentration in the vegetative tissue at R_7 than the low protein cultivar.

For the comparison of Maple Presto with Sioux, the results were consistent over both years and densities. At R_7, the percent nitrogen remaining in the vegetative tissues was significantly greater for Sioux than for Maple Presto.

A comparison of the percent nitrogen data at R_2 and R_7 (Tables 31 and 32), indicated that percent nitrogen in the vegetative tissues decreased during seed development in all three cultivars, and under both planting densities. The net decrease in percent nitrogen was not, however, found to be correlated with protein percentages in the seed. A comparison of the percent nitrogen data at R_2 and R_7, indicated that cultivars which had a higher nitrogen percentage in the vegetative tissue at R_2 also had a higher nitrogen percentage in this tissue at R_7. The net changes in percent nitrogen during seed development were not consistently greater in the high protein cultivars than in the low protein cultivar and therefore, no general conclusion with regards to the efficiency of redistribution of vegetative tissue nitrogen could be drawn from this data.

The data comparing nitrogen percentages in the pod wall tissue at R_4 are presented in Table 33. Again, the results indicated that the high protein cultivars had a higher nitrogen percentage in this tissue at the onset of seed development than the low protein cultivars.

TABLE 33. Pairwise comparisons of percent nitrogen in the pod wall tissue at R_4, of high (H) and low (L) protein cultivars, under spaced (S) and plot (P) conditions.

Cultivar	Protein	Planting density	Nitrogen (1981)[1]	Nitrogen (1982)[2]
			%	
Maple Presto	L	S	1.36±.05	1.89±.06
X446-2-1	H	S	2.31±.07*	2.17±.07*
Maple Presto	L	P	1.30±.19	1.25±.07
X446-2-1	H	P	1.91±.12	1.54±.09
Maple Presto	L	S	1.36±.05	1.89±.06
Sioux	H	S	2.42±.06*	2.42±.10*
Maple Presto	L	P	1.30±.19	1.25±.07
Sioux	H	P	2.18±.16*	2.20±.12*

[1]Mean based on 4 replicates
[2]Mean based on 6 replicates
*Means significantly different at P=.05.

Under spaced conditions, X446-2-1 had a significantly higher nitrogen percentage in the pod wall than did Maple Presto. Under plot conditions, although the difference was not significant at P=.05, the mean nitrogen percentage for X446-2-1, in both experimental years, tended to be higher than that for Maple Presto and were significantly higher at P=.10.

In both experimental years and under both planting densities, the comparison of Maple Presto with Sioux indicated that the high protein cultivar had a significantly higher nitrogen percentage in the pod wall tissue at R_4 than the low protein cultivar.

The determinate cultivar consistently had the highest nitrogen concentration in the pods, of the three cultivars under study. In addition, the nitrogen concentration in Sioux varied less with planting density than it did in either of the other two cultivars. This suggested that the pods of the determinate cultivar may have a more important role accumulating nitrogen for later remobilization to the seed than the pods of the indeterminate cultivars.

Nitrogen percentages in the pod wall tissue at R_7 were compared and the results are presented in Table 34.

Under spaced conditions, the results of the comparisons of Maple Presto and X446-2-1, showed that the nitrogen percentage in the pod wall was significantly higher in the high protein cultivar than in the low protein cultivar. Under plot conditions, the means were not significantly different.

Results for the comparison of Maple Presto with Sioux, also indicated that under spaced conditions, the nitrogen percentage in the

TABLE 34. Pairwise comparisons of percent nitrogen in the pod wall tissue at R_7, of high (H) and low (L) protein cultivars, under spaced (S) and plot (P) conditions.

Cultivar	Protein	Planting density	Nitrogen (1981)[1]	Nitrogen (1982)[2]
			%	
Maple Presto	L	S	0.67±.06	0.54±.01
X446-2-1	H	S	1.24±.05*	1.13±.07*
Maple Presto	L	P	0.50±.02	0.50±.03
X446-2-1	H	P	0.67±.04	0.54±.03
Maple Presto	L	S	0.67±.06	0.54±.01
Sioux	H	S	1.07±.04*	0.69±.04*
Maple Presto	L	P	0.50±.02	0.50±.03
Sioux	H	P	0.84±.05*	0.57±.01

[1]Mean based on 4 replicates
[2]Mean based on 6 replicates
*Means significantly different at P=.05.

pod wall of the high protein cultivar was significantly greater than in the low protein cultivar. The mean data taken of plot samples, also suggested that Sioux had a higher nitrogen percentage in the pod wall at R_7, although differences in 1982 were not significant.

Comparisons of the nitrogen percentages in the pod wall tissue at R_4 and R_7 (Tables 33 and 34), indicated clearly that the net decrease in percent nitrogen in the pod wall tissues during seed development is greater in the high protein cultivars than in the low protein cultivar and was greatest, regardless of planting density, in the determinate cultivar Sioux. This reinforces the conclusion drawn earlier, that the pods may play an important role in the high protein cultivars in accumulating nitrogen for subsequent redistribution to the developing seed. This role appeared to be of greatest importance in the determinate high protein cultivar, Sioux.

The results of comparisons of Sioux and X446-2-1 for percent nitrogen in the vegetative and pod wall tissues at R_2 and R_4 respectively are presented in Table 35. These two cultivars were compared because they were both high protein cultivars, but differed in growth habit.

Comparisons of nitrogen percentages in the vegetative tissues of the two cultivars at the onset of pod development showed no significant differences in vegetative nitrogen concentration between the cultivars. Comparisons of nitrogen percentages in the pod wall tissue at the onset of seed development showed no significant cultivar differences under spaced conditions but suggested a higher pod wall nitrogen concentration in the determinate cultivar under plot conditions when nitrogen

TABLE 35. Pairwise comparisons of percent nitrogen in the vegetative tissue at R_2 and the pod wall tissue at R_4, of indeterminate (I) and determinate (D) high protein cultivars, under spaced (S) and plot (P) conditions.

Cultivar	Growth type	Planting density	Nitrogen (1981)[1]	Nitrogen (1982)[2]
			%	
Vegetative				
X446-2-1	I	S	3.97±.05	4.27±.11
Sioux	D	S	3.94±.05	4.13±.14
X446-2-1	I	P	3.23±.10	3.60±.11
Sioux	D	P	3.33±.05	3.71±.07
Pods				
X446-2-1	I	S	2.31±.07	2.17±.07
Sioux	D	S	2.42±.06	2.42±.10
X446-2-1	I	P	1.91±.12	1.54±.09
Sioux	D	P	2.18±.16	2.20±.12*

[1]Mean based on 4 replicates
[2]Mean based on 6 replicates
*Means significantly different at P=.05.

may be limiting. The mean pod wall nitrogen percentage for Sioux was marginally higher under plot conditions in 1981 than for X446-2-1 and was significantly higher than X446-2-1 in 1982.

Comparisons of vegetative and pod wall nitrogen concentrations for these two cultivars were also made at R_7 and the results are presented in Table 36. The data for vegetative nitrogen concentration were not conclusive but suggested a higher nitrogen percentage in the vegetative tissues of X446-2-1 under spaced conditions. This reflected the presence of the dark green leaves of this cultivar at physiological maturity, noted earlier. Under plot conditions, again the data were not conclusive but suggested that Sioux retained a higher nitrogen percentage in the vegetative tissues than X446-2-1.

Examination of the data on nitrogen concentrations in the pod wall tissue at R_7 indicated that under spaced conditions, X446-2-1 retained a higher nitrogen percentage in the pod wall at physiological maturity than did X446-2-1, while under plot conditions, the cultivars were not significantly different. Calculations made from Tables 35 and 36, of the net decrease in pod wall nitrogen during seed development (R_4 through to R_7) showed that Sioux consistently had the greatest decline in percent nitrogen in the pod wall tissue over this period. This advantage is consistent over both planting densities and years.

Finally, the results of the study of intragenotypic differences under spaced and plot conditions, are presented in Table 37. It was apparent from these results, that there were significant environmental effects conditioning the expression of percent nitrogen in all of the parental cultivars. In both experimental years, percent nitrogen was

TABLE 36. Pairwise comparisons of percent nitrogen in the vegetative and pod wall tissue at R_7, of indeterminate (I) and determinate (D) high protein cultivars, under spaced (S) and plot (P) conditions.

Cultivar	Growth type	Planting density	Nitrogen (1981)[1]	Nitrogen (1982)[2]
			%	
Vegetative				
X446-2-1	I	S	2.59±.11	2.95±.19*
Sioux	D	S	2.53±.05	2.43±.07
X446-2-1	I	P	1.66±.17	0.57±.09
Sioux	D	P	2.09±.07	1.00±.13*
Pods				
X446-2-1	I	S	1.24±.05*	1.13±.07*
Sioux	D	S	1.07±.04	0.69±.04
X446-2-1	I	P	0.67±.04	0.54±.03
Sioux	D	P	0.84±.05	0.57±.01

[1]Mean based on 4 replicates
[2]Mean based on 6 replicates
*Means significantly different at P=.05.

TABLE 37. Influence of spaced (S) or plot (P) density planting on percent nitrogen in the vegetative tissue at R_2, and the pod wall tissue at R_4.

Cultivar	Planting density	Nitrogen (1981)[1]	Nitrogen (1982)[2]
		%	
Vegetative			
Maple Presto	S	3.42±.13*	3.57±.14*
	P	2.88±.02	3.07±.12
X446-2-1	S	3.97±.05*	4.27±.11*
	P	3.23±.10	3.60±.11
Sioux	S	3.94±.06*	4.13±.14*
	P	3.33±.05	3.71±.07
Pods			
Maple Presto	S	1.36±.05	1.89±.06*
	P	1.30±.19	1.25±.07
X446-2-1	S	2.31±.07*	2.01±.13*
	P	1.91±.12	1.54±.09
Sioux	S	2.42±.06	2.42±.10
	P	2.18±.16	2.20±.12

[1]Mean of 4 replicates
[2]Mean of 6 replicates
*Means significantly different at P=.05.

higher in the vegetative tissue of plants grown under spaced conditions, than of plants grown at plot density. Intragenotypic competition had a significant effect on the expression of this trait.

An examination of the data for percent nitrogen in the pod wall tissue at R_4, also presented in Table 37, suggested that the environment was a smaller source of variation in the expression of percent nitrogen in the pod wall tissue than it was in the vegetative tissue. Although in all cases, nitrogen percentage in the pod wall tissue was less under plot conditions, spacing primarily influenced the X446-2-1 cultivar where differences were significant in both experimental years. This again reflected the "evergreen" nature of this cultivar when it was grown under spaced conditions.

Similar comparisons made at R_7 (Table 38) indicated that the environmental component of the variability is consistent through the period of reproductive development. At physiological maturity, spacing resulted in all three cultivars retaining significantly greater nitrogen percentages in the vegetative tissues. Planting density did not have as pronounced an effect on the nitrogen concentration in the pod wall tissue as it influenced only nitrogen percentage in the pod wall tissue of the cultivar X446-2-1.

Throughout the period of reproductive development then, the expression of percent nitrogen in the pod wall tissue appeared to be more independent of the environment than percent nitrogen in the vegetative tissues. Because it also was positively correlated with protein in the seed, nitrogen percentage in the pod wall tissues may be a useful selection criterion when selecting for high protein lines.

TABLE 38. Influence of spaced (S) or plot (P) density planting on percent nitrogen in the vegetative and pod wall tissues at R_7.

Cultivar	Planting density	Nitrogen (1981)[1]	Nitrogen (1982)[2]
		%	
Vegetative			
Maple Presto	S	1.83±.12*	1.27+.10*
	P	1.06±.11	0.41±.04
X446-2-1	S	2.59±.11*	2.95+.19*
	P	1.66±.17	0.57±.09
Sioux	S	2.53±.05*	2.43±.07*
	P	2.09±.07	1.00±.13
Pod Wall			
Maple Presto	S	0.67±.06	0.54±.01
	P	0.50±.02	0.50±.03
X446-2-1	S	1.24±.05*	1.13±.07*
	P	0.67±.04	0.54±.03
Sioux	S	1.07±.04	0.69±.04
	P	0.84±.05	0.57±.01

[1]Mean based on 4 replicates
[2]Mean based on 6 replicates
*Means significantly different at P=.05.

4.3.3 Harvest Index and Harvest Nitrogen Index

Comparisons of harvest index and harvest nitrogen index for the three cultivars under plot conditions, were made throughout the reproductive period, from R_4 to R_8 and are presented graphically in Figure 7 and Figure 8. The 1981 data are summarized in Figure 7 while the 1982 data are summarized in Figure 8.

Both of the indices for the harvest sample are "apparent" indices as no effort was made to collect abscised leaves. The correlation coefficient for apparent harvest index, was found to be .96 (Schapaugh and Wilcox 1980) and it was assumed that the correlation coefficient for harvest nitrogen index would also be high. This assumption was based on reported nitrogen concentrations in the abscised leaves as low as 0.09 percent (Streeter 1978).

It was clear from the results of both experimental years, (Figures 7 and 8), that the rate of increase in the harvest nitrogen index for all three cultivars was greater than the rate of increase of harvest index. Harvest index ranged from .09 to .18 at R_4 for the three cultivars and increased to a range of .45 to .57 at R_8. Harvest nitrogen index, on the other hand, increased from a range of .07 to .19 at R_4 to a range of .85 to .95 at R_8. All cultivars were more efficient, therefore, at remobilizing nitrogen than dry matter.

The results of the characterization of the parental material for harvest index and harvest nitrogen index, and their relationship to seed protein concentration and nitrogen content, are presented in Tables 39 and 40. The 1981 data are presented in Table 39 while the 1982 data are presented in Table 40.

Variation was noted among the three cultivars for both harvest index and harvest nitrogen index, over both experimental years. Harvest index values ranged from .34 to .61 and .24 to .48 under spaced

Figure 7. Harvest nitrogen index (—) and harvest index (--) for Maple Presto (●), X446-2-1 (■) and Sioux (▲), during reproductive development. (1981)

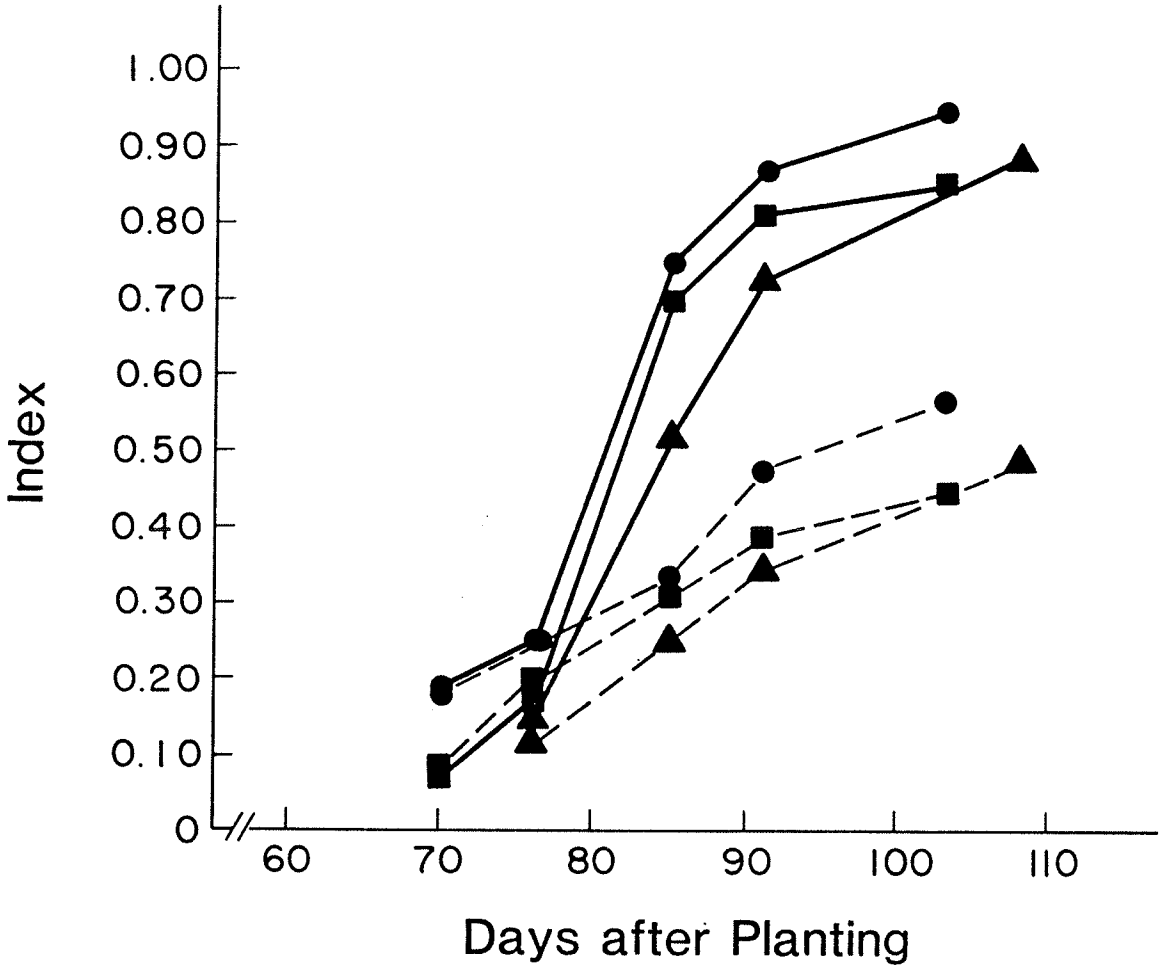

1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
Index
60
70
80
90
100
110
Days after Planting

Figure 8. Harvest nitrogen index (—) and harvest index (--) for Maple Presto (●), X446-2-1 (■) and Sioux (▲), during reproductive development. (1982)

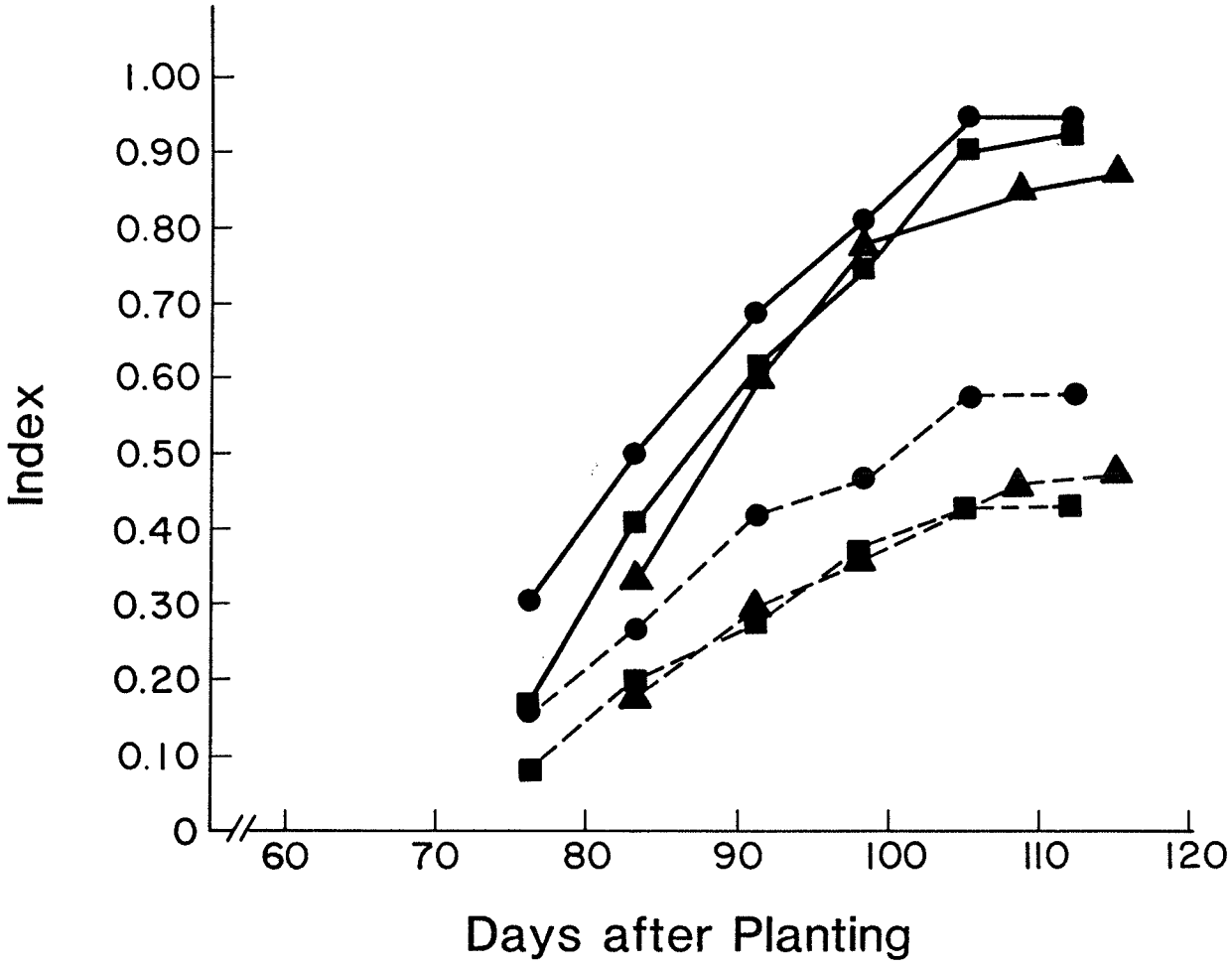

1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
Index
60
70
80
90
100
110
120
Days after Planting

TABLE 39. Characterization of parental cultivars at R_8 for seed nitrogen traits, harvest index (HI) nitrogen index (HNI) and their ratio (HNI/HI), under spaced (S) and plot (P) conditions. (1981)

Cultivar	Planting density	% Seed protein[1]	Seed TNG(g)[2]	HI	HNI	HNI/HI
Maple Presto	S	41.0	2.86	.61	.95	1.56
	P	42.2	1.50	.57	.95	1.67
X446-2-1	S	59.4	1.90	.34	.63	1.85
	P	59.3	1.66	.45	.85	1.88
Sioux	S	53.6	1.23	.43	.77	1.79
	P	48.4	.74	.49	.89	1.82

[1]Mean based on 4 replicates.
[2]Mean total nitrogen content in the seed based on 4 replicates.

TABLE 40. Characterization of parental cultivars at R_8 for seed nitrogen traits, harvest index (HI), harvest nitrogen index (HNI) and their ratio (HNI/HI), under spaced (S) and plot (P) conditions. (1982)

Cultivar	Planting density	% Seed protein[1]	Seed TNG(g)[2]	HI	HNI	HNI/HI
Maple Presto	S	41.4	3.68	.48	.85	1.77
	P	38.6	.91	.58	.95	1.64
X446-2-1	S	57.0	2.48	.24	.49	2.04
	P	50.8	.90	.43	.93	2.16
Sioux	S	50.3	2.00	.38	.72	1.89
	P	45.7	.59	.48	.88	1.83

[1]Mean based on 6 replicates.
[2]Mean total nitrogen content in the seed based on 6 replicates.

conditions, and from .45 to .57 and .43 to .58 under plot conditions, in 1981 and 1982 respectively. Harvest nitrogen index values ranged from .63 to .95 and .49 to .85 under spaced conditions and from .85 to .95 and .88 to .95 under plot conditions, in 1981 and 1982 respectively.

The similar ranking of the genotypes over years and planting densities suggested that the variation observed was in part, genetic and agreed with the results of Jeppson et al. (1978) for nodulating and non-nodulating soybean lines. An environmental component was also noted as there appeared to be a greater proportion of the vegetative dry matter and nitrogen redistributed to the seed under plot conditions than under spaced conditions. This was indicated by the higher values for harvest index and harvest nitrogen index under plot conditions in both experimental years. There was a more pronounced difference in 1982 than in 1981 however, between spaced and plot indicies for all cultivars and in particular, for X446-2-1. This was thought to be due to a more marked effect of the frost at R_6 on X446-2-1, than on either Maple Presto or Sioux.

In examining the relationship between harvest index, harvest nitrogen index and protein percentage in the seed, it was clear, that both of these indices were negatively correlated with percent protein in the seed, under both spaced and plot conditions. Harvest index and harvest nitrogen index were both highest for the low protein cultivar and lowest for the cultivar with the highest percentage of nitrogen in the seed. This relationship was consistent over both experimental years. There was no clear relationship between either harvest index or harvest nitrogen index and the total nitrogen content in the seed.

The ratio of harvest nitrogen index and harvest index, varied consistently with percent protein over both experimental years. The ratio increased with increased percent nitrogen under both spaced and plot conditions. The consistency of the relationship over planting density suggested that there was a genetic component to the variability, which could therefore be selected for. This ratio therefore, suggested that the two high protein cultivars remobilized nitrogen more efficiently than dry matter.

5. GENERAL DISCUSSION AND CONCLUSIONS

5.1 Inheritance Study

The extent of possible improvement in percent protein and percent oil in early maturing soybeans, depends on a knowledge of the amount of genetic variation present in relation to environmental variation. The genetic variability and in particular, the additive genetic variance, sets the upper limit on the progress that can be made. In turn, the strategy the breeder uses to improve these traits, is dependent on the type of gene action governing the phenotypic expression of the trait. Both the extent of genetic variance and gene action can change with the genetic material under consideration. Therefore, a knowledge of the inheritance of percent protein and percent oil in adapted material is essential to developing a strategy for improving these important economic traits in cultivars being developed for Manitoba growing conditions.

For both crosses, gene action for percent protein was primarily additive with a smaller, yet significant, dominance component for low percent protein. Epistasis may also have contributed to the expression of percent protein as an additive X dominance interaction component was significant. The importance of this interaction term was not clear however, as it was not consistently present in the simplest model over years. In addition it was consistently associated with a large standard error.

A large additive component of the genetic variance in percent protein was also noted for both crosses. This was reflected in the moderately high narrow sense heritability estimates for percent protein in both the Maple Presto/Sioux and the Maple Presto/X446-2-1 crosses.

From the genetic analyses of percent oil in the two crosses, it was clear again that the phenotypic expression of the trait was conditioned largely by additive gene action. There appeared to be partial dominance for low oil in the Maple Presto/Sioux cross in 1981, but dominance was not significant in 1982 in this cross. For the cross Maple Presto/X446-2-1, there was a partial dominance for high percent oil in 1981 but again in 1982 the analysis failed to detect a significant dominance component. Since the standard error associated with the estimate was large in both crosses in 1981, it can be assumed that the role of dominance in the expression of genes for percent oil is minor in the genetic material under investigation. Similarly, the role of epistasis was not clear as a significant additive X additive interaction component was detected for percent oil in both crosses in 1982 but not in 1981.

For both crosses, additive genetic variance was the only significant component of the heritable variance. This sole component of the genetic variance was reflected in the broad sense and narrow sense heritabilities being equal for both crosses over the two experimental years. For both crosses, the heritability estimates were moderately high.

The genetic analyses for the sum of percent protein and percent oil resulted in the conclusion that this "sum" was conditioned solely

by additive gene action. There were no significant dominance or epistatic components for either cross over the two experimental years.

The genetic variance for this trait contained both an additive or fixable component and a dominance or an unfixable component. The additive component was large relative to the dominance component in 1981 as evidenced by the moderately high narrow sense heritability estimates. In 1982, the narrow sense heritability estimates for both crosses, were lower but still large enough to predict significant improvement with selection.

The large additive component of the gene action conditioning the phenotypic expression of these important economic traits, means that improvement can be made in each of the traits by using a breeding strategy that would accumulate favorable alleles. Many of the conventional breeding methodologies would achieve this end, but recurrent selection, which allows for generations of random mating in the breeding cycles, would be most efficient. This would allow for the break-up of linkage groups that would in turn enhance the possibility of accumulation of favorable alleles. Recurrent selection has proved to be an effective technique for improving percent protein (Brim and Burton 1979) and percent oil (Burton and Brim 1981). This technique would also be particularly effective if the objective was to improve protein and oil simultaneously, as these two traits are negatively correlated. If the negative association is due to linkage, this additional opportunity for recombination could result in some break-up of linkage groups and the increased chance of accumulating favorable alleles for both traits simultaneously.

Indices for improving more than one trait at a time have been proven effective in soybeans for the simultaneous improvement of yield and protein (Brim et al. 1959). The sum of protein and oil could therefore prove an effective index for improving these two traits concurrently. The fact that the trait appeared to be solely conditioned by additive gene action, suggests that improvement in the "sum" could be achieved through conventional breeding methods that allow for the accumulation of favorable alleles. Again, recurrent selection should be a particularly effective technique because of the new gene combinations that are possible.

The predominance of additive genetic variance in the heritable portion of the observed variation is also important for improving these traits. In the absence of the resources required to accomplish the crossing necessary for recurrent selection techniques, the predominance of additive genetic variance in the traits under investigation, suggests that single seed descent would be an effective breeding strategy for improvement of percent protein, percent oil and their "sum". It has proven to be an effective technique when compared to early generation testing and the pedigree method in selection for yield (Boerma and Cooper 1975) and therefore could also be effective for quality traits where much of the genetic variance is additive.

The large additive component reflected in the moderately high narrow sense heritabilities for these traits, is also important to the breeder because it represents fixable genetic variation and suggests that significant improvement can be made in each trait with selection. In a practical sense, the narrow sense heritability represents the

percentage of the selection differential that can be gained through selection.

The estimates of heritability made through the conventional parent-progeny regression analyses and the standard unit regression analyses, represent narrow sense heritability. They however, were higher than the narrow sense estimates calculated from the components of variance. This was due to the inherent bias in early generation parent-offspring regression analyses which results from heterozygosity, and therefore dominance variance in the generation for which the estimates are made. Consequently, the presence of dominance variance results in the F_2/F_3 estimate being closer to a broad sense estimate than a narrow sense estimate. Therefore, parent-offspring regression in later generations would provide a closer approximation of the useful heritability than the F_2/F_3 regression (Hanson and Weber 1961).

Of the two regression methods used in this study, the standard unit method (correlation coefficient) has two advantages over the conventional method. It sets a ceiling for heritability at 100 percent whereas the conventional method has no such ceiling. Until this upper limit is set, it is difficult to interpret a value as high, medium or low. Secondly, it has been shown to predict more accurately, the actual gain from selection (Frey and Horner 1957) which, in fact, is the practical use of heritability estimates. In the absence of factors influencing the range of phenotypic variability however, the conventional regression technique adequately estimates the narrow sense heritability.

In conclusion, the regression heritability estimates indicated that much of the variation is useful, fixable genetic variation and predicted that successful gains for selection could be made in breeding programs aimed at increasing percent protein, percent oil or their "sum" in early maturing soybeans.

5.2 Physiology

Improvement in the protein percentage in the seed of early maturing soybeans while maintaining or improving yield levels requires a better understanding of the underlying factors causing the negative genetic relationship between these two traits. This genetic correlation between seed protein percentage and yield, probably results from more basic genetic associations between nitrogen and carbon supply.

From a physiological viewpoint, one of the keys to increasing the percentage of protein in the seed of early maturing soybeans must be to increase the accumulation of nitrogen in the seed. The two components of this process would appear to be to increase the whole plant accumulation of nitrogen and/or to increase the redistribution of accumulated nitrogen from the vegetative and pod wall tissues to the developing seed. The extent of success in using either of these components as a selection criterion in the improvement of protein percentage is dependent on a positive relationship between percent protein and accumulated nitrogen, nitrogen redistribution and/or complementary traits that could be selected for such as sustained nitrogen fixation. The extent of possible improvement is also dependent on the nature and extent of genetic variability for each of the traits.

This preliminary study attempted to identify cultivar differences in nitrogen accumulation and remobilization that are genetically related to seed protein in early maturing soybeans and therefore potentially useful in a breeding program designed to improve seed protein percentage.

Cultivar comparisons were made of accumulated nitrogen in the vegetative tissues by the onset of pod development (R_2) with the assumption that nitrogen accumulated by this developmental stage, was available for remobilization to the developing reproductive tissues. Although the data were confounded by dry matter differences and variability that resulted from sampling technique and storm damage, comparisons of high and low protein cultivars tended to show cultivar differences for vegetative nitrogen accumulation. The high protein cultivar tended to have accumulated more nitrogen per unit dry matter of vegetative tissue by R_2 than the low protein cultivar. The data indicated that this trend was consistent over both planting densities and years and therefore suggested that it was under genetic control. This is in agreement with work in corn (Hoener and DeTurk 1938) and soybeans (Carter et al. 1982, Israel 1981).

Greater nitrogen accumulation in the vegetative tissues by R_2 however, was not consistently coupled with a greater amount of nitrogen remobilized to the developing seed as both high protein cultivars retained a greater amount of nitrogen in the vegetative tissues at R_7 than the low protein cultivar. The indeterminate high protein cultivar X446-2-1 however, did accumulate and remobilize more nitrogen under plot conditions than did the low protein cultivar Maple Presto.

Cultivar comparisons of the amount of nitrogen accumulated in a sub-sample of the pod tissue by the onset of seed development (R_4) again showed cultivar differences that were consistent over planting densities and years and therefore under genetic control. Both high protein cultivars accumulated more nitrogen per unit dry matter with differences between the determinate cultivar, Sioux and the indeterminate cultivar, Maple Presto being significant. The comparison of nitrogen levels in the pod wall tissue at R_2 and R_7, indicated that the determinate cultivar both accumulated a greater amount of nitrogen in the pod wall and remobilized a greater amount of pod wall nitrogen to the developing seed. The consistency of these results indicated the importance of the pods in the high protein determinate cultivar as a source of seed nitrogen.

The data for accumulated nitrogen was confounded by dry matter differences among the three cultivars and therefore comparison of percent nitrogen in the vegetative and pod wall tissues were made. The results reinforced the conclusions drawn from the analyses of accumulated nitrogen.

Both high protein cultivars had a significantly higher nitrogen concentration in the vegetative tissues at R_2 than the low protein cultivar. This was consistent over both planting densities and years. At R_7 however, the high protein cultivars also retained a higher nitrogen concentration in the vegetative tissues and therefore the net decline in vegetative nitrogen concentration was not larger for the high protein cultivars than for the low protein cultivar.

At R_4, both high protein cultivars had a greater nitrogen concentration in the pod wall tissue than the low protein cultivar and although they retained a higher nitrogen concentration in the pod wall at R_7, both high protein cultivars had a greater net decline in percent nitrogen in this tissue during seed development. Since it was assumed that nitrogen lost from the pod wall tissue was remobilized to the developing seed, these results again indicated the importance of the pods as a source of seed nitrogen in the high protein cultivars. The consistency of the results, in particular for the determinate cultivar Sioux, suggested that genetic and therefore heritable variation for percent nitrogen in the pod wall tissue was present which could be selected for in a program designed to increase protein percentage in the seed.

Differential strategies were also apparent for remobilizing nitrogen from the pod wall tissue to the developing seed. Sioux, the high protein determinate cultivar accumulated a higher percentage of nitrogen in the pod wall tissue by the onset of seed development than the other two cultivars and the subsequent rate of decrease in percent nitrogen in the pod wall was also significantly greater than for the two indeterminate cultivars. This was interpreted as a significant difference in the rate of remobilization of nitrogen from the pod wall tissue to the developing seed. No significant differences in the rate of decrease in nitrogen content in the vegetative tissue were detected for any of the cultivars although genetic variability for nitrogen remobilization from the vegetative tissues has been noted in soybeans (Israel 1981).

When dealing with a trait measured as a concentration, such as percent nitrogen, it is always difficult to know if a change in the concentration of the element is actually related to that element, or to some other cell component. This is of course due to the fact that the concentration of, in this case nitrogen, is a function, not only of the nitrogen but of the amount of the other constituents present, in particular the carbohydrates. It was assumed here however, that the differences detected in percent nitrogen, were due, at least in part, to a differential ability of the cultivars compared, to accumulate and redistribute nitrogen.

The validity of this assumption was in part upheld by the results of comparisons of the proportion of seed nitrogen derived from remobilized nitrogen for the three cultivars. Sioux, accumulated a higher percentage of nitrogen in the pod wall tissue and remobilized it at a faster rate, than the indeterminate cultivars. The proportion of seed nitrogen that was derived from remobilized nitrogen from the pods, indicated that the pod wall of the high protein cultivar, Sioux, provided three times more nitrogen to the developing seed, than was provided by the pod wall of either of the indeterminate cultivars. These data suggested, therefore, that the decline in percent nitrogen in the pod wall represented, at least in part, the remobilization of nitrogen. The positive correlation of these two traits with protein percentage in the seed also suggested that selection for a high percentage of nitrogen in the pod wall at the onset of seed development and a high rate of remobilization to the developing seed, could enhance the accumulation of protein in the seed.

A range of genetic variability was also noted for the percentage of nitrogen in the seed that was derived from nitrogen remobilized from the vegetative tissues (RN/SN), but it was not consistently related to either the amount of nitrogen accumulated in the vegetative tissues or to the percentage of protein in the seed. Sioux, the high protein, determinate cultivar, accumulated more nitrogen per unit mass in the vegetative tissue than the low protein cultivar. It also relied heavily on the remobilization of tissue nitrogen in order to maintain high protein levels in the seed. X446-2-1, the high protein, indeterminate cultivar, also accumulated more nitrogen in the vegetative and pod wall tissues prior to seed development than the low protein cultivar, but only remobilized a small portion of this nitrogen to the developing seed. This implied a different strategy for accumulating nitrogen in the seed. It suggested that X446-2-1 had sustained nitrogen assimilation during reproductive development under spaced conditions, in order to maintain high percent protein in the seed. However, seasonal nodule activity would have to be monitored to conclusively determine this and although an attempt was made to measure this, the acetylene reduction technology available did not generate data that could be used.

A wide range of variation for harvest index and harvest nitrogen index was also observed for the three cultivars. The similar ranking of the genotypes for these indices over the two experimental years, suggested that at least part of this variability was genetic and therefore could be selected for. Although independently, the indices were negatively related to percent protein, the ratio of harvest nitrogen

index to harvest index increased with increased percent protein in the seed. This relationship was consistent over years and planting densities and therefore indicated that much of the variability for this ratio was genetic. Since the data were based on single plants, this consistency also suggested that selection for this trait could be effective on a single plant basis.

Rao et al. (1977), working on wheat, suggested that selection of genotypes with a high nitrogen-use efficiency was not yet possible on the basis of any single physiological trait. Rather, he suggested, selection should be based on two or more components involved in nitrogen utilization. The results of this study suggest that the ratio of harvest nitrogen index and harvest index, might serve as one component for plant breeders to use in the improvement of nitrogen-use efficiency, as it is positively related to percent protein and the variability appears to be, at least in part, genetic.

In summary, a range of genetic variability appears to exist for the accumulation and redistribution of nitrogen to the developing grain, although in this study, interpretation was often confounded by dry matter differences and poor precision in the estimates. Identifying the variable strategies that exist and subsequently determining their heritability and the nature of the gene action controlling them, is necessary before they can be incorporated into a breeding program. Once this preliminary genetic work is completed, it should be possible to use conventional breeding methodologies to select for complementary strategies for the accumulation of nitrogen in the seed, within a single cultivar. For example, it could be possible to breed for

the capability for sustained nitrogen assimilation during the reproductive period, that appeared to be present in the indeterminate cultivars in this study, in combination with the high percentage of nitrogen in the vegetative and pod wall tissues and the increased capability to remobilize this nitrogen to the seed, that was evident in the determinate cultivar in this study. These complementary strategies should enhance nitrogen accumulation in the seed and subsequently, percent protein in the seed.

The success of breeding for nitrogen accumulation has already been noted in soybeans by Carter et al. (1982). Recurrent selection techniques were used successfully to breed for increased nitrogen accumulation in the vegetative plant parts. The increased nitrogen in the vegetative tissue that resulted from this breeding strategy, was the apparent cause of increased protein in the seed.

Breeding for the physiological components of percent protein therefore, should prove to be a useful technique for enhancing protein concentration in the seed of early maturing soybeans.

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APPENDIX

APPENDIX TABLE 1. Coefficients of the components of means (Mather and Jinks 1977).

Generation	m	[d]	[h]	[i]	[j]	[l]
P_1	1	1	0	1	0	0
P_2	1	-1	0	1	0	0
F_1	1	0	1	0	0	1
F_2	1	0	.50	0	0	.250
F_3	1	0	.25	0	0	.063
B_1	1	.50	.50	.25	.250	.250
B_2	1	-.50	.50	.25	-.250	.250
$B_{1(s)}$	1	.50	.25	.25	.125	.063
$B_{2(s)}$	1	-.50	.25	.25	-.125	.063

APPENDIX TABLE 2. Coefficients of the components of variance (Mather and Jinks 1977).

Generation	Component			
	D	H	F	E
P_1	0	0	0	1
P_2	0	0	0	1
F_1	0	0	0	1
F_2	.500	.250	0	1
F_3	.750	.188	0	1
B_1	.250	.250	-.500	1
B_2	.250	.250	.500	1
$B_{1(s)}$	.500	.188	-.250	1
$B_{2(s)}$	.500	.188	.250	1

APPENDIX TABLE 3. F_2 parental values and F_3 family means for parent-progeny regression analyses for percent protein, percent oil and their "sum" for the cross Maple Presto/Sioux.

% Protein		% Oil		Sum	
F_2	F_3[1]	F_2	F_3	F_2	F_3
44.4	44.7	18.3	14.9	62.7	59.6
44.6	42.9	18.6	16.4	63.2	59.3
47.2	46.5	17.1	15.4	64.3	61.9
44.3	45.0	17.9	16.3	62.2	61.3
44.5	42.8	17.5	16.5	62.0	59.2
47.6	46.3	17.8	14.6	65.4	60.9
45.6	42.8	17.5	16.1	63.1	58.9
45.1	46.0	18.3	14.4	63.4	60.4
46.7	46.3	16.1	14.3	62.8	61.6
45.6	45.2	16.8	14.2	62.4	59.4
46.9	46.2	16.2	14.4	63.1	60.6
45.5	44.0	18.0	16.3	63.5	60.3
46.9	45.8	17.0	15.0	63.9	60.8
44.3	42.7	18.2	17.1	62.5	59.9
48.3	45.8	16.1	15.7	64.4	61.5
48.2	46.0	17.8	15.8	66.0	61.8
48.7	48.2	17.1	15.0	65.8	63.2
44.3	43.9	18.8	15.4	63.1	59.3
46.2	47.6	18.0	14.3	64.2	62.0
48.6	48.0	16.8	13.8	65.4	61.8
45.8	45.7	17.9	15.5	63.7	61.2
44.4	46.3	17.5	14.0	61.9	60.3
45.1	44.6	16.6	15.4	61.7	59.9
45.3	44.5	17.2	15.3	62.5	59.8
46.7	45.2	18.3	15.6	65.0	60.8
43.6	42.6	18.6	15.5	62.2	58.0
47.8	46.9	16.8	14.6	64.6	61.5
43.9	44.0	18.5	15.9	62.4	59.9
45.8	45.2	18.2	15.0	64.0	60.2
46.6	47.0	17.7	14.4	64.3	61.4

[1] F_3 family means based on 5 plants per family.

APPENDIX TABLE 4. F_2 parental values and F_3 family means for parent-progeny regression analyses for percent protein, percent oil and their "sum" for the cross Maple Presto/X446-2-1.

% Protein		% Oil		Sum	
F_2	F_3[1]	F_2	F_3	F_2	F_3
48.9	48.7	17.5	15.2	66.4	63.8
47.7	47.8	16.7	14.9	64.4	62.7
45.8	45.3	17.8	15.5	63.6	60.9
44.6	40.8	17.5	17.2	62.1	58.0
47.2	44.7	18.1	16.6	65.3	61.3
48.7	49.0	16.2	14.7	64.9	63.7
49.0	46.9	16.1	15.0	65.1	61.9
45.3	45.8	18.5	16.0	63.8	61.9
47.5	46.7	16.0	15.5	63.5	62.1
48.0	47.7	17.6	15.1	65.6	62.8
47.1	45.8	17.5	16.5	64.6	62.3
50.1	49.8	15.7	14.2	65.8	64.0
45.7	44.3	17.7	16.3	63.4	60.6
45.1	42.9	19.1	17.9	64.2	60.8
47.1	47.0	16.7	14.9	63.8	61.9
48.4	48.0	17.3	15.2	65.7	63.2
49.6	48.2	16.3	15.1	65.9	63.4
49.9	49.2	16.2	14.9	66.1	64.1
46.9	43.8	17.0	17.7	63.9	61.6
48.3	46.2	16.6	15.8	64.9	62.0
48.5	44.8	17.0	16.2	65.5	61.0
47.8	47.7	17.1	15.2	64.9	62.9
49.4	50.4	15.6	14.1	65.0	64.5
45.8	45.6	18.0	15.8	63.8	61.4
49.2	48.8	16.2	14.8	65.4	63.6
46.6	44.8	17.7	17.2	64.3	62.0
46.3	43.4	17.7	16.5	64.0	59.9
47.7	45.7	16.5	15.9	64.2	61.6
47.4	48.8	16.8	14.9	64.2	63.7
48.7	46.5	17.5	16.0	66.2	62.5

[1] F_3 family means based on 5 plants per family.