

# Soybean Nitrogen Acquisition and Utilization<sup>1</sup>

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## ABSTRACT

Because of its high seed protein concentration soybean has a huge nitrogen demand, about 350 lbs/A for a 65-bu/A crop. The nitrogen is supplied by two acquisition systems that operate in tandem. In early season the plant uses soil nitrate; later its nitrogen is acquired largely through N<sub>2</sub> fixation. On highly fertile, Midwestern soils nitrate utilization peaks at about R3 and then declines sharply. With heavy nitrogen fertilization peak nitrate utilization can be extended through R5, but ordinarily there is no yield benefit from heavy nitrogen fertilization because nitrate interferes with infection by *Bradyrhizobium* and so delays and suppresses nodule formation. On soils low in nitrogen, a low rate of N fertilization at seedling can benefit yield through production of a vigorous vegetative plant capable of supporting a large N<sub>2</sub> fixing association. Usually, the plant does not obtain significant benefit from N<sub>2</sub> fixation for several weeks, so there is need for some soil nitrogen to stimulate early season growth. For most Midwestern soils this need is accommodated by annual mineralization and carry-over. N<sub>2</sub> fixation usually peaks at or slightly after R5 and then declines rapidly through seed filling. This decline is primarily a function of nodule senescence and is not reversible. Protein synthesis in the seed depends upon mobilization of vegetative nitrogen for half or more of the seed's needs. Soybean varieties don't differ much in the proportion of seed-N derived from mobilization, or in mobilization efficiency. There is a direct relationship between vegetative nitrogen acquired by R5 and final seed yield.

## INTRODUCTION

Soybean (*Glycine max* L. Merr.) has a very large nitrogen requirement. A 65-bu/A crop of 38% protein seed requires 240 lbs of N/A solely to satisfy seed needs. The nitrogen harvest index (NHI) of soybean is about 0.7, making the total nitrogen requirement about 350 lbs/A.

Up to 50% of the total requirement may be supplied from N<sub>2</sub>-fixation (Harper, 1987), leading to 175 lbs/A needing to be supplied from mineral sources—either fertilizer, residual-N from a previous crop, or from mineralization of soil organic matter. It is generally conceded that the capacity for N<sub>2</sub>-fixation is greater than often realized on Midwestern soils simply because those soils' high mineral-N content delays and suppresses nodulation. Though N<sub>2</sub>-fixation capacity may be higher than often realized, it is also clear that a soybean crop relying solely on N<sub>2</sub>-fixation never could attain yields of 65 bu/A, or perhaps even 50 bu/A. That is because soybean doesn't form an effective N<sub>2</sub>-fixation system for several weeks after emergence.

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## SEASONAL PROFILES OF NITROGEN ACQUISITION

### *Nitrate Utilization*

Soybean has dual nitrogen acquisition systems that operate in tandem. In the first half of the season soybean relies primarily on nitrate-N, and in the second half on N<sub>2</sub>-fixation (Fig. 1). The process of nitrate utilization is regulated by the activity of one enzyme, nitrate reductase, which is present in the cytoplasm of leaf mesophyll cells. Nitrate reductase converts nitrate to nitrite in the cytoplasm. Then, in a series of steps driven directly by photosynthetic energy, nitrite is reduced in the chloroplast to ammonium which is incorporated into amino acids. Sulfate is reduced and incorporated into amino-N compounds in the same manner albeit with a different complement of enzymes.

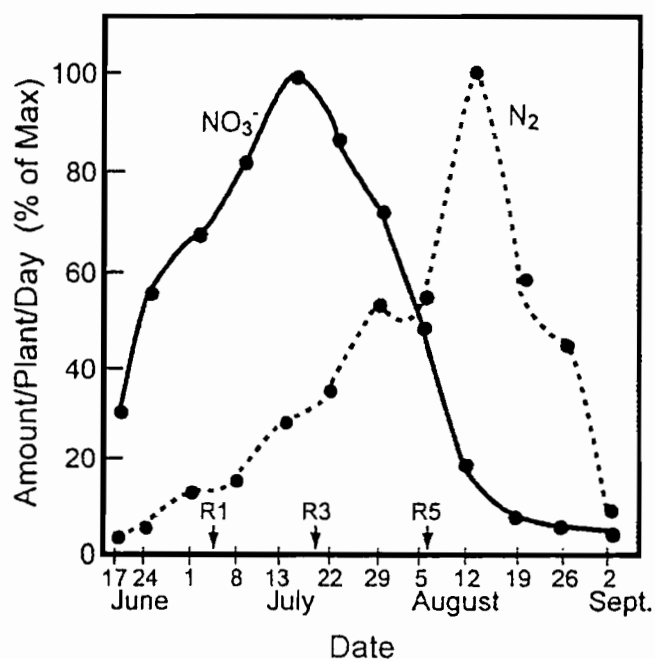


Figure 1. Seasonal patterns of nitrate and N<sub>2</sub> utilization by soybean as estimated from leaf nitrate reductase activity for nitrate, and by acetylene reducing activity of nitrogenase using intact root systems for N<sub>2</sub>. (Adapted from Harper and Hageman, 1972).

The reduction of nitrate to nitrite is rate-limiting, and therefore the major regulatory step in mineral-N utilization. This regulation is performed largely through the amount of nitrate reductase, which is continually being synthesized and degraded. Except for a small amount of the enzyme that functions all the time and is called 'constitutive nitrate reductase,' the enzyme virtually disappears at night because sunlight is required for its synthesis. Nitrate reductase is a substrate inducible enzyme, meaning that its synthesis is strongly related to the availability of nitrate to the leaf. In leaves of otherwise high physiological capacity, the amount of the enzyme and thus the capacity for reduction, is strongly related to nitrate supply. Enzyme synthesis is very rapid in young leaves with a strong protein metabolism system, and peak capacity for nitrate reduction in a leaf is attained at about the same time as the leaf reaches full expansion. Thereafter it declines due to a decline in transcription of the enzyme.

In fertile, but nonfertilized, Midwestern soils soybean capacity for nitrate reduction peaks around R2 to R3 (early to mid-podding) and then declines sharply (Fig. 1). The decline is probably a reflection of low nitrate supply, the crop having used most of the available soil mineral-N by that time. If fertilizer-N is supplied at seeding or in early growth the peak capacity for nitrate utilization can be increased and the duration extended. Because new leaves continue to be produced by indeterminate soybean up to R5 (beginning of rapid seed fill) and leaf expansion does not cease for another week to 10 days one can envision that, given an adequate supply of mineral-N, nitrate reductase activity potentially could be extended to R5 or perhaps even a few days beyond. After that nitrate utilization would decline because of declining ability to synthesize nitrate reductase due to leaf aging. The peak capacity for and duration of (up to R5 at least) mineral-N utilization is essentially completely dependent upon nitrate supply.

### *N<sub>2</sub>-Fixation*

Under the best of circumstances—an adequate population of *Bradyrhizobia* and low mineral-N content of the soil—the plant does not obtain significant quantities of nitrogen from N<sub>2</sub>-fixation much before R1 (onset of flowering), and does not attain full capacity much before R5 (Fig. 1). The presence of free nitrate in the soil interferes with the infection process, and on soils of even moderate mineral-N content nodulation may be delayed and total nodule mass reduced resulting in a decrease in both peak and total seasonal capacities (compare hydroponic culture and soil check in Fig. 2). If large amounts of nitrogen fertilizer are applied to the crop at time of seeding, N<sub>2</sub> fixing capacity may be virtually wiped out (200 lbs/A in Fig. 2).

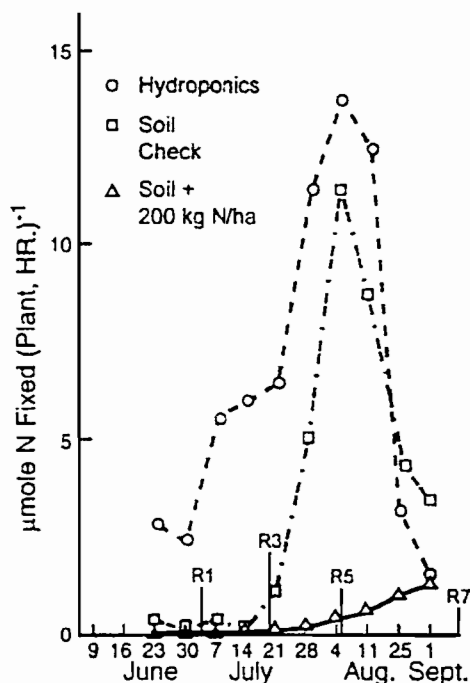


Figure 2. Seasonal profiles of N<sub>2</sub>-fixation for soybean grown outdoors in hydroponic gravel culture with 1.5 mM nitrate, on an unfertilized soil following corn (soil check), and on soil following corn and fertilized with 200 kg N/ha. (From Harper, 1974).

The reason that most Midwestern soybean crops fail to respond to “starter-N” lies in that the added mineral-N suppresses nodulation. Yield is not reduced because nitrate merely supplants  $N_2$  as the N source. Clearly, there is need for some mineral-N early in the season to stimulate growth prior to formation of an effective  $N_2$  fixing association. On a soil of low mineral-N content supplementation with some fertilizer nitrogen results in production of a larger nodule mass and greater  $N_2$ -fixation than would otherwise occur. The exact amount of mineral-N needed to give a soybean crop its best start would differ for each soil type and previous cropping practice. It is sufficient to say that most Midwestern soils generally have more than adequate N from mineralization and carry-over to get the soybean crop off to a good start.

$N_2$  fixing capacity begins to decline rapidly a week or so after R5 (Fig. 1). Thus, at the time of peak nitrogen demand for protein synthesis in seeds the nitrogen supplying mechanism loses capacity rapidly. It has been popular to ascribe this loss of  $N_2$ -fixation capacity to competition for photosynthetic assimilates. The notion is that as seed filling progresses the increasing seed demand for carbon leaves progressively less for roots and to maintain nitrogen fixing capacity. This may account for part of the decline, but it seems doubtful that it is the sole, or perhaps even the major factor involved.

A more likely explanation for the rapid decline in  $N_2$ -fixing capacity during seed filling is simply natural senescence of the nodules. We now know that plant organs have a natural peak of activity followed by a senescence phase, which occurs over time and is characterized by decline in physiological activity. Though senescence may be slowed to a certain extent, it is not reversible. That is, irrespective of energy and substrate carbon supply to nodules the capacity for  $N_2$ -fixation declines principally due to genetically directed nodule senescence.

Where then does the seed obtain its nitrogen needed for such large quantities of protein? Soybean is the highest seed protein yielding crop. And, as previously mentioned, in a 65 bu-crop about 240 lbs of nitrogen are removed per acre with seed.

### MOBILIZATION OF VEGETATIVE NITROGEN

Well over half of the nitrogen needs for protein synthesis are satisfied by mobilization of vegetative nitrogen (Table 1). Most of the nitrogen is mobilized from leaves, with pod tissue acting as a secondary source. Stems contribute some, and what little evidence is available suggests that roots contribute little. Leaves of soybean may contain up to 6% nitrogen, about 38% protein.

Although lower, early-produced leaves on soybean begin to senesce and yield up their nitrogen not long after attaining full expansion, most of this nitrogen is utilized in the production of upper leaves and other nonseed tissues. So, the effect on a whole canopy basis is that net mobilization of nitrogen from vegetation organs begins at about 7 to 10 days after R5, and the loss continues gradually and concomitant with accumulation of nitrogen in seeds right up until R7 (physiological maturity). When leaves abscise they still contain about 1.3 to 1.5% nitrogen (Hanway, 1971; Loberg et al., 1984).

In parallel with the decline of nitrogen in leaves there is decline in photosynthetic capability of the canopy. This occurs, of course, because most of nitrogen in leaves is in physiologically active proteins—rubisco, nitrate reductase, etc. And, as the leaves senesce these

Table 1. Mobilization characteristics of six diverse soybean lines.

Soybean line	Seed Protein	Protein Yield	Veg-N		Seed-N from Mob
			R5	NME	
	%	g/m <sup>2</sup>	g/m <sup>2</sup>	%	%
Century-84	42.1	116	16.4	68.3	56.4
Newton	39.2	99	13.4	63.2	49.4
Expt-1	46.7	109	15.7	66.9	56.8
Kenwood	41.2	117	15.3	66.5	50.3
IA 2010	45.0	117	17.2	67.0	57.7
Exptl-2	41.2	121	16.0	67.1	58.7

Veg-N R5. Nitrogen content of aboveground vegetation at R5

NME. Nitrogen mobilization efficiency. The proportion of vegetative nitrogen that is mobilized.

Seed-N from Mob. Proportion of seed-N potentially derived from mobilization of vegetative-N.

proteins are disassembled. It has not been possible to reverse or retard senescence. Hormone therapy has not worked. Application of foliar nitrogen does not retard senescence or nitrogen mobilization from the leaf (Sesay and Shibles, 1980). Moreover, depodding, either wholly or partially, fails to stop the decline of photosynthesis. The nitrogen still is lost from active proteins and merely accumulates in the leaf in inactive proteins—vegetative storage protein and/or an inactive form of rubisco (Crafts-Brandner et al., 1991; Lauer and Shibles, 1987; Staswick, 1994). This leads to the conclusion that senescence is a genetically directed program with limited response to sink-source relationships. Chlorophyll levels in leaves remain high in depodded plants giving the visual impression that physiological activity continues, and indeed it may be extended for a few days, but in this circumstance chlorophyll is not a general indicator of photosynthetic capability.

Soybean genotypes don't differ very much in the proportion of seed nitrogen derived from mobilization or mobilization efficiency, even among genotypes with striking differences in seed protein content (Table 1). Although varieties and genotypes differ in the absolute amount of nitrogen available for seed protein, these differences are primarily related to amount of leafage and nitrogen concentration in the leafage, rather than to differences in mobilization efficiency.

### MOBILIZATION AND SEED YIELD

Over a wide range in yielding capacity, from 30-60 bu/A, seed yield is directly related to amount of nitrogen accumulated in leaf tissue by R5 (Fig. 3). That is, seed yield is directly related to amount of potentially mobilizable nitrogen. The coefficient of determination suggests that about 37% of the yield variation among the 64 genotypes investigated by Shibles and

Sundberg (1998) could be accounted for by differences in leaf nitrogen content at R5. This study, along with Imsande's hydroponic studies (1989, 1992) are strongly suggestive that, in terms of yielding capacity, the soybean is a nitrogen-limited plant. Moreover, it now must be clearly recognized that there is no one stage in the life of the soybean more important than another in nitrogen metabolism. Clearly, the acquisition of nitrogen prior to the onset of seed growth is just as important to yielding potential as is the acquisition of nitrogen by N<sub>2</sub>-fixation after the onset of seed growth. To maximize yield of high protein varieties it will be necessary to maximize nitrogen acquisition by both the nitrate reduction and N<sub>2</sub>-fixation mechanisms.

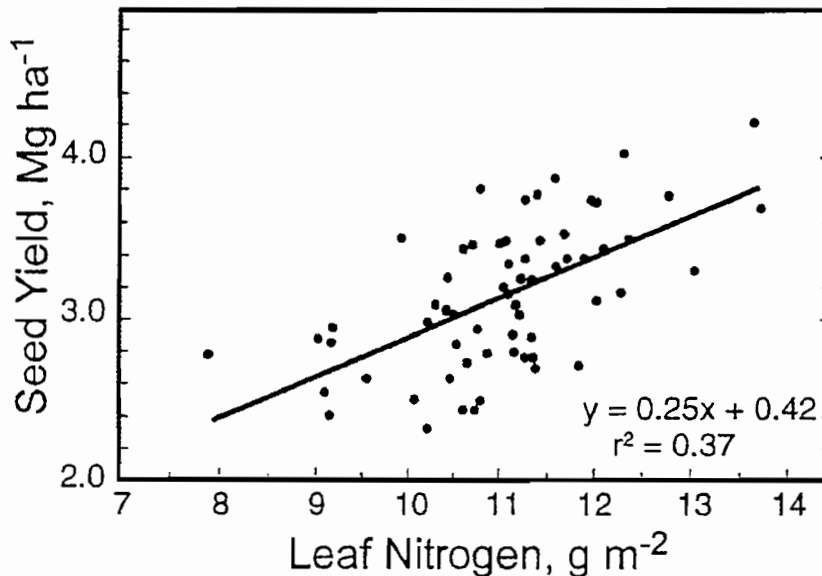


Figure 3. Seed yield of 64 soybean lines as a function of their leaf nitrogen accumulated by R5, the beginning of rapid seed growth. The 64 lines consisted of ancestral lines and varieties released for production in the Group II area of the Midwest from the 1950s through the 1980s. (From Shibles and Sundberg, 1998).

The irony of soybean nitrogen metabolism is that, although the plant seems to be nitrogen-limited, it does not consistently respond to supplemental nitrogen input. Except on nitrogen-deficient soil, applications of fertilizer nitrogen at seeding delay and depress nodulation. Applications of mineral nitrogen at R5 or later have not given consistent responses because at that stage the plant doesn't have a strong capacity for reducing nitrate, and the plant has responded inconsistently to foliar fertilization with urea. Recently, there have been reports in the popular press of yield responses to nitrogen fertilization at the podding stage, R2 to R3. Though we have no information on the consistency of those responses reported in the popular press, Wesley et al. (1998) recently have reported yield responses to 20 lbs of nitrogen applied at R3 prior to irrigation on soils low in nitrogen. Application of this small amount on a soil low in nitrate probably stimulated the nitrate reducing mechanism of the plant at its time of maximum potential response without interfering significantly with the development of nodules. It remains to be seen whether such a fertilization practice would give a consistent response on soils with higher nitrogen mineralizing capacity.

## REFERENCES

- Crafts-Brandner, S.J., Salvucci, M.E. and Egli, D.B. 1991. Fruit removal in soybean induces the formation of an insoluble form of ribulose-1, 5-bisphosphate carboxylase oxygenase in leaf extracts. *Planta (Berl)* 183:300-306.
- Hanway, J.J. and C.R. Webber. 1971. N, P, and K percentages in soybean, [*Glycine max* (L.) Merrill] plant parts. *Agron. J.* 63:286-290.
- Harper J.E. 1987. Nitrogen metabolism. In J.R. Wilcox (ed.) *Soybean: improvement, production and uses*, 2<sup>nd</sup> edition. *Agronomy* 16:498-533.
- Harper, J.E. and R.H. Hageman. 1972. Canopy and seasonal profiles of nitrate reductase in soybean (*Glycine max* (L.) Merr.). *Plant Physiol.* 62:662-664.
- Imsande, J. 1989. Rapid dinitrogen fixation during soybean pod fill enhances net photosynthetic output and seed yield: A new perspective. *Agron. J.* 81:549-556.
- Imsande, J. 1992. Agronomic characteristics that identify high yield, high protein soybean genotypes. *Agron. J.* 84:409-414.
- Lauer, M.J. and R. Shibles. 1987. Soybean leaf photosynthetic response to changing sink demand. *Crop Sci.* 27:1197-1201.
- Loberg, G.L. R. Shibles, D.E. Green, and J.J. Hanway. 1984. Nutrient mobilization and yield of soybean cultivars. *J. Plant Nutr.* 7:1311-1327.
- Sesay, A. and R. Shibles. 1980. Mineral depletion and leaf senescence in soya bean as influenced by foliar nutrient application during seed filling. *Ann. Bot.* 45:47-55.
- Shibles, R. and D. Sundberg. 1998. Relation of leaf nitrogen content and other traits with seed yield of soybean. *Plant Prod. Sci* 1:3-7.
- Staswick, P.E. 1994. Storage proteins of vegetative plant tissue. *Annu. Rev. Plant Physiol. Plant Molec. Biol.* 45:303-322.
- Wesley, T.L., R.E. Lomond, V.L. Martin, and S.R. Duncan. 1998. Effects of late-season nitrogen fertilizer on irrigated soybean yield and composition. *J. Prod. Agric.* 11:331-336.

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