Effect of Multiple Factor Source-Sink Manipulation on Nitrogen and Carbon Assimilation by Soybean

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ABSTRACT

The objectives of this study were to determine the effect of light enhancement and hastened reproductive development on nitrogen and dry matter accumulation by field-grown soybean (Glycine max [L.] Merr.). The impacts of photosynthetic supply and reproductive development on change in the season-long profiles of in vitro leaf nitrate reductase (NR) activity and root node acetylene reduction (AR) activity were evaluated.

Light enhancement resulted in significant increases in dry matter accumulation, root nodule fresh weight and AR activity. Seed yield was increased in both light enhanced treatments in 1978 and in one in 1979.

Hastened flowering and seed development was accomplished through photoperiod manipulation within a single genotype. Seasonal decline in leaf NR activity was most rapid in plants entering reproductive development early. An early increase in root nodule fresh weight and AR activity was also observed in response to this treatment and was followed similarly by early decline.

The addition of high levels of soil-applied nitrogen increased leaf NR activity and delayed late season decline in NR activity for both control and early reproductive plants. Nitrate supply was therefore implicated as limiting to leaf NR activity during the decline associated with flowering and early seed development. A limited additional increase in leaf NR activity was observed in response to light enhancement plus soil-applied nitrogen. As no significant increase in leaf NR activity was observed in response to light enhancement alone, leaf nitrate supply was further implicated as more limiting to leaf NR activity than was photosynthetic supply during flowering and early seed development.

The uptake of soil nitrate and its reduction via leaf NR2 activity has been cited as the primary means of N assimilation during vegetative growth in soybean (9). During subsequent development, symbiotic N2 fixation via root nodule nitrogenase activity increases to become the dominant source of nitrogen during podfill. Although the relative contributions of these two N sources vary greatly with the N content of the rooting medium (7, 12), both are energy expensive and reliant upon available photosynthetic energy. On a seasonal basis, canopy photosynthetic rates for field-grown soybean have been reported to be at maximum during flowering and early podfill, followed by sharp decline during late podfill (4, 10, 19). Declines in the reduction of nitrate to nitrite via leaf NR activity and maximum rates of root nodule nitrogenase activity have also been associated with flowering and early podfill, respectively (7, 14, 15, 23). While comparisons among genotypes of varying maturity have been made, no attempt has been made to alter the timing of reproductive development within a single genotype and to measure the resultant impact of this change on these nitrogen assimilatory enzyme systems.

The addition of supplemental light to field-grown soybean has previously been reported to increase dry weight accumulation and seed yield (13). Photosynthetic enhancement through CO2 enrichment increased the assimilation of nitrogen by soybean in previous work (5, 6, 8). Partial or complete pod removal has been reported to delay visible leaf and root nodule senescence and result in enhanced total N assimilation by soybean (15). However, subsequent reports have indicated that while visible senescence (leaf yellowing and loss) was delayed in depodded and male sterile soybean, functional senescence (decline in CER) was not delayed and may be enhanced in male sterile and depodded plants relative to seed-bearing controls (1, 10, 22, 25, 26). Consistent with these reports, Crafts-Brandner et al. (2, 3) reported no delay in leaf or nodule functional senescence and no increase in N assimilation by soybean in response to pod removal.

The objectives defined for this study were to determine the effect of light enhancement and hastened reproductive development on nitrogen and dry matter accumulation by field-grown soybean. The impacts of increased photosynthetic supply and altered reproductive timing on the season-long profiles of leaf NR and root nodule AR activity were evaluated. The study was expanded in its second year to include an examination of the effect of soil-applied nitrogen on leaf NR activity in a light-enhanced environment.

MATERIALS AND METHODS

Plant Culture and Experimental Design. Soybean plots [Glycine max (L.) Merr. cv Williams] were planted at the University of Illinois Agronomy and Plant Pathology South Farm at Urbana, IL, on May 27, 1978 and May 22, 1979. Seed was planted in east-west rows 2.4 m long with 0.5-m row spacings, at a density of 26 seeds m−1 of row length. Eight replicates of each treatment were planted with each replicate plot consisting of a single treatment row and three adjacent border rows.

Growth conditions varied substantially between the 2 years. While temperatures were roughly equivalent for the 2 years, two long periods without rainfall occurred with one during critical developmental stages in 1979. The first interval was 23 consecutive days in late May and extending into June. A second more critical rain-free period occurred during podfill in August and September.

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2 Abbreviations: NR, nitrate reductase; CER, carbon exchange rate; AR, acetylene reduction; LAI, leaf area index; GAP, days after planting.
Highly reflective foil panels were placed into treated plots to produce light enhancement in the field environment. The panels were 0.61 m tall and 2.4 m long, placed at a 45° angle (from ground level) on either side of the treatment rows. Rain gutters were installed across the bottom of each panel to divert runoff and prevent treated rows from receiving greater rainfall amounts than control rows.

An additional treatment included artificial short photoperiod exposure for the period of June 18, through July 8 using opaque plastic canopies to regulate daylength and elicit early flowering and early seed development. The plastic canopies were suspended on a framework of galvanized wire under tension each covering two complete plots. Canopies were deployed at 1800 h and withdrawn at 0800 h on the following morning to effect a 10-h photoperiod in lieu of the nearly 15-h ambient photoperiod. Photoperiod treatments were initiated when one to two trifoliate leaves were fully developed and continued until the control population was also flowering. The canopies were withdrawn after sundown when rainfall was imminent to maintain uniform soil moisture for all plots. Short photoperiod treatment was also combined with light enhancement to allow for an evaluation of the effects of early flower and seed development in light-enhanced conditions. An additional treatment in the 2nd year involved the application of 450 kg N ha⁻¹ to the soil surface as urea just after plant emergence and immediately preceding precipitation.

Sampling for determination of in vivo leaf NR activity, root nodule AR activity, and fresh weight, and pod, seed, and total plant dry matter accumulation (not including fallen leaves) was begun at 60 (1978) and 57 (1979) DAP and continued at intervals through each season. Sampling procedures were as previously described (21). Final samples were collected just before upper canopy leaf loss occurred. Sample size was four to eight bordered plants per replication. Leaf NR determination was as described by Nicholas et al. (17). Other determinations were as previously described and referenced (21).

**Statistical Tests of Significance.** F tests for significance (0.05 level) were based upon analysis of variance conducted using the Statistical Package for the Social Sciences (18). Where F tests detected significance, mean separation was accomplished using the Newman-Keuls sequential range test for harvest summary data and LSD values for season profiles. The overall LSD values calculated compare between treatments within sampling dates and across sampling dates.

**RESULTS AND DISCUSSION**

Exposure to a 10-h photoperiod from June 18 through July 8 resulted in flowering 7 d earlier for treated plants than for control plants in each year. Podfill also occurred early (Fig. 1) and maturity was hastened by 17 and 16 d in 1978 and 1979, respectively, as flowering at an early vegetative stage resulted in the early termination of vegetative development. Nearly determine (simultaneous from top to bottom node) flowering and pod set were observed in response to short photoperiod exposure, resulting in the greater difference between control and short photoperiod plants which was noted at maturity than that which was noted at flowering. Pod and seed dry weight accumulation was greater for light-enhanced plants by the final sampling in both years for both control and short photoperiod plants (Fig. 1). Plant dry weight accumulation (stems, pods, seed, and retained leaves) was increased significantly by light enhancement in 1978 and also in 1979 where an equivalent response was obtained both with and without soil-applied nitrogen.

Responding to early flower and seed development, a significantly more rapid decline in leaf NR activity was observed for short photoperiod-exposed plants than for control plants in 1978 (Fig. 2). A similar profile was observed in 1979, but no significant difference was detected between control and short photoperiod plants in the dry 1979 season. Light enhancement delayed loss of leaf NR activity in 1978 (Fig. 2). Similar results were obtained in 1979 but were again not significant in this dry season. The addition of 450 kg of soil-applied N/ha resulted in a substantial increase in leaf NR activity along with a delay in decline during flowering and early podfill in 1979 (Fig. 2). Even though drought may have also reduced photosynthetic activity in 1979, leaf NR activity was still responsive to amended levels of nitrate availability. Light enhancement along with applied N increased leaf NR activity beyond that of N application alone at 78 and 85 DAP in 1979. The effect of nitrate supply on leaf NR activity during flowering and early podfill appears to exert a dominant impact on photosynthetic activity in contributing to decline during reproductive growth. Nelson-Schreiber (16) altered reproductive timing using photoperiod manipulation and isogenic lines of soybean. Her results also demonstrate association between flowering, beginning podfill, and decline in leaf NR activity. Furthermore, a decline in stem nitrate content was also associated with these reproductive stages in her study. Increased leaf NR activity at 78 and 85 DAP under light-enhanced plus N-amended conditions indicates that photosynthetic supply was limiting to
and without light enhancement) in 1979 indicated an increased supply of photosynthate was available to the root nodules during the early portions of these developmental stages. This association may be attributed to decreased competition for photosynthate as leaf NR declines in the absence of sufficient nitrate, sink enhancement of photosynthesis, or to an increased partitioning of photosynthate to the root nodules under hormonal control. At later sampling times, root nodule fresh weight (both years) and AR activity (1979) in short photoperiod plants also declined more rapidly than did controls. This response was associated with early visible and functional leaf senescence and maturity as observed for these plants. Thus, the timing of reproductive development was associated with the rise and decline of nodule development as well as with leaf NR activity. The addition of high levels of soil-applied N almost completely eliminated root nodule development (Fig. 2) as was expected based upon previous reports (8, 12). The repression of root nodule development by soil-applied nitrogen was not overcome by light enhancement indicating controls other than simple competition for photosynthate in high N environments (Fig. 2).

Light enhancement resulted in a significant increase in total N accumulation in normal flowering and early flowering plants relative to their respective controls in 1978 (Table I). This result was consistent with the elevated root nodule fresh weight observed for light-enhanced plants. In agreement with these results, supplemental light and CO₂ enrichment have previously been reported to increase total N assimilation in soybean (5, 6, 8). Light enhancement did not significantly affect total N accumulation in 1979 or the partitioning of nitrogen to the seed fraction (N harvest index) in either year (Tables I and II). Increased plant dry weight at maturity (pods, stem, and seed only) resulted from light enhancement in both normal and early flowering plants in 1978 (Table I) as was indicated by higher rates of accumulation during the season (Fig. 1). The lack of dry weight advantage for light-enhanced plants in 1979 was apparently associated with dry conditions in that year.

Light enhancement resulted in significant seed yield increases in control plants in 1978 and in early flowered plants in both years (Tables I and II). An increased seed dry weight harvest index contributed to the yield increases noted for early flowering reflector-exposed plants in 1979 only (Table II). Elevated temperature may also have contributed to increased seed yield in the light-enhanced environment. Thermister measurements of abaxial leaf surfaces documented temperature increases of up to 1.5°C over that of leaves on controls under midday field conditions. Jeffers and Shibles (11) suggested that soybean leaves light saturate at slightly lower levels when grown at temperatures elevated from 25 to 30°C. Thomas and Raper (24) found that the rate of seed filling was increased at 26 to 30°C when compared with that at 16 to 18°C.

The application of high levels of nitrogen (1979 only) had no significant impact on seed yield or upon any of the harvest characteristics, which were evaluated.

In summary, the timing of both seasonal decline in leaf NR activity and increase in root nodule fresh weight and AR activity were associated with flowering and beginning podfill. Soil-applied nitrogen increased leaf NR activity and delayed decline. Light enhancement had little impact on leaf NR activity unless high levels of nitrate were made available. Nitrate supply was therefore implicated as limiting to leaf NR activity when decline occurs during these reproductive stages. Photosynthate supply was implicated as a limitation to leaf NR during this period only where nitrate supply was nonlimiting.

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Table 1. Effect of Variable Timing of Flowering and Podfill and of Light Enhancement on the Accumulation and Partitioning of Nitrogen and Dry Weight by Soybean in 1978

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total Nitrogen</th>
<th>Harvest Index</th>
<th>Total Dry Wt</th>
<th>Harvest Index</th>
<th>Seed Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g plant⁻¹</td>
<td>seed nitrogen</td>
<td>g plant⁻¹</td>
<td>seed dry wt</td>
<td>g plant⁻¹</td>
</tr>
<tr>
<td>Control</td>
<td>0.9 b</td>
<td>0.86</td>
<td>22.5 b</td>
<td>0.48 b</td>
<td>10.8 b</td>
</tr>
<tr>
<td>Short photoperiod</td>
<td>0.9 b</td>
<td>0.79</td>
<td>20.7 b</td>
<td>0.55 ab</td>
<td>11.4 b</td>
</tr>
<tr>
<td>Reflector</td>
<td>1.7 a</td>
<td>0.82</td>
<td>34.5 a</td>
<td>0.53 ab</td>
<td>12.8 a</td>
</tr>
<tr>
<td>Reflector plus short photoperiod</td>
<td>1.6 a</td>
<td>0.82</td>
<td>32.9 a</td>
<td>0.60 a</td>
<td>19.7 a</td>
</tr>
</tbody>
</table>

* Short photoperiod exposure hastened flowering and podfill by 7 d in both 1978 and 1979 and maturity by 17 and 16 d, respectively, in 1978 and 1979 as pod development proceeded in a near determinate fashion.

Table 2. Effect of Variable Timing of Flowering and Podfill, Light Enhancement, and of Nitrogen Amendment on the Accumulation and Partitioning of Nitrogen and Dry Weight by Soybean in 1979

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total Nitrogen</th>
<th>Harvest Index</th>
<th>Total Dry Wt</th>
<th>Harvest Index</th>
<th>Seed Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g plant⁻¹</td>
<td>seed nitrogen</td>
<td>g plant⁻¹</td>
<td>seed dry wt</td>
<td>g plant⁻¹</td>
</tr>
<tr>
<td>Control</td>
<td>0.8</td>
<td>0.84 ab</td>
<td>21.6 ab</td>
<td>0.44 ab</td>
<td>9.5 ab</td>
</tr>
<tr>
<td>Short photoperiod</td>
<td>0.6</td>
<td>0.87 ab</td>
<td>17.4 b</td>
<td>0.48 ab</td>
<td>8.4 b</td>
</tr>
<tr>
<td>Reflector</td>
<td>0.9</td>
<td>0.84 ab</td>
<td>24.9 b</td>
<td>0.45 ab</td>
<td>11.2 ab</td>
</tr>
<tr>
<td>Reflector plus short photoperiod</td>
<td>1.09</td>
<td>0.91 a</td>
<td>28.2 a</td>
<td>0.54 a</td>
<td>15.2 a</td>
</tr>
<tr>
<td>450 kg N/ha</td>
<td>0.7</td>
<td>0.82 b</td>
<td>22.3 ab</td>
<td>0.42 b</td>
<td>9.4 ab</td>
</tr>
<tr>
<td>N/ha</td>
<td>1.0</td>
<td>0.85 ab</td>
<td>30.1 a</td>
<td>0.44 ab</td>
<td>13.2 ab</td>
</tr>
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</table>

* Treatments as described in Table 1.  

LITERATURE CITED