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EFFECTS OF NITROGEN STRESS ON NITROGEN REDISTRIBUTION AND PHOTOSYNTHESIS IN SOYBEANS

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Abstract

*The effect of N stress on the redistribution of N from soybean leaves (*Glycine max *(L.) Merrill) was investigated by growing soybeans (cv. 'Cutler 71') in nutrient solutions with varying N Levels in the greenhouse and measuring the* $^{14}CO_2$ *uptake rate, the levels of total N, Ribulose 1,5 - bisphosphate carboxylase (RuBPCase) and its activity, and 1 N NaOH extractable protein in the leaves. When the plants were subjected to varying levels of N stress for 63 days after emergence, leaf N levels ranged from 2.7 to 13.6 mg.dm*$^{-2}$ *and the* CO_2 *uptake rate was closely correlated with the variation in leaf N levels. N stress during seed fill (no N in the nutrient media from growth stage R5 to maturity) enhanced the senescence process and the redistribution of N compounds from the leaves. The stressed plants reached physiological maturity 8 days sooner than the control and the levels of N, protein and RuBPCase from growth stage R5 to maturity was similar for both treatments. RuBPCase activity was most sensitive to N stress. Its significant decrease was observed prior to changes in* CO_2 *uptake rate and total protein.*

Introduction

Soybeans *(Glycine max* (L.) Merrill*)* redistribute significant quantities of N from their leaves and other parts of plant to the seed during reproductive growth and this redistributed N can account for from 50 to nearly 100 % of the N in the seed at maturity[1-3]. The decline in the concentration of N in the leaves during reproductive growth has been related to the decline in photosynthetic activity[4-8]. It has been suggested that the loss of N from the leaf and the associated loss of physiological activity may be a cause of senescence[9,10]. The reported associations between leaf N and photosynthesis would be consistent with this hypothesis; however, other workers[1] have suggested that senescence is initiated by a signal from the developing seed.

Nitrogen stress during reproductive growth of soybeans has been shown to accelerate the senescence process as measured by the rate of leaf abscission[2]. However, little information is available on the effects of N stress during reproductive growth on the photosynthetic capacity of the leaf. Thus, the objective of this work was to investigate the effects of N stress

on leaf N status and photosynthetic capacity during vegetative and reproductive growth in soybeans.

Materials and Methods

Soybeans (cv. 'Cutler 71') were grown in the greenhouse in 17.5 cm. pots filled with sand that had been thoroughly washed with deionized water (Experiment I). Four seeds were planted in each pot and they were thinned to 2 plants per pot soon after emergence. Varying levels of N were supplied in the nutrient media to create wide differences in leaf N content. The pots were given 100 ml. of the various nutrient media twice weekly. There were six replications of each treatment as follows. The zero N treatment received a modified 0.5X Hoaglands solution lacking N from emergence until the photosynthetic measurements were made. The high N treatments received the 0.5X modified Hoaglands solution (containing 7.5 mM NO_3^-) for three weeks after emergence and then 2 sets of 6 pots received the modified Hoaglands solution containing either 15.5 or 22.5 mM No_3^- until the photosynthetic measurements were made. The remaining pots received the 0.5X modified Hoaglands solution containing 7.5 mM NO_3^- until they were shifted to a solution lacking N at 1, 2, 3 or 4 weeks prior to the measurement of photosynthesis. The basic 0.5X modified Hoaglands solution had the following composition; 0.5 mM KH_2PO_4, 2.5 mM KNO_3, 2.5 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$,9 µM FeHEDTA and micronutrient (48 µM H_3BO_3, 9 µm $MnCl_2$, 0.8 µM $ZnSO_4$, 0.3 µM $CuSO_4$ and 0.1 µM H_2MoO_4). The solutions containing the high N levels were basically as described except that 2.5 mM $CaSO_4$ and 15.5 mM KNO_3 or 2.5 mM $CaSO_4$ and 22.5 mM KNO_3 were used in place of 2.5 mM $Ca(NO_3)_2$ and 2.5 mM KNO_3. The solution with no N contained 1.25 mM K_2SO_4, 1 mM $MgSO_4$, 0.25 mM $Ca(H_2PO_4)_2$, 1 mM $CaSO_4$, 9 uM FeHEDTA and micronutrients as given above.

When the plants were 63 days old the CO_2 uptake rate was measured on the second and fourth leaves from the growing point on the main stem using a $^{14}CO_2$ method[8,12]. The CO_2 uptake rate was measured on the central leaflet of the trifoliate and the leaf chamber exposed 3.1 cm^2 of both sides of the leaf to a gas mixture containg labeled CO_2 (330 ppm) with a specific activity of 15 $mCi.1^{-1}CO_2$. Measurements were made only when the photosynthetic photon flux density was between 1600 and 1800 $\mu E.m^{-2}.sec^{-1}$. Leaf punches (2.5 cm^2) were taken immediately for measurement of ^{14}C. The samples were freeze-dried, weighed, and oxidized. The CO_2 generated by the oxidizer was collected directly in a scintillation cocktail and the ^{14}C determined with a Packard Tri-Carb scintillation spectrometer. The total N content of the leaves used for the measurement of CO_2 uptake rate was determined with a Coleman nitrogen analyzer. Twenty mg of dried ground leaf tissue was ignited at 800 C and the amount of N_2 gas evolved was determined volumetrically after removing the CO_2 with a 40% KOH solution.

A second experiment (Experiment II) was conducted using the same cultivar (Cutler 71) and a greenhouse gravel culture system. Pots (201) filled with gravel were used and each pot was irrigated every 20 minutes with 2-1 of nutrient solution which drained into a

20-1 reservoir. The nutrient solution was replaced at weekly intervals and water was added daily to maintain the volume in the reservoir. Six seeds were planted per pot on 19 July 1979, and thinned to 3 plants at the 2nd trifoliolate growth stage and to 2 plants at the 6th trifoliolate growth stage.

The plants were exposed to a 0.5X modified Hoaglands solution with 7.5 mM NO_3^-, as described previously, from planting until 61 days after planting at growth stage R4.8 (Fehr and Caviness[13]). At this stage, half of the pots selected at random were shifted to a modified Hoaglands solution lacking N (as described previously) until maturity. The CO_2 uptake rate of the 6th leaf from the growing point on the main stem was measured (as described previously) on 3 plants from each treatment every few days after the minus N treatment was applied until maturity. Measurements were made only when the photosynthetic photon flux density was at least 1200 $\mu E.m^{-2}.\ sec^{-1}$. Leaf samples were also taken from the same position for the measurement of total N, including NO_3^-, by Kjeldahl[14], protein (1 N NaOH extractable) with the Bio-Rad assay[15], ribulose 1,5-bisphosphate carboxylase (RuBPCase)[16] and its activity[17]. Detailed methods of the analysis for protein have been described previously[8]. RuBPCase was extracted from 5 g of fresh leaf material ground in a mortar with 4.8 ml of chilled solution containing 0.1 M Tris-HCL (pH 7.4), 5.0 M NaCl, 0.005 M disodium EDTA and 0.01 M $MgCl_2$ and 0.2 ml of a solution containing 0.5 M Tris, and 2.5 g of acid-washed sand. The mixture was centrifuged at 20,000 g for 20 minutes and the concentration of RuBPCase was determined using a Spinco Model E ultracentrifuge equipped with a phase plate, as described by Dorner *et al.*[16]. RuBPCase activity was measured using a modified version of the technique described by Johnson *et al*[17]. A crude extract was prepared by grinding 3 leaf punches in a mortar with 5 ml of extraction media (50 mM HEPES buffer, pH 8.1, 10 mM $MgCl_2$, 5 mM D-isoascorbate, 0.25 mM EDTA, 5 mM dithiothreitol and 1.0 $g.1^{-1}$ polyvinyl pyrrolidone). After centrifuging at 17000 g for 20 minutes, 0.1 ml of the supernatant was added to 1 ml of assay media containing 50 umoles HEPES buffer (pH 8.1), 5 μmoles $MgCl_2$, 3 umoles dithiothreitol, 0.1 umoles disodium EDTA, 2.5 umoles NaH $^{14}CO_3$ (0.4 $\mu Ci.\mu mole^{-1}$) and 0.1 umole RUBP. The reaction was stopped after 3 minutes by adding 0.1 ml of 6 N acetic acid and the mixture was dried in a vacuum oven to remove any unfixed $^{14}CO_2$. The fixed CO_2 was measured in a Packard Tri-Carb scintillation spectrometer.

Results and Discussion

The variable N treatments in the first experiment resulted in a wide range in total N levels in the leaf (2.7 (1.0%) of 13.6 $mg.dm^{-2}$ (5.3%) when the measurements of CO_2 uptake rate were made (Fig. 1). The CO_2 uptake rate at this time was directly related to the total N in the leaf for both leaf positions although the second leaf from the growing point showed higher CO_2 uptake rates at all N levels than the fourth leaf. The CO_2 uptake rate reached zero at leaf N levels of 2 to 3 $mg.dm^{-2}$ (approximately 1% N) and these levels were lower than the levels (8 to 9 $mg.dm.^{-2}$) found in naturally abscising leaves of this cultivar in field experiments[18]. Whether this difference is a general result of the N stress

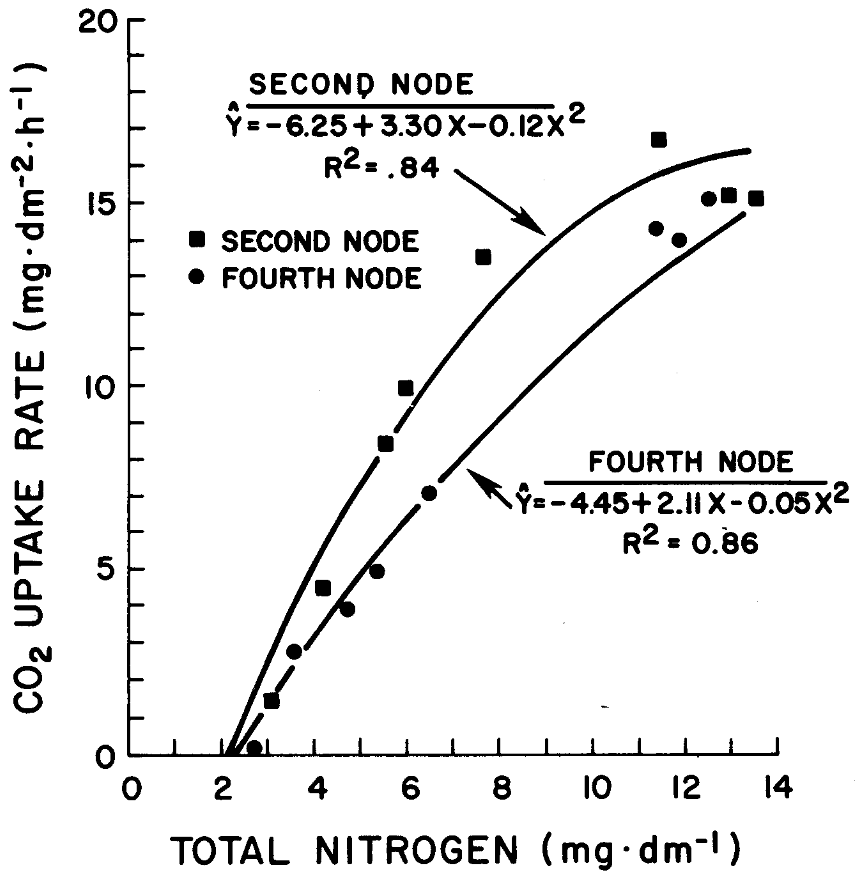

Figure 1. The effect of N stress during vegetative growth on leaf total N and CO_2 uptake. Experiment I.

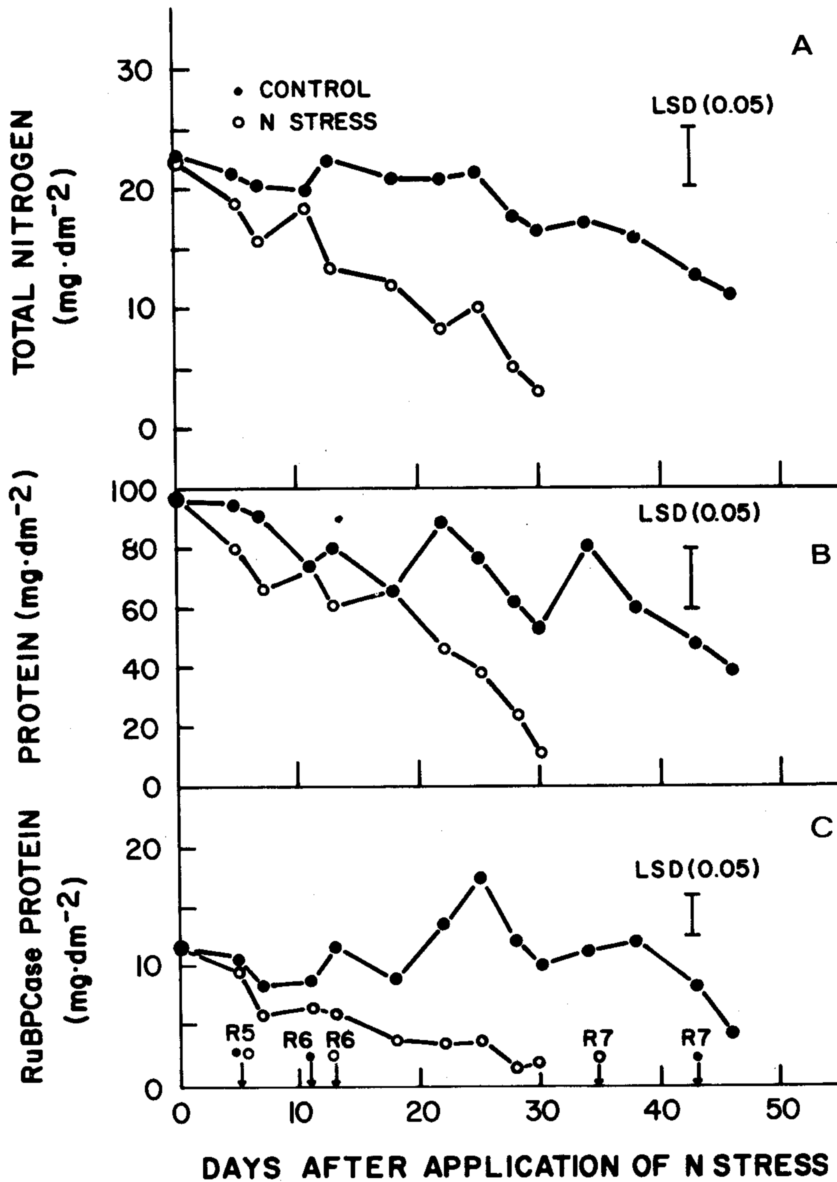

Figure 2. The effect of N stress on leaf total N (A), protein (B), and RuBPCase protein (C), Experiment II, 1979. Reproductive growth stages for the control (●) and N-stressed plants (o) are shown on the x-axis.

or the fact that the leaves in this experiment were vegetative leaves that developed under artificial severe N stress compared to leaves from the field plantings that lost their N naturally as part of the senescence process is not known.

The introduction of N stress at the beginning of seed growth (growth state R4.8) in the second experiment hastened maturity. Te stressed plants reached physiological maturity (growth stage R7) 8 days sooner than the control (Fig. 2) and this was reflected in a significantly lower seed yield (47 $g.plant^{-1}$) for the N stressed plants compared with the control plants (66 $g.plant^{-}$).

The total N levels in leaves of the control plants remained relatively constant through much of growth stage R6 and then declined steadily to a level of 11 $mg.dm^{-2}$ at maturity (Fig. 2A). The protein levels in leaves from the control plants followed a somewhat similar pattern although there was considerable variation before the final decline was initiated late in growth stage R6 (Fig. 2B). The levels of total N and protein in the control leaves at maturity were slightly higher than levels found in abscising leaves of this cultivar in the field[18].

The level of RuBPCase in leaves from the control plants remained relatively constant during much of reproductive growth (expept for a peak midway through growth stage R6), but declined just prior to maturity (Fig. 2C). At maturity the level of RuBPCase was higher than that found in abscissing leaves of this cultivar in the field[18].

The removal of N from the nutrient media accelerated the loss of all three N components from the leaves (Fig. 2). The affect of the N stress on total N and RuBPCase was evident at approximately 13 days after application of the N stress. The effect on leaf protein was not evident until approximately 20 days after the stress was imposed. The levels of all three components in leaves from the stressed plants at the last sample were lower than those in the control leaves and they were also lower than the levels found in abscising leaves of this cultivar in field experiments[18]. The total N content in abscising leaves from the N stress treatment was 3.1 $mg. dm^{-2}$ (1.1 %) which is similar to the level (0.9%) reported by Streeter[19] for abscising leaves from N stressed plants. Since the initial leaves were the same in both treatments, the lower levels at maturity indicated that there was more N redistributed from a leaf on the N stress plants than in the control (Table 1). The N stress plants redistributed 69% more total N from the measured leaf than the control plants. The difference was less for RuBPCase where the N stressed plants redistributed only 28% more from the measured leaf than the controls. The acceleration of senescence by N stress has been previously reported by Egli *et al.*[2]; however, in contrast to the data reported here, they found that leaves from N stressed plants had the same total N concentration at abscission as leaves from control plants. However, Streeter[19] reported that N stress would reduce the N levels in abscising leaves of soybeans below that of abscising leaves that were not stressed.

The CO_2 uptake rate reached a maximum in both treatments at growth stage R6 (Fig. 3A). Similar results have been reported for this cultivar in the field[18] and for other soybeans cultivars[6,20,21], although Lugg and Sinclair[22] reported no such relationship. The CO_2 uptake rate in leaves from the control plants began a steady decline midway through growth

stage R6 (25 days after application of the N stress); however, the decline in CO_2 uptake rate in the N stressed leaves started sooner (18 days after application of the N stress) and the CO_2 uptake rate reached essentially zero approximately 16 days sooner than in the control leaves (Fig. 3A).

The RuBPCase activity in the control leaves also started declining midway through growth stage R6 (Fig. 3B). The RuBPCase activity in leaves from the N stressed plants began to decline immediately after the removal of N from the nutrient media, approximately 18 days before the CO_2 uptake rate was affected by the N stress. The RuBPCase activity reached essentially zero at the same time as the CO_2 uptake rate (Fig. 3A and B). Thus, the RuBPCase activity was affected much more quickly by the N stress than the CO_2 uptake rate. Much of the initial decline in RuBPCase activity was accounted for by the decline in the amount of RuBPCase protein; the specific activity in the stressed leaves was similar to that of the control leaves until 28 days after the imposition of the N stress (Fig. 4). However, after 28 days the specific activity also declined below that of the control, reaching almost zero in leaves from the N stressed plants at the last sample 30 days after the N stress was applied. Thus, the N stress caused a rapid loss in RuBPCase activity; however, there was no measurable effect on the CO_2 uptake rate until 64% of the initial level of RuBPCase and 59% of the initial RuBPCase activity was lost.

The relationship between the leaf nitrogenous components and the CO_2 uptake rate (Fig. 5A, B, and C) was not as close as has been reported in field studies including this cultivar[18]. There was a tendency for a curvilinear relationship in each case with the observations from the N stress treatments providing most of the data points representing the lower levels of the N components and CO_2 uptake rates. Although there was a large amount of scatter in the data points, the regression curves suggest that the relationship between the nitrogenous components and CO_2 uptake rate was similar for leaves from the both N stressed and control plants. The major difference was that the leaves from the N components and lower CO_2 uptake rates (Fig. 5A, B, and C). The curvilinear relationship suggest that a portion of each of the nitrogenous components could be lost from the leaf before the CO_2 uptake rate declined. A similar curvilinear relationship was found for the leaves from the 10th node of field grown plants; however, the relationship in the last fully expanded leaves of field grown plants was essentially linear, suggesting that any loss of the nitrogenous components resulted in a decrease in CO_2 uptake rate[18].

The CO_2 uptake rate of soybean leaves was associated with the N status of the leaf in both leaves developing on the plant before flowering where variations in N levels were created by N stress and in leaves that were naturally redistributing N during normal senescence or where senescence was enhanced by N stress during reproductive growth. Similar relationships have been reported by other workers for soybeans[4-8]. The N stress during reproductive growth accelerated the loss of N from the leaf and the loss of photosynthetic activity. RuBPCase activity was more sensitive to N stress than the CO_2 uptake rate. In a study comparing cultivars with long and short seed filling periods, the level of RuBPCase protein declined

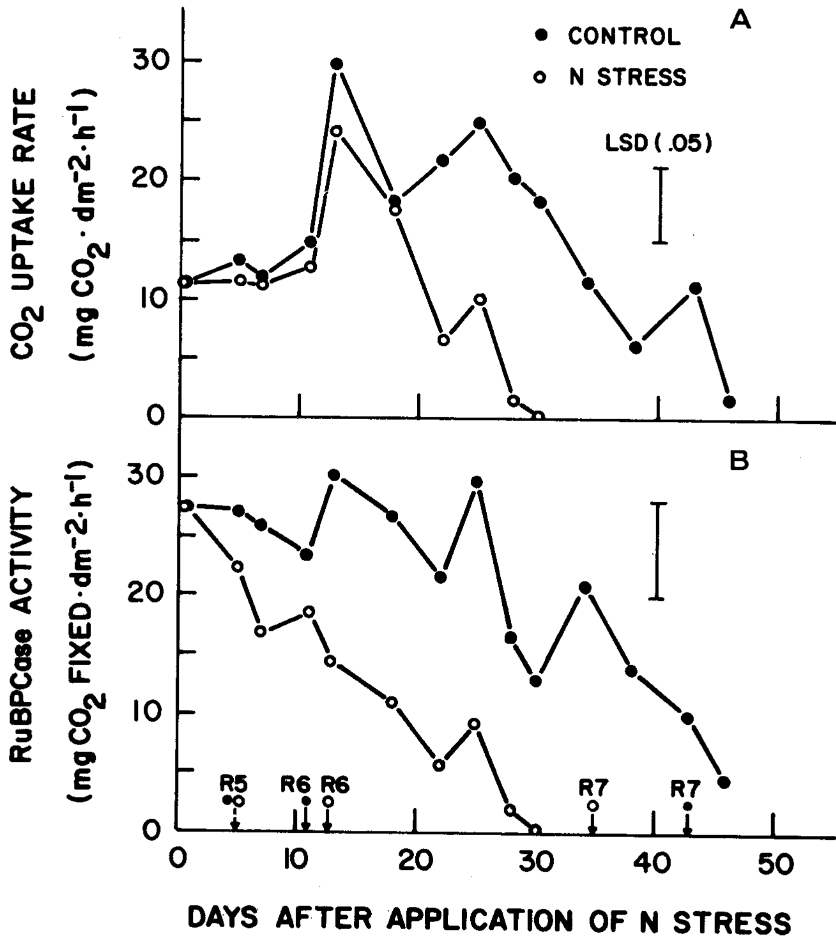

Figure 3 The effect of N stress on CO_2 uptake rate (A), and RuBPCase activity (B), Experimer II, 1979. Reproductive growth stages for the control (●) and N-stressed plant (o) are shown on the x-axis.

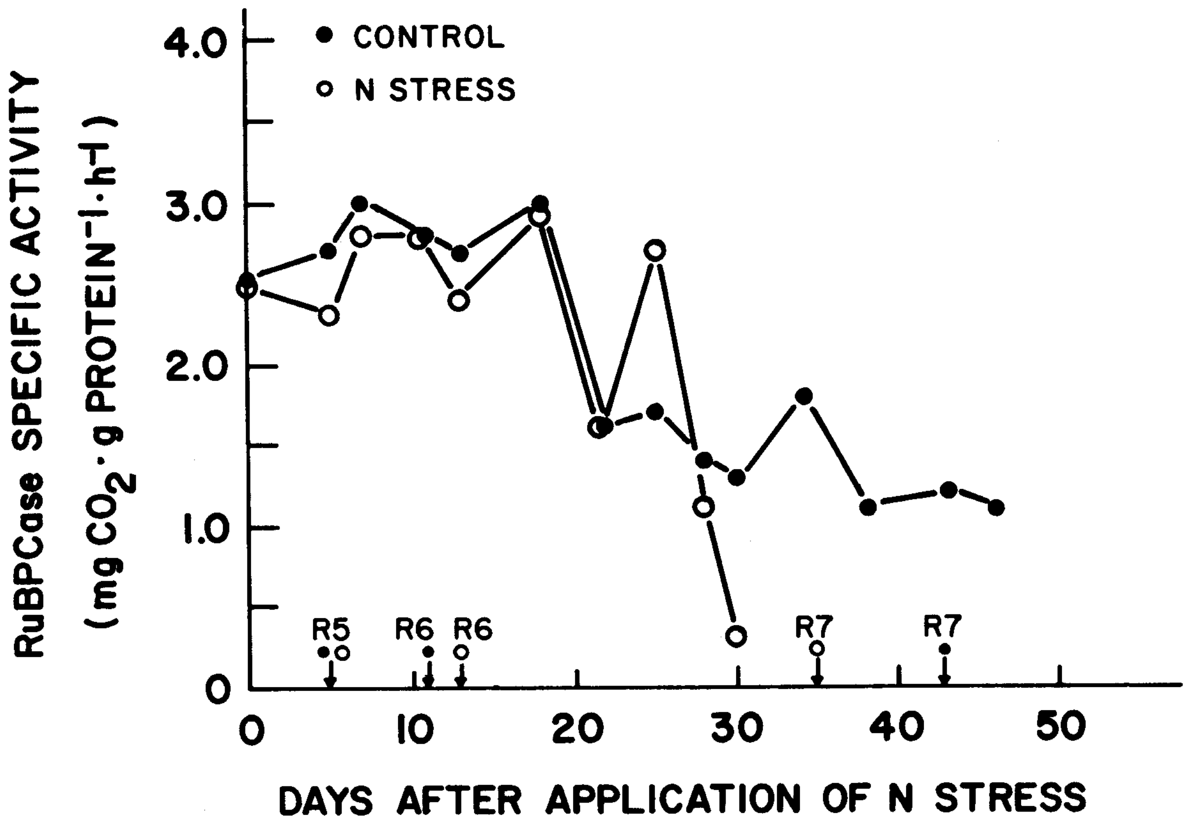

Figure 4 The effect of N stress on the specific activity of RuBPCase, Experiment II, 1979. Reproductive growth stages for the control (●) and N-stressed plants (o) are shown on the x-axis.

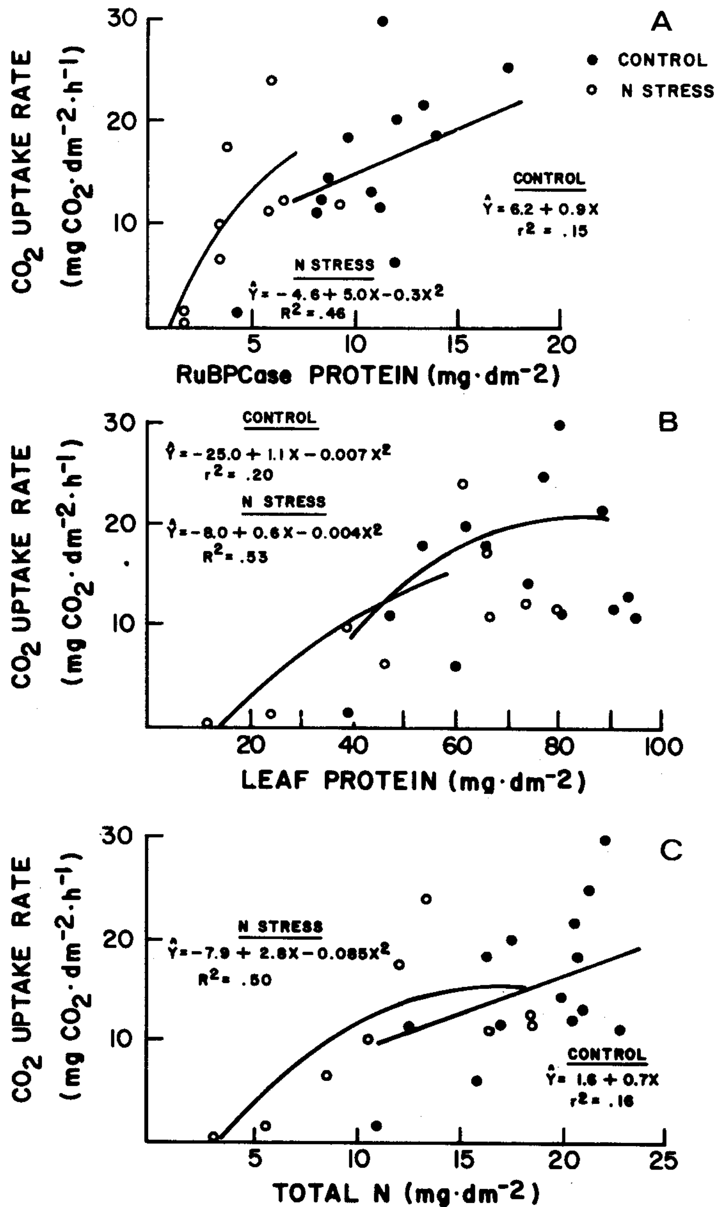

Figure 5 The relationship between RuBPCase protein (A), protein (B), and leaf N (C), and CO_2 uptake, Experiment II.

sooner in the short filling period cultivar but there was no difference in timing of the decline in CO_2 uptake rate between the two cultivars[18]. Thus, the two studies suggest that RuBPCase is a sensitive indicator of the beginning of the senescence process; however, changes in RuBPCase were not immediately translated into changes in CO_2 uptake rate.

The acceleration of the senescence process by N stress and the close association between the N status of the leaf and its photosynthetic activity is consistent with the hypothesis of Sinclair and de Wit[10] that the loss of N from the leaves caused by the inability of the plant to meet the N requirements of the seed is the cause of senescence. However, the loss of nitrogenous components from the leaf and the decline in photosynthetic activity of the leaf also occurred in the control plants which were exposed to a continuous high level of NO_3^- in the nutrient media until maturity. It is possible that the senescence signal suggested by Lindoo and Nooden[11] may have been operating in the control plants.

TABLE I. AMOUNT OF TOTAL N, PROTEIN, AND RuBPCase PROTEIN REDISTRIBUTED FROM THE 6TH LEAF, EXPERIMENT II.

N components	Redistribution of N (mg.dm^{-2})	
	Control	N Stress
Total N	11.6[a]	19.6
Protein	56.6	83.9
RuBPCase Protein	7.5	9.6

[a] Calculated as the difference between level when the N stress was applied and the level in the leaves at the last sample or maturity.

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บทคัดย่อ

ได้ศึกษาผลของความกดดันทางไนโตรเจนต่อการกระจายของไนโตรเจนในถั่วเหลือง (*Glycine max* (L.) Merrill) โดยปลูกถั่วเหลือง (cv. 'Cutler 71') ในน้ำเลี้ยงที่มีระดับไนโตรเจนต่างกันในเรือนทดลอง และวัดอัตราการดูด $^{14}CO_2$ ระดับของไนโตรเจนทั้งหมด ระดับของ ribulose 1,5-bisphosphate carboxylase (RuBP Case) และโปรตีนที่สะกัดได้ด้วย 1N NaOH ในใบไม้ หลังจากกดดันไนโตรเจนได้ 63 วัน นับจากวันที่ขึ้น ระดับของไนโตรเจนในใบอยู่ระหว่าง 2.7 ถึง 13.6 $mg.dm^{-2}$ และอัตราการดูด CO_2 มีความสัมพันธ์อย่างใกล้ชิดกับระดับไนโตรเจนในใบ การกดดันไนโตรเจนในช่วง R5 ถึง maturity เร่งการแก่ และการกระจายของไนโตรเจนจากใบ พืชที่ได้รับความกดดันจะถึง physiological maturity เร็วกว่าปรกติ 8 วัน และระดับของไนโตรเจน โปรตีน และ RuBP Case ในใบจะต่ำกว่าปรกติ พบความสัมพันธ์ระหว่าง การดูด CO_2 และ ไนโตรเจนในใบ โปรตีนและ RuBP Case ในทำนองเดียวกัน ในช่วงการเจริญจาก R5 ถึง maturity RuBP Case ไวต่อการกดดันไนโตรเจนมากที่สุด และจะลดลงก่อนการเปลี่ยนอัตราการดูด CO_2 และระดับโปรตีน