Plant Physiol. (1983) 73, 6–10
0032-0889/83/73/0006/05/$00.50/0

Metabolism of Carbon and Nitrogen by Soybean Seedlings in Response to Vegetative Apex Removal[1]

Received for publication November 1, 1982 and in revised form May 7, 1983

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ABSTRACT

Short-term (31-hour diurnal) growth-chamber studies were conducted to determine the effects of removing the vegetative apex (meristem and developing trifoliolate leaves) on net photosynthesis (changes in plant dry weight), on distribution of metabolites among plant parts, and on nitrate metabolism and reduced-N accumulation by soybean [*Glycine max* (L.) Merr.] seedlings. Roots and stems served as alternate sinks for dry matter accumulation in the absence of the vegetative apex. Sugar concentration in roots increased (42%) within 4 hours of vegetative apex removal, and remained higher than for the controls during the 31-hour experimental period. Nitrate assimilation (nitrate reductase activity and total accumulation of reduced-N) was also enhanced in response to vegetative apex removal. Although dry matter accumulation was similar between treated and control plants (113 *versus* 116 milligrams per plant) over the 31-hour sampling period, more nitrate (1.31 *versus* 0.79 milligrams per plant) and more reduced-N (3.96 *versus* 3.45 milligrams per plant) accumulated in treated plants during the same interval. It was concluded that vegetative apex removal had little effect on overall net photosynthesis of soybean seedlings during the 31-hour treatment period, but did alter partitioning of photosynthate and enhanced uptake, transport, and reduction of nitrate. Implications are that uptake and metabolism of nitrate by soybeans may be limited by flux of carbohydrate to the roots, although hormonal effects due to vegetative apex removal cannot be ruled out.

Experiments involving vegetative source-sink manipulations have typically been designed either to investigate effects on photosynthesis and partitioning of photosynthate within leaves (2, 5, 7, 16), or to study hormonal changes within the plant (15). A general enhancement of metabolic processes has been observed upon decapitation of *Phaseolus vulgaris* (15). Both diurnal (13) and long-term (9) experiments have illustrated the interdependence of C and N assimilation and the resulting influence of assimilation on plant metabolism and growth. The close relationship between shoot photosynthesis and nitrate uptake, assimilation, and transport by roots has been well documented (3). Crop models have utilized N and photosynthetic inputs as major factors involved in crop yield (4, 11).

In order to better understand regulatory mechanisms involved in C and N assimilation and partitioning, more basic information is needed. Knowledge of C and N assimilation and partitioning in response to source-sink manipulations may help to understand the underlying controls of partitioning metabolites to the various sinks.

The purpose of this research was to determine the effect of altering the source-sink ratio of vegetative soybean seedlings (by removal of the vegetative apex) on changes in C and N parameters over a diurnal period.

MATERIALS AND METHODS

Plant Culture. Soybean seeds [*Glycine max* (L.) Merr. cv Williams] were germinated in sand moistened with deionized H_2O. Six DAP[2] seedlings were transplanted into 2-L pots containing half strength modified Hoagland solution (1). Plants were grown in growth chambers under 14 h, 29°C light and 10 h, 19°C dark. Other growth conditions were as described (1). The experimental design was a randomized complete block with three replications. The three growth chambers served as blocks. Experimental units were 2-L pots containing six plants.

Treatment. The vegetative apex (apical meristem and trifoliolate leaves) was removed from half the plants at 14 DAP at the end of a dark cycle. The other plants served as controls. At the time of the vegetative apex removal treatment, the unifoliolate leaves were actively expanding and the first trifoliolate leaf was just emerging and was considered to be a sink rather than a source leaf.

Sampling. Two pots (six plants/pot) of each treatment from each growth chamber were harvested at 0, 4, 7, 14, 24, 28, and 31 h after removal of apical meristems and trifoliolate leaves. Time zero also corresponded to the beginning of a light cycle. The dark period occurred between 14 and 24 h. Eight plants per treatment per chamber were composited for chemical analysis and four separate plants were composited for NR assay at each sampling time.

Sample Preparation. Plants were divided into unifoliolate leaves, stems with petioles, cotyledons, roots, and vegetative apices when present. Fresh weight was determined on all parts except roots. Area of unifoliolate leaves was determined with an area meter. Plant parts were dried in a forced draft oven for 1 h at 100°C followed by 60 h at 70°C. Dried tissue was mechanically ground (20-mesh) and stored for chemical analyses.

Analyses. *Carbohydrates.* Subsamples (50–100 mg) were extracted and assayed for total nonstructural carbohydrates (14) and soluble sugars (reducing sugars and hydrolyzed sucrose) (8). Starch concentration was calculated by subtracting soluble sugars from total nonstructural carbohydrates and multiplying by 0.9 (12).

Nitrate-N. Extracts used for carbohydrate analyses were used to determine nitrate-N as described (14).

Total-N and Reduced-N. Total-N and reduced-N were determined as described (14).

NR Assay. Intact unifoliolate leaves and roots were assayed by an *in vivo* NR assay method which lacked nitrate in the incuba-

[1] Supported in part by Hatch Act appropriations, project ILL-15-0371.

[2] Abbreviations: DAP, days after planting; NR, nitrate reductase; NRA, nitrate reductase activity.

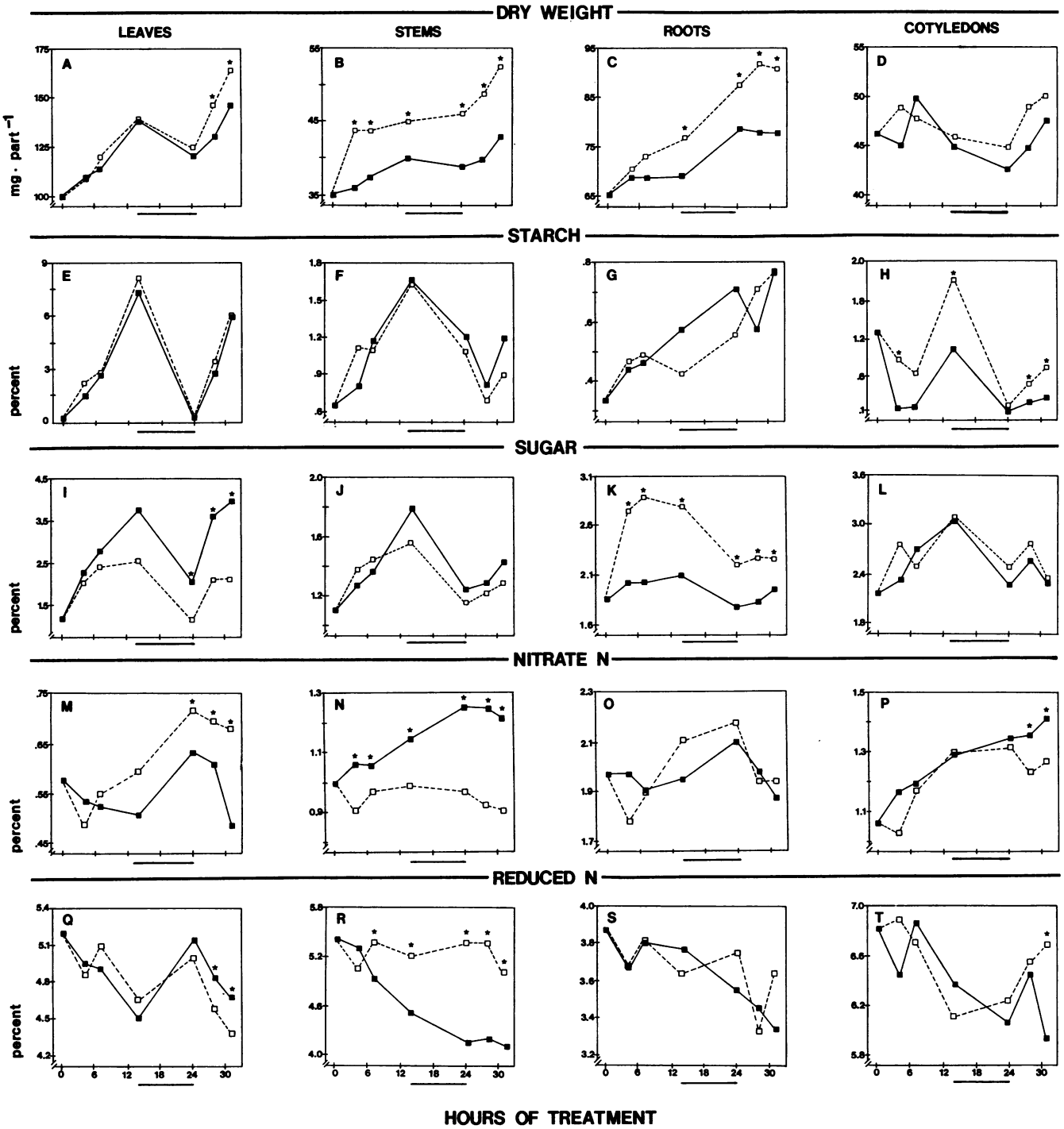

FIG. 1. Effect of vegetative apex removal on diurnal dry weight accumulation (A–D), and percent of starch (E–H), sugar (I–L), nitrate-N (M–P), and reduced-N (Q–T) in soybean plant parts. The vegetative apex removal treatment (□- - -□) was done at time 0 at the beginning of the light period. Controls (■——■) were intact plants. Plants were separated into unifoliolate leaves, roots, stems, and cotyledons. The vegetative apices were removed from controls at each sampling time (Table II). Dark period (14–24 h) is indicated by bold line. Data points accompanied by * indicate significant difference between control and treatment (5% level) within specific sampling time.

tion medium (1).

Statistical Analysis. Analysis of variance procedures were used to compare dry weight and component concentrations in treated and control plant parts, within each sampling time. Comparisons of dry matter and component content changes over specified light and dark periods, within plant parts and over whole plants, were done by paired *t* tests of treated and control plants. The experiment was conducted twice with similar results. The results of one experiment are presented.

RESULTS AND DISCUSSION

Dry Weight and Constituent Concentration. *Unifoliolate Leaf, Stem, Root, and Cotyledon.* The treatment involving removal of the vegetative apex (apical meristem and developing trifoliolate

Table I. *NRA of Unifoliolate Leaves and Roots of Soybean Seedlings*

Treatment (T) involved removal of the vegetative apex (apical meristem and expanding trifoliolates) at time 0, compared with intact controls (C). Growth conditions were light (0–14 h), dark (14–24 h), and light (24–31 h). Values are means ± SE.

Sample Time	Nitrate Reductase Activity			
	Leaves		Roots	
	C	T	C	T
h	*μmol NO_2^- plant^{-1} h^{-1}*			
0	4.8 ± 0.3	4.8 ± 0.3	3.8 ± 0.3	3.8 ± 0.3
4	5.6 ± 0.4	4.1 ± 0.3	3.9 ± 0.4	4.3 ± 0.2
7	5.3 ± 0.5	5.2 ± 0.5	4.1 ± 0.5	4.4 ± 0.4
14	2.1 ± 0.3	4.8 ± 0.3	4.0 ± 0.4	4.6 ± 0.3
24	5.1 ± 0.2	7.3 ± 0.5	4.4 ± 0.7	4.7 ± 0.4
28	6.7 ± 0.2	9.9 ± 0.4	4.6 ± 0.4	5.7 ± 0.3
31	5.7 ± 0.3	9.8 ± 0.5	4.7 ± 0.6	5.0 ± 0.4

Table II. *Dry Matter Content and Constituent Concentration of Vegetative Apices Removed at Various Sampling Times from Soybean Seedlings*

Other experimental details as given in Table I legend.

Sample Time	Dry Wt	Starch	Sugars	NO_3^--N	Reduced-N
h	*mg plant^{-1}*			%	
0	22.4	0.00	0.93	0.13	7.08
4	26.5	0.00	1.09	0.12	6.52
7	31.4	0.03	1.12	0.16	6.20
14	46.5	0.40	1.59	0.17	5.76
24	50.0	0.04	1.00	0.22	6.27
28	60.9	0.28	1.50	0.22	5.80
31	70.1	0.87	1.51	0.26	5.31

leaves) from 14-d-old soybean seedlings resulted in greater dry matter accumulation in the stem, root, and unifoliolate leaf fractions, relative to comparable plant parts of control plants, over the ensuing 31-h observation period (Fig. 1, A–D). There was no significant effect of treatment on cotyledon dry weight. Stems were most responsive to the treatment, with significant dry weight increases occurring within the first 4 h after treatment. After 31 h, unifoliolate leaf area was also increased by treatment (64 *versus* 59 cm^2/plant), relative to controls. Within the dark phase of the 31-h sampling period, dry weight of cotyledons and unifoliolate leaves decreased, that of stems was unchanged, and that of roots increased, in both control and treated plants. This indicated that translocation of photosynthate to the roots was occurring in the dark, and presence or absence of the vegetative apex did not affect this translocation.

Removal of the vegetative apex from the plant did not have marked effects on starch concentrations within the plant parts, relative to controls, although the cotyledons of treated plants were higher in starch than were cotyledons of controls at four sampling times (Fig. 1, E–H). For both control and treated plants, the starch concentration increased in the roots and decreased in all other plant parts during the dark. The increase in starch concentration in the roots indicated that the root served as a sink for storage of reserves. Starch concentration in all plant parts increased during periods of light, with the unifoliolate leaf showing the largest increase. The unifoliolate leaf also showed the largest decrease in starch concentration during the dark period.

Sugar concentration was lower in leaves and higher in roots in response to removal of the vegetative apex, relative to controls (Fig. 1, I–L). No treatment effect on sugar concentration was observed in the stems and cotyledons. Sugar concentration in all plant parts increased during the light period (0–14 h) and decreased during the following 10-h dark period for both control and treated plants. The rapid (within first 4 h) and marked effect of treatment on sugar concentration in the roots showed that, in the absence of the vegetative apex, more photosynthate was transported to the root.

Nitrate concentration decreased in all plant parts during the initial (4-h) light period following removal of the vegetative apex (Fig. 1, M–P). Subsequently, nitrate concentration of roots, unifoliolate leaves, and cotyledons of treated plants increased through the end of the dark period and then remained relatively unchanged in leaves and cotyledons but decreased in roots during the second period of illumination. Control plants exhibited the expected diurnal pattern of change in nitrate concentration in roots and leaves (increased in dark, decreased in light). Removal of the vegetative apex resulted in lower nitrate concentration in the stems at all samplings, compared with controls (Fig. 1N). The lower nitrate concentration in stems was in large part due to the marked increase in stem dry weight associated with vegetative apex removal (Fig. 1B). Vegetative apex removal was also associated with lower nitrate concentrations in the cotyledons during the second period of illumination, relative to controls (Fig. 1P). After the second sampling (4 h), unifoliolate leaf nitrate concentrations of treated plants were enhanced, being significantly higher than controls on the last three sampling times. The significant differences in nitrate concentration and dry weight content of the unifoliolate leaves by the end of the experimental period indicated that removal of the vegetative apex resulted in increased translocation of nitrate to the unifoliolate leaf, relative to controls. Transpirational differences did not appear to account for this difference inasmuch as transpiration was slightly greater for control plants (211 ± 13 g H_2O/pot of six plants) than for treated plants (184 ± 19 g H_2O/pot), measured by weighing the pots.

Unifoliolate leaf NRA was significantly higher in plants with vegetative apices removed than in control plants, during the last four sampling periods (Table I). These results were consistent with the higher nitrate concentration in unifoliolate leaves of treated plants, relative to controls, during the same sampling periods (Fig. 1M). Although the NR activities were closely related to nitrate concentrations, the activities may reflect a differential flux of nitrate into the various plant parts as suggested by Shaner and Boyer (10). Such observations provide support for the view that vegetative apex removal enhances transport of nitrate to the leaves. The NRA in roots was consistently higher in treated plants than in control plants throughout the experimental period (Table I). However, the differences between control and treated plants were seldom significant within any particular sampling time.

The concentration of reduced-N in the stems of treated plants remained constant over the 31-h sampling period in contrast to a steady decline in reduced-N concentration in stems of control plants (Fig. 1, Q–T). Treatment had little effect on the concentration of reduced-N in cotyledons or roots, while the reduced-N concentration in unifoliolate leaves was significantly less at 28 and 31 h after treatment initiation, compared with controls. These differences in concentration are related, at least in part, to differences in leaf dry weight of treated and control plants (Fig. 1A).

Vegetative Apex (Apical Meristem and Trifoliolate Leaves). The dry weight of the vegetative apices (removed from control plants at each harvest) increased 3-fold over the 31-h sampling period (Table II). The average rate of increase in dry weight during the first illumination period (14 h) was 1.7 mg plant^{-1} h^{-1} as compared with 0.35 mg plant^{-1} h^{-1} during the dark period (10 h) and 2.9 mg plant^{-1} h^{-1} during the second illumination period (7 h). The changes in concentration of starch, sugars, and reduced-N in the vegetative apex during the 31-h period (Table II) were similar to changes of comparable components in the

Table III. *Incremental Changes in Dry Weight and Content of Sugars, Starch, Nitrate-N, and Reduced-N of Various Plant Parts of Soybean within Four Time Periods*

Values presented were calculated from Figure 1 by determining the absolute change in component content from the beginning to the end of each of the following time periods; first light period (0–14 h after treatment), dark period (14–24 h after treatment), second light period (24–31 h after treatment), and total period (0–31 h after treatment). Treatment (T) = removal of vegetative apex, *i.e.* meristem and expanding trifoliolate leaf at time 0; control (C) = intact control plants. Values without sign indicate gain while values with negative sign indicate loss during that time period.

Plant Part	Dry Wt		Sugars		Starch		NO_3^--N		Reduced-N	
	C	T	C	T	C	T	C	T	C	T
	Δ *mg plant*$^{-1}$·*time period*$^{-1}$						Δ *μg plant*$^{-1}$·*time period*$^{-1}$			
First light period (14 h)										
Unifoliolate leaves	38.0	39.0	4.1	2.4*[a]	11.1	12.4	113	250	1025	1275
Roots	2.8	11.8*	0.2	0.9*	0.2	0.1	50	325*	38	275*
Stems	5.0	10.2*	0.3	0.3	0.5	0.6	113	88	−75	463*
Cotyledons	−0.8	0.7	0.4	0.4	0.0	0.5	25	113	−263	−288
Vegetative apex	24.1		0.5		0.6		49		1092	
Dark period (10 h)										
Unifoliolate leaves	−16.8	−14.2	−2.7	−2.1	−10.8	−12.1	75	63	−13	−225
Roots	10.8	11.6	0.0	−0.2*	0.2	0.2	313	325	125	575*
Stems	−0.7	1.0	−0.2	−0.2	−0.2	−0.4	25	−13	−175	125*
Cotyledons	−2.6	−2.4	−0.4	−0.3	−0.6	−0.4	13	−13	−263	−88
Vegetative apex	3.5		−0.3		−0.4		30		463	
Second light period (7 h)										
Unifoliolate leaves	26.7	39.6	3.6	2.0*	9.3	10.7	−50	225*	700	1013*
Roots	−0.7	2.7	0.1	0.2	0.1	0.2	−189	−150	−113	−25
Stems	2.6	6.6	0.1	0.2	0.1	0.0	38	38	150	163
Cotyledons	4.3	6.8	0.1	0.1	0.2	0.4	88	63	175	700*
Vegetative apex	20.1		0.6		1.1		74		588	
Total period (31 h)										
Total plant	116.3	113.4	6.5	3.9*	11.3	11.5	792	1314*	3524	3963*

[a] *, significant differences at 5% confidence level between control and treatment, within a plant part and measured component.

unifoliolate leaves (Fig. 1, E, I, Q). In contrast, nitrate concentration in the vegetative apex increased throughout the 31-h period (Table II) while nitrate concentration in the unifoliolate leaves declined during the second period of illumination (Fig. 1M). These data indicated that the rate of nitrate flux to the vegetative apex increased faster than did development of nitrate assimilation capabilities.

Incremental Changes in Dry Weight and Constituent Content. Because changes in concentration of components in various plant parts within the 31-h experimental period reflect changes in both dry weight accumulation and uptake, metabolism, and/or synthesis of the various components measured, calculation of changes in content of components provides an alternative way of evaluating the effects of vegetative apex removal. The absolute gain (or loss) in content of the various components measured are presented in Table III; with reference to the three consecutive time periods (14-h light, 10-h dark, and 7-h light) and to the entire 31-h experimental period.

First Light Period (14 h). The effect of vegetative apex removal on changes in dry matter and constituent content was most evident with roots and stems (Table III). Roots of treated plants showed greater increases in dry weight (4.2-fold), sugar (4.5-fold), nitrate-N (6.5-fold), and reduced-N (7.2-fold) than controls. The stems of the treated plants also showed greater increases in dry weight and reduced-N content than stems of control plants. In the absence of the vegetative apex, it appeared that the phloem transport components were shunted to root and stem. Treatment had little effect on changes in dry weight or reduced-N contents of unifoliolate leaves and cotyledons relative to controls. However, the increase in nitrate content of the unifoliolate and cotyledonary leaves was slightly greater (significant at the 10% level) in treated than in control plants. Whether the increased nitrate uptake and transport to the shoot was a direct effect (enhanced uptake and transport), an indirect effect (root growth), or due to hormonal changes is not clear.

Dark Period (10 h). During this period, the increase in reduced-N content in the root was greater for the treated than for the control plant (Table III). This increase in reduced-N is consistent with the view that nitrate reduction would be enhanced by the higher initial sugar concentration, the subsequent marked loss of sugar during the dark period (Fig. 1K), and an adequate supply of nitrate (Fig. 1O) in the root of the treated plant. Except for the roots, all parts of treated and control plants showed a decrease in carbohydrate (sugar and starch) content during the dark period (Table III). The loss of carbohydrates from the aerial portions of all plants can be attributed to dark respiration and translocation. In the treated plants, the loss of reduced-N from the unifoliolate and cotyledonary leaves was only partially accounted for by the gain in reduced N by the stems. Whether some of this leaf-N was transferred to the roots is not known. For the control plants, the loss of reduced-N from the aerial parts (unifoliolate leaves, stems, and cotyledons) was balanced by the gain of reduced-N by the vegetative apex. The gain in amount of reduced-N (0.463 mg N) by the apex equates to 3.96 mg amino acids. Thus, the gain in dry weight of the apex appeared to be associated with a gain in reduced-N (organic forms) rather than carbohydrates *per se.*

Second Light Period (7 h). The unifoliolate leaves of both treated and control plants showed increases in dry weight, sugar, starch, and reduced-N during the second light period. The unifoliolate leaves of the treated plants accumulated more dry weight (significant at the 10% level), nitrate, and reduced-N than leaves of the control plants. The greater accumulation of nitrate and reduced-N, in conjunction with the higher level of NRA, indicated that nitrate flux to leaves was greater in treated than in control plants. Such changes infer that photosynthesis and nitrate reduction in the leaves of treated plants were not impaired by vegetative apex removal. What portion of the relatively large increase in reduced-N content of the cotyledons of the treated

plant was derived from *in situ* reduction of nitrate is not clear. Comparison of the changes in reduced-N content of the cotyledons of treated and control plants indicated that the cotyledons of the treated plant were acting as an alternate sink.

Total Sampling Period (31 h). Despite marked differences between treated and control plants in distribution of dry weight within plant parts during light and dark periods, the total plant dry matter and carbohydrate contents (including vegetative apices for controls) were similar after 31 h (Table III). In contrast, nitrate uptake and reduction over the 31-h sampling period was increased by the vegetative apex removal treatment (Table III).

CONCLUSIONS

The initial response to removal of the vegetative apex was a rapid (first 4 h) increase in sugar concentration of the root (Fig. 1K). Although the initial movement of sugar to the root, in response to treatment, appeared to be the initiating factor in a sequential chain of events, the measurements made do not define cause and effect factors. The removal of the vegetative apex is a drastic treatment and may invoke changes other than those parameters measured (*e.g.* hormonal balance). The increase in root sugar concentration was followed by a significant increase in root dry weight (Fig. 1C) and nitrate content (Table III), relative to controls over the first 14-h light period. Nitrate reduction also increased during the dark and second light periods in response to vegetative apex removal (Table III). The increase in root, unifoliolate leaf, and stem dry matter content of the treated plants (Fig. 1, A–C), relative to controls, indicated that these structures served as alternate sinks following removal of the vegetative apex. Over the 31-h period, we concluded that vegetative apex removal had little effect on dry weight accumulation by the whole plant, but did enhance nitrate uptake, transport, and reduction (Table III). The changes in metabolic activities of the soybean seedlings in response to removal of the vegetative apex was consistent with the improved plant vigor achieved by the established practice of pruning horticultural and household plants. Additional implications are that nitrate uptake and metabolism in soybeans may be limited by flux of carbohydrate to the roots, although a hormonal effect due to vegetative apex removal cannot be ruled out.

Acknowledgment—The authors wish to express their gratitude to Donna Suarez for graphics.

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