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Carbon and Nitrogen Assimilation and Partitioning in Soybeans Exposed to Low Root Temperatures[1]

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ABSTRACT

Low root temperature effects on vegetative growth of soybean (Harosoy 63 × *Rhizobium japonicum* USDA 16) were examined in 35 day old plants exposed to temperatures of 15°C (shoots at 25°C) for an 11 day period. Duing this period various aspects of C and N assimilation and partitioning were monitored including shoot night and nodulated root respiration, C and N partitioning to six plant parts, C_2H_2 reduction, H_2 evolution, leaf area, transpiration, net photosynthesis, and N_2 fixation. The low temperature treatment resulted in a decrease in the net rate of N_2 fixation but nitrogenase relative efficiency increased. In response, the plant retained N in the tissues of the nodulated root and decreased N partitioning to young shoot tissues, thereby inducing the remobilization of N from older leaves, and reducing leaf area development. The leaf area specific rate of net photosynthesis was not affected over the study period; however, shoot and nodulated root respiration declined. Consequently, C accumulated in mature leaves and stems, partly in the form of increased starch reserves. Three possibilities were considered for increasing low temperature tolerance in nodulated soybeans: (a) decrease in temperature optima for nitrogenase, (b) increased development of nodules and N_2 fixation capacity at low temperature, and (c) alterations in the pattern of C and N partitioning in response to low temperature conditions.

The introduction of soybeans and other crops of subtropical origin into cool temperate regions has prompted numerous studies into the effects of photoperiod and temperature on the growth and yield of these plants (3, 11). In Canada, early vegetative growth of soybean occurs under elevated shoot temperatures but low soil temperatures. Relatively little is known of the way in which root temperature adversely affects plant growth and N assimilation at temperatures greater than those known to disrupt membrane integrity (17). To date most studies have been restricted to an examination of the short term effects of low temperature on individual physiological processes. For example, previous studies have reported on the immediate effects of root temperature on nitrogenase activity (2, 6, 15), nodule development (8, 22), transpiration (18), photosynthesis (12), dark respiration (4), dry matter partitioning (20), or N transport (25). However, a full appreciation of the cause/effect relationships associated with low temperature can be obtained only in longer term studies in which there is an attempt to integrate these physiological processes with an analysis of whole plant growth and C and N partitioning.

In the present study, this integrative approach is employed using vegetative plants reared at 25°C before being exposed to a root chilling treatment of 15°C. Therefore, the effect of root chilling was examined on vegetative growth as distinct from germination, emergence, flowering, or pod filling.

MATERIALS AND METHODS

Plant Material and Experimental Treatments. Seeds of soybean (*Glycine max* L. Merr) cv Harosoy 63 were inoculated at sowing with *Rhizobium japonicum* USDA 16 (a strain lacking uptake hydrogenase activity [1, 15]) and cultured in Turface (I.M.C., Des Plaines, IL) as described previously (15). Plants were maintained at 25°C and were provided with 0.5 mM KNO_3 in nutrient solution for the first 4 weeks of growth and subsequently with nutrient solution lacking combined nitrogen. The nutrient solution also contained (μM): K 2750, Ca 1500, Mg 980, P 630, S 1500, Na 0.1, Fe 75, Cl 3980, B 31, Mn 10, Zn 1.0, Cu 1.0, Mo 0.5, and Co 0.2. Thirty-five d after sowing, with all plants at the same developmental stage (*i.e.* late vegetative growth, unfolding of the fifth trifoliate leaf) 70 pots were chilled to 15°C as described by Layzell *et al.* (15) and maintained at this temperature for the following 11 d. The shoot temperature of the treated plants and the shoot and root temperatures of the control plants were maintained at 25°C throughout the 11 d study period.

Plant Harvest and Tissue Analysis. At 35, 39, and 46 d after sowing, 15 plants were harvested from each treatment, and were separated into eight organ classes, including roots (Rt[2]), nodules (Nd), primary stems + petioles of the PL (PS), primary leaves (PL), other stem and petioles (MS) and leaves (ML) present at 35 d, and new stem + petiole (NS) and leaves (NL) produced during the course of experiment.

Samples were dried for at least 48 h at 70°C, cooled in a desiccator before weighing, and ground to pass a 40 mesh sieve in a Wiley mill (model 3381-610). Subsamples (2–3 mg dry weight) were analyzed for total C and N content using a CHN Elemental Analyzer (Controlled-Equipment Corp. model CEC 240). The increments in whole plant N between two successive harvests were used as measures of N_2 fixation. Aliquots of 20 to 50 mg of plant material were extracted with 90% ethanol and the residues analyzed for starch content. Starch was gelatinized and free sugars eliminated by exposure to 0.1 N NaOH for 3 h at 95°C. After adjusting the pH to 5.5, the samples were digested overnight with α-amylase and amyloglucosidase at 37°C and glucose estimated enzymically as described by Jones (13).

Gas Exchange Analysis. During the study period (35–46 d

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[2] Abbreviations: Nd, nodules; Rt, roots; PS, primary stem and petioles; PL, Primary leaves; MS, mature stem and petioles; ML, mature leaves; NS, new stem and petioles developed during the study period; NL, new leaves; StR, shoot night respiration; RtR, nodulated root respiration; RE, relative efficiency.

from sowing) four randomly selected plants from each treatment were continuously monitored for root CO_2 exchange and H_2 production using an automated 12-channel gas exchange system as described previously (15). H_2 was detected using a continuous flow, solid state detector (16).

An additional plant from each treatment was used to measure CO_2 exchange at night by IR gas analysis (ADC, model 225) from entire, intact shoots on a continuous basis using a Teflon (0.1 FEP Type A film, Dupont, DE)-walled 27 L aluminum-framed cuvette equipped with a rotary fan for air circulation. While the cuvette was not temperature controlled, the Teflon wall ensured that temperature differentials were less than 1.5°C. Plants were replaced every 2nd d and were harvested upon removal. Air flow rates were adjusted to ensure CO_2 differentials in the shoot chamber of 20 to 40 $\mu l \cdot L^{-1}$ at night, and in the roots of 100 to 200 $\mu l \cdot L^{-1}$.

Acetylene reduction was assessed in a continuous flow-through system using a four channel flow diverter and a 1 ml autoinjection valve (Carle, model 5618/4201) on a gas chromatograph equipped with a flame ionization detector (Shimadzu Corp., model GC 8AIF). Ethylene separation was achieved on a Poropak N column (⅛ × 2 m) at 100°C and N_2 as the carrier gas. Each day the root systems of two to four plants were flushed with about 200 ml/min of 10% C_2H_2 in air, which resulted in an ethylene differential between inlet and outlet gas streams of up to 200 $\mu l \cdot L^{-1}$.

The relative efficiency of the symbiosis was calculated as either:

$$RE(C_2H_2) = 1 - \frac{(H_2 \text{ production in air})}{(C_2H_2 \text{ reduction})}$$

or (24)

$$RE(N_2) \frac{\text{N increment}}{(\text{N increment} \times 3 + H_2 \text{ evolution})}.$$

In *Rhizobium* strains lacking uptake hydrogenase activity such as USDA 16 (15), these estimates of RE were considered to be measures of the electron allocation coefficient of nitrogenase.

Each day during the study period whole plant transpiration rates were determined gravimetrically using ten randomly chosen plants per treatment (19).

Statistical Analysis. All raw data are presented as mean ± SE and were statistically compared ($P>0.05$) using the Student's *t* test. The errors associated with the calculated values (*e.g.* C and N increments; C and N partitioning patterns; development of new N fixation capacity) were determined using standard statistical procedures which assumed that the raw data values were independent of one another. However, since most of the data used in these calculations were highly interdependent (*e.g.* C [or N] contents at subsequent harvest days in calculating the C [or N] increment), it is likely that the errors associated with these values greatly overestimated the true error (*cf.* 19). Nevertheless, these statistical criteria were applied to the data and unless otherwise noted all comparisons stated in the text were statistically different at $P>0.05$.

RESULTS

Plant Growth. Over the first 4 d of growth the relative growth rates of the 15°C treated plants were 1.5 times that in the control (25°C) plants (Table I), but in the 2nd growth period (39–46 d) the growth rates of the plants from the two populations were not significantly different. Much of the greater dry matter accumulation in the treated plants relative to the control plants was attributed to the growth of the MS or ML tissues (Fig. 1). Over the entire study period, the NL and NS tissues accumulated less dry matter in the treated plants while the other plant fractions accumulated slightly more dry matter at root temperatures of

Table I. *Relative Growth Rate of Organ Groups from Soybean Plants Exposed to Root Temperatures of 25 or 15°C Over Two Subsequent Periods in Late Vegetative Growth*

Values were determined from three sequential harvests of 15 to 30 plants each. Student's *t* test was used to compare temperature treatments and values which were significantly different are marked with an asterisk.

Plant Organ Group	Relative Growth Rate			
	35 to 39 d		39 to 46 d	
	25°C	15°C	25°C	15°C
	g dry wt·g⁻¹ dry wt·d⁻¹			
Whole plant	0.061*	0.092	0.073	0.067
Leaves	0.052*	0.096	0.085	0.074
Stem + petioles	0.109*	0.135	0.081	0.092
Nodules	0.075	0.092	0.049	0.061
Roots	0.047	0.064	0.056	0.040

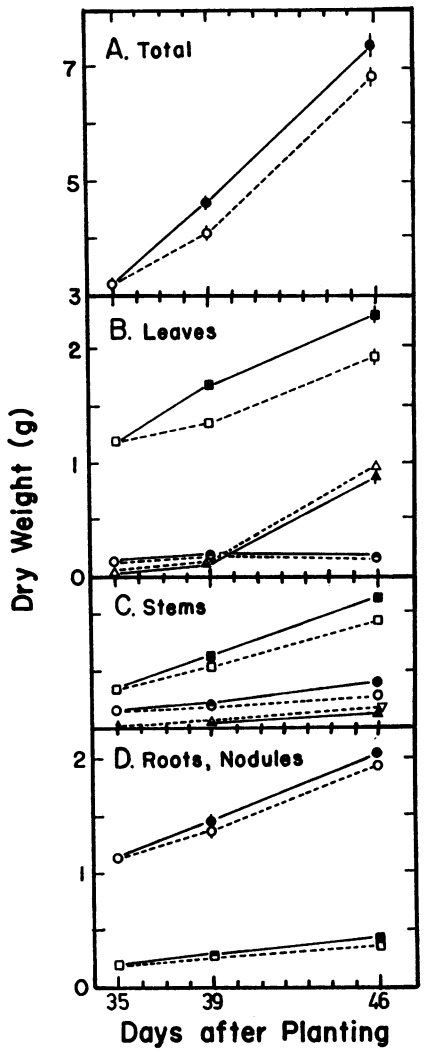

FIG. 1. Dry weight accumulation in soybean plants in which the nodulated roots were exposed to temperatures of 15°C (——, ●, ■, ▲) or 25°C (- - - - -, ○, □, △). A, Whole plant (●, ○). B, Leaf fractions including the PL (●, ○); ML at 35 d (■, □); and NL (▲, △). C, Stem and petiole fractions including PS (●, ○) MS at 35 d (■, □) and NS tissue (▲, △). D, Nodulated root fractions, including Nd (■, □) and Rt (●, ○). Values are presented as mean (n=15) ± SE.

15°C than at 25°C (Fig. 1).

Leaf Area. Despite the greater dry matter accumulation in the ML of the treated plants (Fig. 1B) the leaf area of this plant fraction was not different from the control (Fig. 2A). Consequently, the specific leaf weight of the ML in the treated plants was 1.2 to 1.3 times that in the control plants (Fig. 2B). In contrast, the leaf area of the NL in the treated plants was, at 46 d, only 66% of that in the control plants, while the specific leaf weight of these NL was also greater than in the control plants.

Respiration. A sharp decrease in nodulated root respiration (mg C evolved.plant^{-1}·d^{-1}) was measured following transfer to 15°C and a slow recovery in respiration reflecting the steady growth of this organ over the 11 d study period (Fig. 3). Expressed on a specific activity basis, RtR averaged 196 ± 19 μmol CO_2·g^{-1} dry weight·h^{-1}, in the control plants and 108 ± 8 μmol CO_2·g^{-1} dry weight·h^{-1} in the 15°C treated plants over the study period (Q_{10} = 1.81 ± 0.07). A recovery in the specific respiration rate of chilled plants was not apparent during the 11 d study period.

Despite similar shoot temperatures, the plants exposed to lower root temperatures displayed shoot night respiration rates which averaged 73% of that in the control plants in the 11 d study period. Expressed per plant (Fig. 3), rates of respiratory C loss increased over the study period, reflecting the increased biomass of the shoot organs.

Net Photosynthesis and Transpiration. Estimates of the percent C in dry matter ranged from 38 to 42% in Rt, MS, PS, NS, and ML and from 43 to 44% in Nd and NL tissues. These values were combined with measurements of plant dry weight to obtain a measure of the C increment in each plant organ. The sum of the C gained by the whole plant and the C lost through respiration over a given period (Fig. 3) was used as a measure of net photosynthesis. Using this approach net photosynthesis in root chilled plants were 99 and 87% of that in the control plants over the first 4 d and subsequent 7 d study periods, respectively. However, when expressed on a leaf area basis, no significant differences were observed between the whole plant photosynthetic rate in the control and the treated plants in any growth interval.

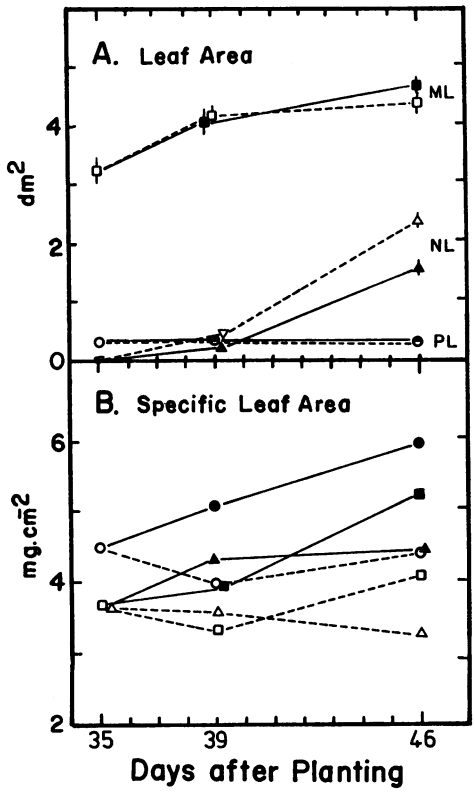

FIG. 2. Leaf area development (A) and specific leaf weight (B) in soybean plants in which the nodulated roots were exposed to temperatures of 15°C (●, ■, ▲) or 25°C (○, □, △). The leaf fractions included the PL (●, ○), the ML at 35 d (■, □), and the NL tissue (▲, △). Each value in part A represents the mean (n=10) ± SE.

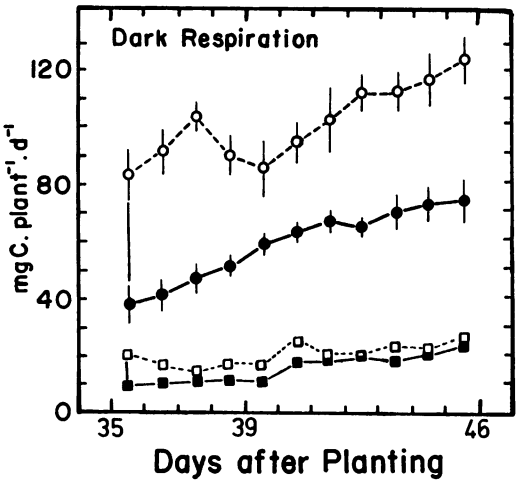

FIG. 3. Whole plant dark respiration rates of nodulated roots (●, ○) and shoots at night (■, □) in soybean plants in which the nodulated roots were exposed to temperatures of 15°C (●, ■) or 25°C (○, □). Values for nodulated root respiration were presented as the mean (n=4) ± SE while those for shoot night respiration were an integrated measure from a single plant selected at random from the population each day.

Following transfer to low root temperatures, whole plant transpiration immediately declined to 72% of the control rates and remained approximately at this level over the 11 d study period (data not shown).

N_2 Fixation, C_2H_2 Reduction and H_2 Evolution. After 4 and 11 d exposure to low root temperatures the N content of dry matter was significantly lower than in the control plants. The greatest differences in the N content were observed in shoot tissues which at 46 d after sowing, contained 2.9% N in dry matter in the control plants, but only 2.1% N in the treated plants. By comparison, the average N content of the nodulated root at these harvest dates was not significantly different between treatments. By combining these data on N content with the information on dry weight increment (Fig. 1) it was possible to determine the N increment and this was used as a measure of the N_2 fixation rate. Using this approach the 15°C treated plants were found to fix an amount of N which was 80% and 62% of that in the control plants over the 35 to 39 d and 39 to 46 d periods, respectively. However, due to the compounded errors inherent in these calculations, it was not possible to prove significant differences ($P>0.05$) in the N increment between the two temperature treatments. Nevertheless, at 46 d, N content per plant was significantly lower in the treated plants.

In addition to the direct measure of N_2 fixation by nitrogen increment, the rates of C_2H_2 reduction and H_2 evolution were measured throughout the study period (Fig. 4). The specific activity of nodule C_2H_2 reduction declined with root chilling to about 45% of the control rate resulting in a Q_{10} value averaged over the period 39 to 46 d of 2.2 ± 0.2 (Fig. 4A). Also, root chilling resulted in a large reduction in H_2 evolution resulting in an average Q_{10} over the 11 d period of 3.7 ± 0.44 (Fig. 4B). Consequently, the RE(C_2H_2) increased with root chilling from 0.51 to 0.67 at 39 d and 0.78 at 46 d. Similarly, the RE(N_2) values were higher in the treated plants (0.83 ± 0.07 and 0.64 ±

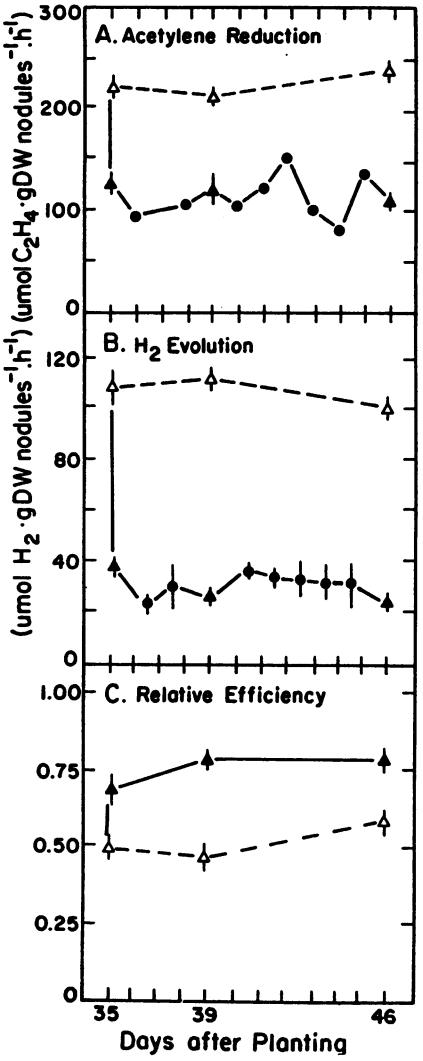

FIG. 4. Time course of the specific rates of C_2H_2 reduction (A) and H_2 evolution in air (B) and the resulting Relative Efficiency (C) in root chilled (●, ▲) and control (△) soybean plants. Data points denoted by triangles (▲, △) represented samples in which H_2 evolution and C_2H_2 reduction measurements were carried out on the same plants. These data were used to calculate the RE(C_2H_2) values (C). Data points with SE bars represent the mean of four samples; other data points are the mean of two samples.

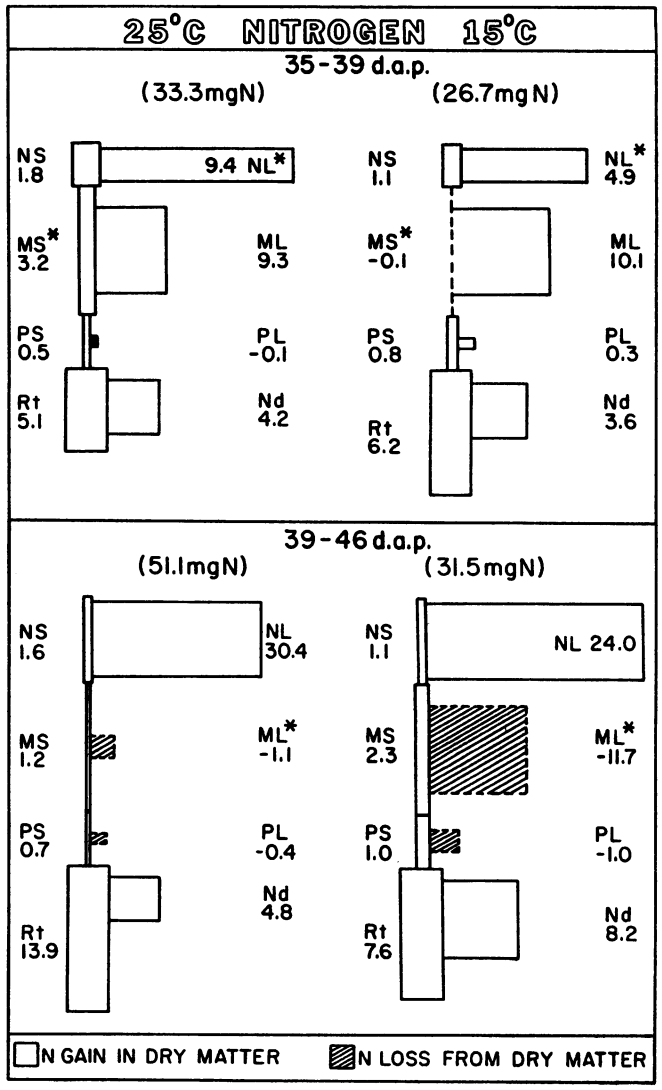

FIG. 5. The pattern of N partitioning in soybean plants in which the nodulated roots were exposed to temperatures of 15 or 25°C during the growth periods 35 to 39 d and 39 to 46 d. The N increment or decrement of each plant part is depicted as a series of rectangles; the areas of which are proportional to the N_2 fixation rate (values in parenthesis) for each population of plants. All values presented on the diagrams are as mg N per plant per growth interval and an asterisk denotes those N increments which were significantly different between treatments ($P>0.05$).

0.07 for the 35–39 d and 39–46 d study periods, respectively) than in the control plants (0.67 ± 0.09 and 0.57 ± 0.05 for the 35–39 d and 39–46 d study periods, respectively).

N Partitioning. In addition to the direct effects on N_2 fixation, low root temperatures dramatically altered the way in which N was partitioned within the whole plant (Fig. 5). In the 25°C control plants over the period 35 to 39 d, the ML and NL were the major sinks for N, together consuming 56% of the N_2 fixed (Fig. 5). Over the subsequent 7 d period (39–46 d) the NL fraction alone attracted 60% of the N_2 fixed while the ML ceased to be a sink for N. It was against this background of ontogenetic changes in N partitioning that the low root temperature treatment was imposed.

Three major effects of low root temperature on N partitioning were observed (Fig. 5):

(a) N retention by the nodulated root. Despite the lower N fixation rate in the treated plants, the N increment of the nodulated root was not different from that in the control population over either growth interval. Therefore, as a percentage of the total N fixed, the N increment of the Nd plus Rt tissue of the treated plants accounted for 37% to 50% of the recently fixed N but only 28 to 37% of the N fixed in the control plants (Fig. 5). Consequently, in the 15°C treated plants the shoot systems relied on only 16 to 17 mg N per growth interval while 24 to 32 mg N per growth interval was used to support shoot growth in the control plants.

(b) Decrease in N partitioning to young shoot tissues. Over the period 35 to 39 d, the NS and NL in the treated plants acquired only 61% and 52%, respectively, of the N increment of similar plant organs in the 25°C treated plants (Fig. 5). During the same period, the ML accumulated a similar amount of N in the treated (10.1 mg N; 38% of N fixed) and control (9.3 mg N; 28% of N fixed) plants. Therefore, during the first 4 d of exposure to low root temperatures, the shoots of the treated plants not only received proportionally less of the N fixed but the ML gained a disproportionate share of the available N at the expense of the NS, NL, and MS.

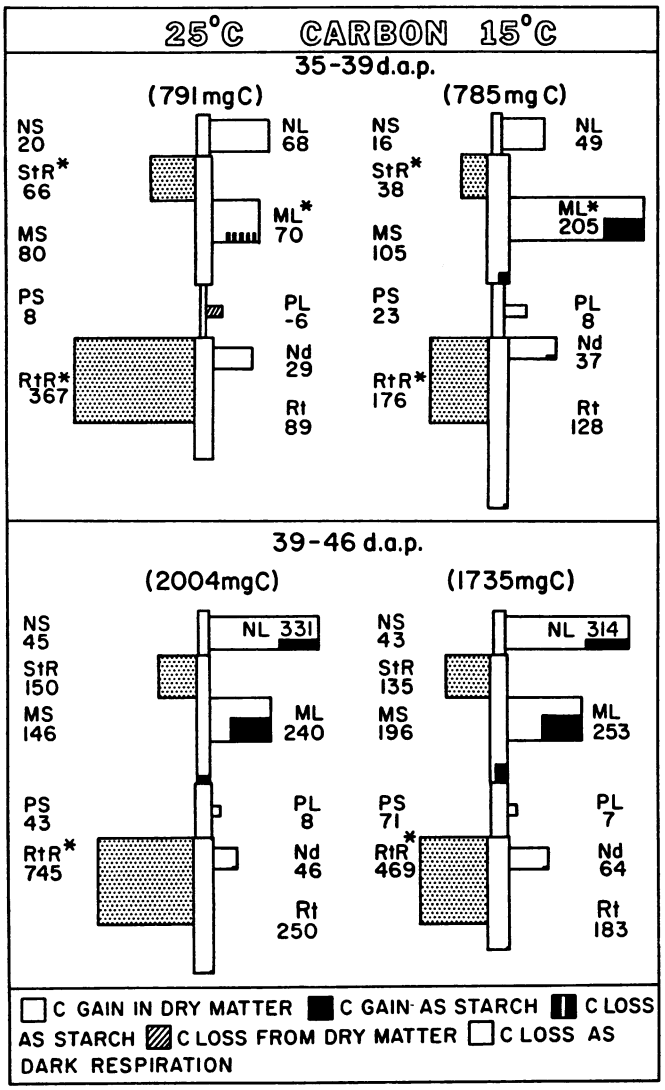

FIG. 6. The pattern of C partitioning in soybean plants in which the nodulated roots were exposed to temperatures of 15°C or 25°C over the growth periods 35 to 39 d and 39 to 46 d. The C increment (or decrement) of each plant part; the C increment in the starch pool of NL, ML, NS, MS, Rt, and Nd; and the C respired by the shoot (StR) or nodulated root (RtR) are depicted as a series of rectangles; the areas of which are proportional to the net photosynthetic rate (values in parenthesis) for each population of plants. All values presented on the diagrams are as mg C per plant per growth interval and an asterisk denotes those C increments which were significantly different between treatments ($P>0.05$).

(c) Remobilization of N from older leaves. Over the period 39 to 46 d, the N stress induced by the low root temperature treatment resulted in N remobilization from the ML such that by 46 d they lost an amount of N equivalent to 22% of the N initially present in the tissues at 39 d. By comparison, the N lost by the ML in the control plants was equivalent to only 2% of the N present at 39 d. Presumably, in the treated plants, this N was transported predominantly to the NS and NL tissues which became major sinks for N over this period, acquiring an amount equivalent to 80% of the N fixed. Despite the remobilization of N from the ML over the 11 d study period, the NL of the treated plants received only 29 mg N compared with the 40 mg N received by the NL in the control plants.

C Partitioning. The pattern of C partitioning in the control and treated plants over the two study periods is shown in Figure 6. In the 25°C plants there was little change with time in the proportion of net photosynthate utilized in the growth of ML, MS, Nd, and Rt tissues, while the C utilized for NL and NS growth increased from 11 to 19% over the initial 4 and subsequent 7 d study periods, respectively. The low temperature effects on C partitioning and utilization were classified into three groups (Fig. 6):

(a) Decrease in respiratory C evolution. Following transfer to low root temperatures the specific activity of nodulated root respiration declined to 55% of the control rate and the C saved (equivalent to up to 24% of net photosynthesis) accumulated in dry matter. Shoot night respiration also declined following exposure to low root temperature and made available for growth an amount of C equivalent to 1 to 3% of net photosynthesis.

(b) Carbon accumulation in shoots. Since less C was lost in dark respiration in the treated plants, more was available for partitioning to dry matter in both shoot and root tissues. Of this extra C in the treated plants, the shoot organs received the greater proportion (Fig. 6), resulting in an increase in the shoot:nodulated root ratio. This was the opposite pattern to that observed for N partitioning in which the below ground parts retained the majority of the available N while the shoot organs became N limited.

(c) Increased starch deposition. In Nd, Rt, and MS, starch deposition was greater in the treated plants than in the control plants, but did not account for more than 10% of the C incremented in either treatment. However, within the ML of the root chilled plants starch deposition accounted for 15 and 41% of the C increment over the periods 35 to 39 d and 39 to 46 d, respectively. In contrast, starch reserves were depleted (equivalent to 21% of the C increment) in the leaves of control plants over the first 4 d period, while in the subsequent 7 d, starch deposition accounted for 36% of the starch increment.

DISCUSSION

The range of physiological measurements which was carried out in the present study permitted the reconstruction of a sequence of events which may be used to describe the effects of low root temperature on C and N assimilation and partitioning in these plants.

Direct Low Root Temperature Effects on Photosynthesis, Transpiration, Respiration and Nitrogenase Activity. Even though transpiration in the treated plants was inhibited to 72% of the rate in the control plants, the photosynthetic rate on a leaf area basis was not affected significantly over the study period. Consequently, as reported previously (11) the temperature stress resulted in an increased water use efficiency.

In contrast to C fixation, nodulated root respiration was inhibited to 55% of the control rate, giving a Q_{10} value (1.8) which was similar to that obtained in other studies of temperate or tropical legumes over the temperature range employed (4, 17). The reduction observed in shoot night respiration was thought to be a reflection of either a lower rate of protein synthesis in the N stressed shoot, or more starch deposition at low temperatures rather than the production of new cell and tissue biomass.

During the first 4 d of low temperature treatment total electron flow through nitrogenase as measured by C_2H_2 reduction or as (1.5 × N increment + H_2 evolution) was inhibited to 47 and 65% of the rates in the control plants, respectively. The Q_{10} values (2.1 and 1.5, respectively) are similar to other studies in which the short term effects of low temperatures were reported on C_2H_2 reduction activity (2, 15, 21).

Increase in Nitrogenase Relative Efficiency. Following transfer to low root temperatures, an apparent increase was observed in the RE(C_2H_2) of the symbiosis (Fig. 4). Similar results have been reported elsewhere in studies which have used either C_2H_2 reduction (2, 15), or H_2 evolution in Ar:O_2 (21) as a measure of

total electron flow through nitrogenase. However, to this date some questions remain concerning whether the observed increases in RE with low temperature reflected real changes in nitrogenase efficiency or inaccurate estimates of total electron flow through nitrogenase. For example, Bertelsen (3), working with excised pea nodules, and Thorneley and Eady (26) using purified *Klebsiella* nitrogenase reported that H_2 evolution in argon and C_2H_2 reduction were affected differently by temperature change. While there are inconsistencies in the results of these studies, both would predict an apparent increase in nitrogenase RE at low temperatures.

In the present study, when N increment was used as a direct measure of N fixation, the calculated RE(N_2) values were greater at 15°C than at 20°C (Fig. 4). These results strongly suggested that the changes observed in nitrogenase RE at low temperature were indeed associated with real changes in nitrogenase efficiency. The physiological basis for this phenomenon remains to be determined. In studies with isolated enzymes, a number of factors have been shown to affect the nitrogenase RE, including ADP/ATP ratio, ATP concentration, pH, ratio of nitrogenase subunits, or the rate of electron flow through the enzyme (10, 26). In addition, studies with whole plants or intact nodules have shown nitrogenase RE to be affected by both carbohydrate supply (7) and O_2 concentration (5), either of which likely would change in plants exposed to low root temperatures.

The net result of the change in nitrogenase RE was that instead of inhibiting N_2 fixation by 52%, over the period 35 to 39 d, as suggested by the C_2H_2 reduction measurements, it was inhibited by only 20% in the 15°C-treated plants compared with the 25°C plants.

Proximity to Source Determines Initial N Partitioning. Assimilate partitioning patterns have frequently been explained on the basis of a 'hierarchy of demand' in which the nutrient requirements of sinks closest to the source are satisfied first, and only excess nutrients pass on to other sinks (23). This approach may be used to explain the pattern of N partitioning over the period 35 to 39 d (Fig. 5). The increment of N and Nd and Rt tissues was similar in treated and control plants, while the shoots of treated plants received only 70% of the N received by the control shoots. Further, those sinks (PS, PL, ML, and MS) in the shoot physically near the source organ, accumulated N at a rate similar to that in the counterpart organs in control plants. Therefore, the N available for NS and NL growth in the treated plants was equivalent to only 30 and 52%, respectively, of that in the control plants.

Sink Limitation Affects C Partitioning Pattern. The reduction in nodulated root respiration following the low temperature treatment provided for growth an amount of C equivalent to an additional 24% of net photosynthesis. However, since the NS and NL were N limited, the growth of these two plant fractions was inhibited and the extra C was forced to accumulate in other plant organs. As the source of fixed C, the ML of the treated plants accumulated the greatest proportion, displaying a C increment over the period 35 to 39 d which was three times that observed in the ML of the control plants. Other workers have also reported excess C supply under low temperature conditions (3, 12, 20).

N Stress in Young Shoot Tissues Induces Remobilization of N from Older Leaves. An apparent N limitation of new shoot growth preceded the remobilization of the N reserves of ML. Apparently this N supported new shoot growth, for the amount of N received over the second growth period by the NS and NL tissues in the treated plants was, at 79% of that in the control plants, not significantly different from that in the control.

Poor Development of New N_2 Fixation Capacity Limits Future Plant Growth. Despite the low root temperature treatment the Nd were well supplied with both C and N. In the period 35 to 39 d, the Nd of the treated plants gained 126% of the C and 87% of the N partitioned to Nd dry matter in the control plants. In the subsequent 7 d period they accumulated 139% of the C and 171% of the N accumulated by the Nd in the control plants. However, despite the availability of nutrients, relatively little new N_2 fixation capability developed over the 11 d study period. For example, when whole plant N increments were compared over the initial 4 d and subsequent 7 d study periods, the rate of N accumulation in the treated plants increased by only 18% while in the control plants the N increment was 53% higher in the second growth period than in the initial growth period. Therefore, at low root temperatures, the production of new N_2 fixation capacity in the nodulated roots held at 15°C was only 34% of that in the nodulated roots growing at 25°C. Unfortunately, due to the compounded errors associated with these calculations, statistical differences between treatment and control could not be demonstrated. However, previous studies have demonstrated that low root temperatures may adversely affect the process of infection, bacteroid proliferation and nodule tissue differentiation (8, 22), even though nodules exposed to suboptimal temperatures may be larger than those grown at higher temperatures (8, 22, 25).

Potential for Improvement of Low Temperature Tolerance in Nodulated Soybeans. The sequence of events outlined above was consistent with the concept of a limitation of biosynthesis and cell division at low temperatures resulting from N limitation. Using this interpretation it was possible to identify specific aspects of the plants' response which may account for the plants' relative intolerance to low temperatures and subsequently to propose where improvements may be possible.

(a) Nitrogenase activity and efficiency. The effect of temperature on nitrogenase (C_2H_2 reduction) activity was only partially offset by an increase in the efficiency of N_2 fixation, and resulted in a net 25% inhibition in the rate of N_2 fixation at low temperatures compared with the control plants. Since the optimal temperature for C_2H_2 reduction in *Rhizobium* has been shown to vary with strain (15, 22), soybean symbioses may exist in which temperature changes of 25 to 15°C will have little adverse effect on nitrogenase activity. Alternatively, an acclimation of N_2 fixation to low temperatures may be possible as suggested by previous studies (6, 15) in which soybean C_2H_2 reduction activity recovered to control levels after 1 to 2 d of exposure to low root temperatures. However, it would be unlikely that further increases in nitrogenase RE are possible since, in the present study, the RE observed at 15°C was near the theoretical maximal value of 0.75 (24).

(b) Nodule development and N_2 fixation. At root temperatures of 15°C, the development of new N_2 fixing capacity between the two subsequent study periods was only 34% of that in plants in which roots were held at 25°C. C supply to the nodules was maintained over this period as reflected by high rate of nodule dry matter accumulation. Consequently, it may be possible to select for *Rhizobium* strains which are better able to develop within nodules of selected soybean cultivars, to utilize the available carbohydrate for the fixation of N_2 under low temperatures.

(c) C and N Partitioning. In the present study, the plants responded to the temperature stress by maintaining N supply to the nodules and roots while the shoot apical regions were N stressed. A similar N partitioning pattern was described in root chilled soybean dependent upon nitrate N (23) and in N_2 fixing *Trifolium subterraneum* (9). This phenomenon resulted in the accumulation of excess carbohydrate in the older shoot tissues, and the subsequent remobilization of N from the older leaves.

Thomas and Sprent (25) have shown that compared to a cold-sensitive variety of *Phaseolus vulgaris*, a tolerant cultivar partitioned proportionally more dry weight into shoots than into roots. Increased partitioning to shoots has also been documented

in arctic sedges, when exposed to lower growing temperatures (14). These results would suggest that tolerance to low temperature stress may be achieved in plants which are able to maintain or increase C and N supply to young shoot organs. However, in the present case this would have to be done at the expense of the nodule and root tissues which were not able to maintain the production of new N_2 fixation capacity despite the maintenance of N partitioning to below ground parts. Perhaps a partitioning pattern in which more N is proportioned to the shoots resulting in a N stress in below ground parts at low temperatures would result in an increased rate of nodule development and an increased capacity for N_2 fixation.

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