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Effect of Light, Dark, and Temperature on Root Nodule Activity (Acetylene Reduction) of Soybeans

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ABSTRACT

The objectives of this study were (*a*): to define the effects of light, dark, and temperature on nodule activity (acetylene reduction), and (*b*) to establish the contributions of reserve carbohydrate and recent photosynthate to the support of nodule function. An *in situ* assay of nodule activity was developed for use with intact, hydroponically grown soybeans (*Glycine max* [L.] Merr. cv. Calland).

Nodule activity of 35-day-old plants grown in controlled environment chambers decreased during a 10-hour dark period at 18 C, compared with activity during the preceding and subsequent 14-hour light periods at 27 C. In contrast, plants that were maintained at a constant 27 C did not vary in nodule activity during diurnally varying dark and light exposure. Nodules of plants exposed to diurnal 18 and 27 C in 24-hour continuous dark were less active at 18 C than at 27 C. At constant 27 C, nodule activity was sustained throughout the 24-hour dark period. Thus, nodule activity was independent of short term dark periods but dependent on temperature; nodule activity was decreased at the lower temperature.

Temperature also affected the nodule activity of plants maintained in the light. Exposure of shoots and roots of intact plants to the lower temperature (5 hours at 18 C) during the light period resulted in a marked decrease in nodule activity, compared with that of plants maintained at 27 C. Exposure of only the shoot portion to 18 C (roots were maintained at 27 C) resulted in a similar decrease in nodule activity.

Nodules of plants exposed to 10 days of diurnally variable dark, light, and temperature had high activity in the light at 27 C and low activity in the dark at 18 C. Nodule activity of plants at a constant 27 C was not affected by diurnally variable dark and light exposure throughout the 10-day period, although activity generally increased with time due to increased nodule mass. At a constant 27 C, nodules of intact plants in continuous dark sustained activity through 72 hours before declining to zero by 7 days. At diurnally varying 18 and 27 C in continuous dark, peak diurnal nodule activity was sustained through 5 days, and then declined to zero by 8 days. Analyses of the carbohydrate content of tissue harvested from this study suggested use of reserve photosynthate (primarily of shoot origin) in support of nodule activity in the absence of concurrent photosynthesis.

The nitrogenase-catalyzed reduction of N_2 by root nodules of soybean (*Glycine max* [L.] Merr.) depends upon host-plant photosynthesis to provide substrate for the generation of energy and reductant (3). Fixed carbon must also be supplied for the incorporation of reduced nitrogen into amino acids (19). Consistent with these requirements, Hardy and Havelka (8) concluded that photosynthate availability may limit nitrogenase activity in the *Rhizobium*-soybean association.

Recent reviews (5, 8) have summarized the effects of altered photosynthate production and nodule sink strength on nodule activity (C_2H_2 reduction). Enhancement of net photosynthesis (CO_2 enrichment, supplemental light) produced increased nodule activity. Nodule activity was also enhanced in studies that decreased competition by alternate sinks for limiting photosynthate (partial pod removal). Consistent with these results, treatments that decreased net photosynthesis (defoliation, shading, lodging) reduced nodule activity significantly. In addition, seasonal profiles of soybean root nodule activity have generally illustrated diminished activity during reproductive growth (*i.e.* periods of high competition for photosynthate) (6, 10, 18). In these studies the effect of extended change in photosynthate availability on nodule activity has been demonstrated. However, the response of nodule activity to short term (*e.g.* diurnal) change in photosynthetic activity has not been well defined. Hardy *et al.* (9) and Bergersen (1) have reported diurnal variation in soybean root nodule activity (C_2H_2 reduction), with observed maximal levels at periods of maximum light intensity in the field. In contrast, Fishbeck *et al.* (4), Hardy *et al.* (9), and Hart (12) have observed no diurnal change in the nodule activity of soybeans cultured in controlled environment conditions. Other studies have demonstrated diurnal variation in nodule activity (C_2H_2 reduction) to be dependent upon diurnal change in air temperature as well as light treatment (18).

Mederski and Streeter (16) have also observed diurnal variation in nodule activity (C_2H_2 reduction); activity in the dark was at or above 50% of the maximum rates measured during illumination. Other diurnal studies have also demonstrated the maintenance of a basal level of nodule activity during dark exposure (1, 4, 9, 12, 18). These results suggest that diurnal fluctuation in nodule activity (C_2H_2 reduction) may reflect a change in the composite contribution of recently produced and reserve photosynthate to the support of nodule activity. The significant input of reserve photosynthate in support of nodule activity has been further demonstrated by numerous studies in which the C_2H_2 reduction activity of detached nodules and of excised nodulated roots has been measured (1, 2, 7, 10, 15, 18).

The objectives of the present study were to define the effects of diurnal variation in light, dark, and temperature on soybean root nodule activity (C_2H_2 reduction) and to establish the contribution of reserve carbohydrate to the support of nodule function in the absence of concurrent host photosynthetic activity.

MATERIALS AND METHODS

Plant culture. Seeds of soybean (*G. max* [L.] Merr. cv. Calland) were inoculated with a commercial preparation of *Rhizobium japonicum* (Nitragin Co.,[1] Milwaukee, Wis.) and germinated in sand trays. At 7 days, seedlings were removed from the sand and

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

transplanted into a hydroponic rooting medium. Plants were suspended through the lids of 7-liter polyethylene containers and secured in split rubber stoppers with sponge fill. Plants were supplied with a modified Hoagland nutrient solution containing 2 mM $CaCl_2$, 1 mM $MgSO_4$, 1.25 mM K_2SO_4, 0.03 mM K-phosphate (pH 6.5), 12 μM Fe as sodium ferric diethylenetriamine pentaacetate, 7.75 μM H_3BO_3, 0.15 μM $CuSO_4$, 1.5 μM $MnSO_4$, 0.6 μM $ZnSO_4$, and 5 nM $(NH_4)_6Mo_7O_{24}$. Nitrogen, when included, was supplied as 4 mM urea (8 mM N). Solution pH was maintained near 6.4 by the inclusion of ion exchange resin columns as described by Harper and Nicholas (11). The air-driven pump action of these columns provided continuous aeration of the aqueous rooting medium. Plants were cultured in controlled environment chambers that provided a 14-h photoperiod at 27 C. Sixteen 1,500-mamp cool-white fluorescent and six 40-w incandescent lamps supplied an irradiance of 750 μE m^{-2} s^{-1} (400–700 nm wavelength as measured by LI-170 quantum sensor, Lambda Instruments Co., Lincoln, Neb.) at terminal leaf level during the photoperiod. During 10-h dark periods, chamber temperature was maintained at 18 C.

Measurement of Acetylene Reduction. An *in situ* method was developed to allow the measurement of root nodule nitrogenase activity (C_2H_2 reduction) without removal of the intact plant from its growth environment. During measurement, 2 liters of nutrient solution were removed from the 7-liter containers, exposing the well nodulated upper portions of the roots in a gaseous phase above solution level. Aeration was discontinued, and the containers were sealed to provide an air-tight incubation chamber around the nodulated roots of the four plants grown in each container. Acetylene (prepurified specialty grade) was passed through a H_2SO_4 trap and injected by syringe into the gaseous phase surrounding the nodulated roots, to a partial pressure of 0.1 atm. Polyethylene bags were fitted to the containers to maintain ambient atmospheric pressure throughout the assay. Samples of the incubation atmosphere were withdrawn at intervals (10 and 20 min) with 1-ml syringes. Following withdrawal of the second sample, the containers were opened to the atmosphere, nutrient solution was readded, aeration initiated, and plant growth allowed to continue. Ethylene production (C_2H_2 reduction) was determined from the samples by hydrogen flame gas chromatography as described by Hardy *et al.* (9), with column and chromatographic conditions as outlined by Sloger (17). No ethylene production was detectable in the system in the absence of added acetylene.

Diurnal Variation in Root Nodule Activity (C_2H_2 Reduction). Soybeans were grown in controlled environment chambers under the conditions described above. Urea nitrogen (8 mM N) was supplied in the nutrient solution from transplanting through 14 days. Subsequently, no source of combined nitrogen was available in the otherwise complete nutrient solution. At 35 days, root nodule activity (C_2H_2 reduction) was measured; all plants had 14 h of exposure to light (750 μE m^{-2} s^{-1} at plant top) at 27 C. Following initial measurement, four combinations of light and temperature treatments were initiated. These treatments were: (*a*) diurnal variation in dark, light, and temperature (10 h of dark at 18 C, 14 h of light at 27 C); (*b*) diurnal variation in dark and light at a constant 27 C; (*c*) constant dark with diurnal variation in temperature; and (*d*) constant dark with constant 27 C.

Specified temperature treatments in this and subsequent experiments (unless otherwise stated) were air temperature in the controlled environment chambers. Air temperature in the shoot environment required 10 min to stabilize following a temperature transition (from 18 to 27 C or 27 to 18 C). Solution temperature in the hydroponic root environment required 3.5 h to equilibrate to air temperature following an equivalent temperature transition.

Acetylene reduction activity was determined as previously described, and was repeated at 2- to 4-h intervals throughout the 24 h of a complete diurnal cycle. All measurements represented the average nitrogenase (C_2H_2 reduction) activity of four plants replicated three times for each of the four treatments. Values obtained from acetylene reduction measurement were expressed as μmol C_2H_4 produced $plant^{-1}$, h^{-1}.

Effect of Extended Dark and Temperature Treatments on Root Nodule Activity (C_2H_2 Reduction). Plants from the diurnal study previously described were also used to measure the effect of extended dark and temperature treatments on root nodule activity (C_2H_2 reduction). In this study the four light, dark, and temperature treatments imposed for the previous diurnal study were continued for a 10-day period. Root nodule activity (*in situ* C_2H_2 reduction) was monitored throughout the 10-day period by measurements made twice daily. Measurements were made as previously described, at 5 and 19 h during each 24-h period (*i.e.* at 5 h after the beginning of 18 C and at 5 h after the beginning of 27 C).

Carbohydrate Determinations. Plants harvested from the extended light and temperature study (at 10 days) were separated into nodule, root, and shoot fractions. Tissue was dried to constant weight in an oven at 70 C and ground through 20-mesh screen in a Wiley mill. Subsamples (0.1 g) were taken from the homogeneously ground tissue and extracted with 80% (v/v) ethanol. A phenol-sulfuric determination of sucrose equivalent content was conducted using the method described by Upmeyer and Koller (20). Following ethanol extraction, the tissue was air-dried at room temperature. Starch in the dried tissue was hydrolyzed with a preparation containing amyloglucosidase (EC 3.2.1.3., Sigma) in acetate buffer (pH 4.5) after the methods of Upmeyer and Koller (20). Phenol-sulfuric determination was used to quantitate the resultant sugar and derive the starch equivalent fraction (20). Values obtained for sugar and starch equivalent fractions and total carbohydrates were expressed as mg per g of tissue dry weight. Data were analyzed statistically by analysis of variance for the two-way factorial design.

Effects of Shoot Temperature on the Root Nodule Activity of Soybeans. Soybeans (cv. Calland) were germinated and grown in controlled environment chambers as previously described. Combined nitrogen was supplied as urea (8 mM N) through 24 days; no combined nitrogen was supplied subsequently. At the beginning of the photoperiod on day 35, plants were exposed to one of three shoot/root temperature treatments (27/27 C, 18/27 C, and 18/18 C). Independent control of root and shoot temperature was achieved by the use of a recirculating water bath (Masterline circulating bath, model 2095, Masterline, Inc., Marietta, Ohio). An insulated reservoir was added to the recirculating system and placed inside a controlled environment chamber. The 7-liter polyethylene containers used for *in situ* measurement of C_2H_2 reduction were partially submerged in the reservoir to facilitate independent temperature control of the rooting medium. Shoot temperature was maintained at the desired level by regulation of the air temperature in the controlled environment chamber.

RESULTS AND DISCUSSION

***In Situ* C_2H_2 Reduction.** The *in situ* assay procedure developed for the present study was found to be nondestructive. The intermittent 20-min exposures to C_2H_2 (0.1 atm) used for the measurement of nitrogenase activity (C_2H_2 reduction) were insufficient to impede nodule function. Mederski and Streeter (16) have previously shown that continuous nodule exposure (*in situ*) to 0.07 atm of C_2H_2 decreased activity appreciably. Linear ethylene production was observed immediately following the injection of acetylene into the incubation atmosphere. The production of ethylene by nodulated roots of soybeans exposed *in situ* to 0.1 atm (saturating) acetylene remained linear through 60 min (Fig. 1). Fishbeck *et al.* (4) reported a lag period preceding linear ethylene production during *in situ* exposure of nodulated roots to C_2H_2 in a diffusion-resistant medium (*e.g.* soil).

Effect of Diurnal Variation in Dark, Light, and Temperature.

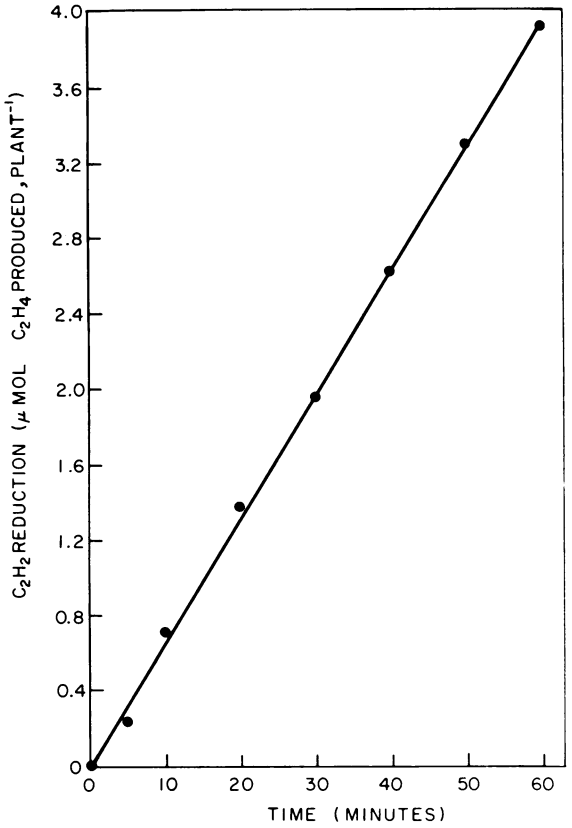

FIG. 1. Linearity of *in situ* acetylene reduction assay of nodulated soybean roots. Intact plants were maintained in the growth chamber in the light at 27 C and the nodulated roots were exposed to 0.10 atm of acetylene.

Nodules of intact plants exposed to diurnally varying dark, light, and temperature had decreased C_2H_2 reduction activity during exposure to 10 h of dark at 18 C (Fig. 2). Subsequent reexposure of the intact plants to light at 27 C caused a rapid increase in nodule activity to the level measured for the preceding photoperiod. Consistent with these results, diurnal variation in soybean root nodule activity (C_2H_2 reduction) has been measured in several other studies (1, 9, 16, 18).

In contrast, no diurnal variation in activity was observed for the root nodules of intact plants exposed to diurnal variation in dark and light at a constant 27 C (Fig. 2). Thus, in response to constant temperature, root nodule activity of intact soybeans was sustained at a constant level, independent of diurnal variation in dark and light treatments imposed. These results suggest that the nodule activity of intact soybeans may be effectively supported during diurnal exposure to dark, apparently by the use of photosynthate reserves. In addition, these results suggest that temperature may regulate the use of these reserves in support of the nitrogenase-catalyzed reduction of acetylene in the short term absence of concurrent photosynthesis. Other investigators have noted a similar lack of response of nodule activity (C_2H_2 reduction) to diurnal change in dark and light for nodulated roots of intact soybeans at constant temperature (4, 12).

Effect of Diurnal Variation in Temperature in Constant Dark. In a separate study, intact soybeans were exposed to 24 h of continuous dark at either a diurnally varying (18/27 C) or a constant (27 C) temperature. Consistent with results of the previous diurnal study (Fig. 2), exposure to plants to 10 h of dark at 18 C resulted in a continuous decline in nodule activity (Fig. 3). Reexposure of the same plants, still in the dark, to 27 C resulted in a rapid recovery of nodule activity to a level equivalent to that observed during the preceding photoperiod. Nodules of intact plants exposed to 24 h of continuous dark at constant 27 C maintained undiminished activity at a level equivalent to that measured for the preceding photoperiod (Fig. 3). Thus, exposure of plants to 27 C (the temperature to which the plants were exposed during the light period of the preceding 35-day growth period) resulted in comparable nodule activity (C_2H_2 reduction) irrespective of whether plants were exposed to diurnally varying dark and light or to continuous dark. These results further support the suggestion that nodule activity may be effectively supported in the short term absence of concurrent photosynthesis by the use of carbohydrate reserves. The use of these reserves in support of nodule function appears to be temperature-mediated.

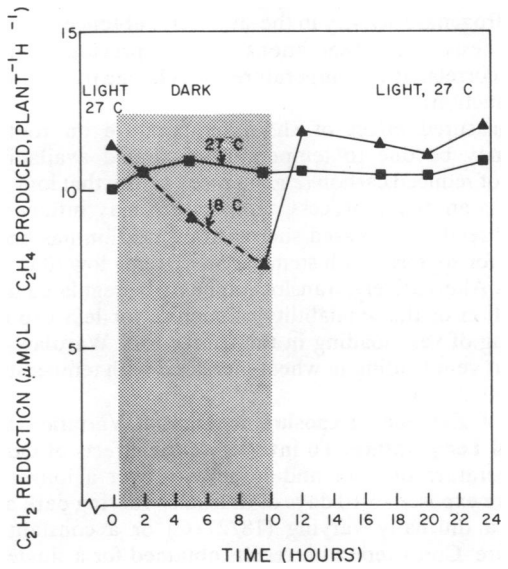

FIG. 2. Effect of diurnal variation in light and temperature on *in situ* acetylene reduction by nodulated roots of intact soybeans. Plants were grown for 35 days at the standard conditions indicated under "Materials and Methods" and were given a 14-h pretreatment in the light at 27 C prior to the start of the assay. During the assay, plants were exposed to 10 h of dark at either 18 or 27 C, followed by 14 h of light at 27 C.

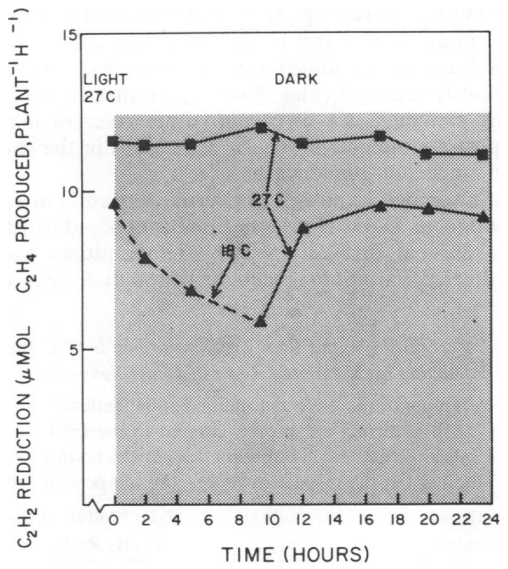

FIG. 3. Effect of diurnally varying *versus* constant temperature on *in situ* acetylene reduction by nodulated roots of intact dark-exposed soybeans. Growth and pretreatment were as described for Figure 2. Plants were assayed during exposure to 10 h of dark at either 18 or 27 C followed by 14 h of dark at 27 C.

Effect of Shoot and Root Temperature on Nodule Activity. To define more closely the effect of temperature on soybean root nodule activity we studied the independent effects of shoot and root temperature on the root nodule activity of intact plants. Nodules of plants with both shoot and root temperatures at 18 C had marked reduction in activity relative to that of the nodules of control plants with shoot and root temperatures at 27 C (Table I). A similar reduction in activity of the root nodules of intact plants was observed when shoot temperature was reduced to 18 C and root temperature was maintained at 27 C. These results suggest that shoot temperature is critical to the temperature effect on nodule activity observed in the previous diurnal studies (Figs. 2 and 3). In addition, shoot tissue is implied as a primary source of carbohydrate reserves for the apparent temperature-mediated support of nitrogenase activity in the short term absence of concurrent photosynthesis. The observations support previous studies (18) that have correlated air temperature with change in nodule activity (C_2H_2 reduction).

The measured effect of shoot temperature on root nodule activity may be due to temperature-mediated availability and transport of reduced carbon (*e.g.* sucrose). Given that long distance transport is an active process, temperature may influence translocation directly. Decreased sucrose translocation has been demonstrated for soybean with stems at an extreme low (0 C) temperature (21). Alternatively, translocation may be regulated indirectly by restriction of the availability of sucrose for leaf export or by the slowing of vein loading in the source leaf. Wardlaw (22) has shown that vein loading in wheat increased with temperature over the range 0 C to 30 C.

Effect of Extended Exposure to Diurnal Variation in Dark, Light, and Temperature. To investigate the effects of dark, light, and temperature on root nodule activity over a longer period, plants were exposed to 10 days of diurnally varying dark and light at either a diurnally varying (18/27 C) or a constant (27 C) temperature. Consistent with results obtained for a single diurnal cycle (Fig. 2), nodules of plants exposed to 10 days of diurnal variation in dark, light, and temperature had corresponding variation in activity (Fig. 4). Activity was high at 27 C in the light and low during exposure to 18 C in the dark.

In contrast, nodules of plants exposed to diurnally varying dark and light at a constant temperature (27 C) maintained a relatively constant level of activity through a 24-h period, independent of dark and light exposure (Fig. 4). The general increase in activity of the root nodules over the 10-day period in this environment reflected an increase in nodule mass. These results, consistent with those previously reported (Figs. 2 and 3), further suggest that the temperature-mediated use of carbohydrate reserves may effectively support nodule activity (C_2H_2 reduction) in the short term absence of concurrent photosynthesis.

Effect of Extended Exposure to Diurnal Variation in Temperature in Continuous Dark. Nodules of plants exposed to 10 days of continuous dark at diurnally varying temperatures had corresponding diurnal variation in activity through 5 days (Fig. 5).

Table I. *Effect of Shoot and Root Temperature on in Situ Acetylene Reduction by Nodulated Roots of Intact Soybeans*

Shoot/root temperatures were maintained independently at 27/27 C, 18/27 C, or 18/18 C during a 5-h pretreatment in the light as described under "Materials and Methods." Subsequently, *in situ* nodule activity was measured in the light at the respective shoot/root temperature regimes.

Shoot/Root Temperature		Root Nodule Activity (C_2H_2 Reduction)
C		*μmol plant*$^{-1}$ *h*$^{-1}$
27/27		14.54
18/27		5.74
18/18		7.83
	LSD 0.05	4.79

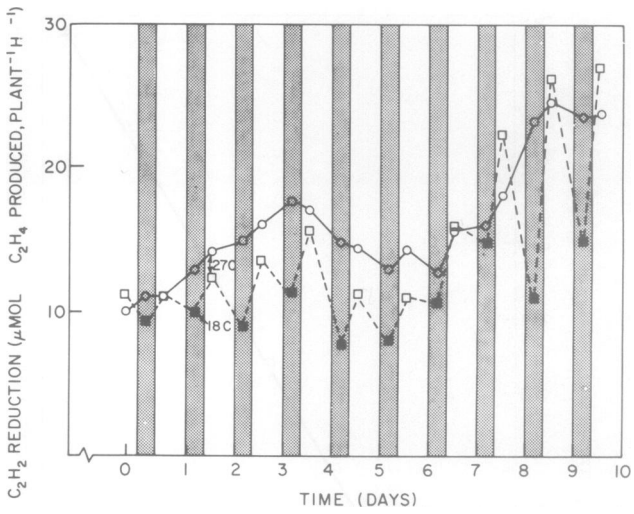

FIG. 4. Effect of 10-day exposure to varying temperature and light regimes on *in situ* acetylene reduction by nodulated roots of intact soybeans. Plants were grown for 35 days at standard conditions. Light treatment was 10 h of dark (shaded area) followed by 14 h of light (white area) during each 24-h period. Temperatures were either 18/27 C or 27/27 C as indicated on figure. Assays were conducted after 5 h of pretreatment in each dark and light period.

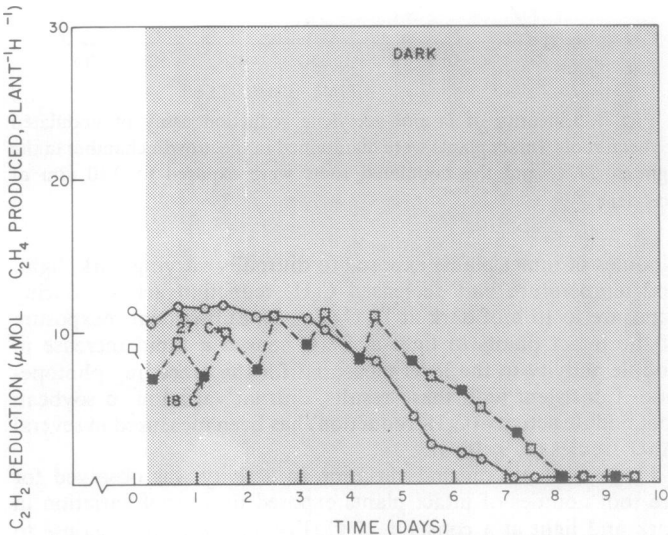

FIG. 5. Effect of 10-day exposure to varying temperature and continuous dark on *in situ* acetylene reduction by nodulated roots of intact soybeans. Growth conditions (through 35 days), temperature treatments, and assay procedure were as indicated for Figure 4.

Nodule activity during 27 C exposure periods was sustained for 5 days at the level measured for the photoperiod preceding dark exposure and then steadily declined to zero at about 8 days.

In contrast, root nodules of intact plants exposed to 10 days continuous dark at constant 27 C sustained the level of activity measured for the preceding photoperiod through only 3 days (Fig. 5). Subsequently, nodule activity rapidly declined to zero at 7 days. The more rapid decline of nodule activity observed for dark-exposed plants at constant 27 C temperature suggests a more rapid depletion of reserve carbohydrate than that occurring in plants in continuous dark at diurnally varying temperatures. This suggestion is consistent with the elevated rates of dark respiration generally associated with sustained high temperature. Hofstra and Hesketh (13) have shown that the rate of dark respiration for soybeans (cv. Biloxi) increases over the range of 15 to 50 C.

Factors such as plant age and cultural conditions that affect the

soluble (sugar) and reserve (starch) carbohydrate content of shoot tissue also affect the expression of diurnal variation in nodule activity; *i.e.* previously noted differences in the response of nodule activity to diurnally varying dark and light (1, 4, 9, 16, 18) may reflect differences in levels of carbohydrate reserves accumulated in different growth environments. Similarly, the response of nodule activity to continuous dark may reflect the level of carbohydrate reserves previously accumulated by shoot tissue in a given growth environment. The current study has shown nodule activity to be sustained for more than 72 h during continuous dark exposure (Fig. 5). In plants cultured in different environments, nodule activity declined after only a few hours of dark exposure (1, 9).

Dry Matter Accumulation and Carbohydrate Analyses of Harvested Tissue. Exposure of plants to 10 days of continuous dark significantly decreased dry matter accumulation relative to that in plants exposed to diurnally varying dark and light (Table II). The detrimental effect of continuous dark on dry matter accumulation was evident within both the constant (27 C) temperature treatments (diurnally varying light and dark *versus* constant dark) and in the diurnally varying (18/27 C) temperature treatments (diurnally varying light and dark *versus* constant dark). In addition, levels of total (nonstructural) carbohydrates were decreased in shoot, root, and nodule tissue of plants exposed to constant dark, relative to that of plants exposed to diurnally varying dark and light (Table II).

The effects of temperature, dark, and light treatments were found to interact significantly in mediating the accumulation of sugar and starch in shoot tissue harvested from the extended (10-day) studies (Table II). The 10-day exposure to continuous dark resulted in decreased sugar and starch content in dark-treated shoots relative to that measured in the diurnal dark/light-treated controls. Decreased sugar and starch content was observed at both the constant (27 C) and the diurnally variable (18/27 C) temperature regimes.

The sugar content of root tissue harvested from plants exposed to diurnally varying dark and light at a constant 27 C was markedly decreased relative to that of comparable tissue harvested from plants exposed to diurnally varying temperatures (Table II). A similar response to temperature was measured for sugar accumulation in root tissue harvested after 10 days of continuous dark (Table II). In contrast, starch concentration in the root tissue of plants exposed either to diurnally varying dark and light or to continuous dark did not differ significantly with temperature treatment over the 10-day period. These results imply a minimal contribution from starch of root origin for the support of nodule activity during short term dark exposure.

Exposure of soybeans to 10 days of continuous dark resulted in a decrease in nodule mass compared with that of plants exposed to diurnally varying dark and light (Table II). No significant difference was observed in the sugar or starch content of nodule tissue harvested from plants in either continuous dark or diurnally varying dark and light exposure over the 10-day period (Table II). This observation is notable in view of the wide disparity between levels of nodule activity measured for intact soybeans in these environments (Figs. 4 and 5). These results suggest that a pool of soluble and reserve carbohydrate not directly involved in support of nodule activity may be maintained in soybean root nodules.

In work with young alder plants, Wheeler (23) observed that diurnal fluctuation in nitrogenase activity was not correlated with a diurnal influx of carbohydrate to the nodule. Based on this evidence, Wheeler suggested that carbohydrates present in the nodule may function in roles other than for the support of $N_2(C_2H_2)$ reduction (*e.g.* growth and maintenance of nodule bacteroids). Lawrie and Wheeler (14) observed that the main sites of accumulation of ^{14}C-labeled photosynthates do not correspond to the most active sites of pea root nodules. They demonstrated a continued transport of ^{14}C-labeled photosynthate to the nodules of darkened plants after nodule activity had been greatly reduced. Their data suggest that photosynthate used in support of nodule activity may be rapidly metabolized rather than accumulating at the nodule level.

CONCLUSIONS

At constant temperature conditions the root nodule activity (*in situ* C_2H_2 reduction) of intact soybeans was unaffected by diurnal

Table II. *Effect of Light, Dark, and Temperature on Dry Weight, Sugar, and Starch Accumulation in Shoot, Root, and Nodule Tissue of Soybean*
Plants were harvested at the end of the extended (10-day) light, dark, and temperature studies (Figs. 4 and 5).

	Treatment				Significant Effect or Interaction ($P = 0.05$)[c]
	Dark/light regime[a]		Dark/dark regime[b]		
	18/27 C	27/27 C	18/27 C	27/27 C	
Shoot tissue					
Dry weight, g (plant)$^{-1}$	5.4	4.8	1.9	1.7	L
Sugar, mg (g dry wt)$^{-1}$	104.5	94.1	27.7	46.9	T × L
Starch, mg (g dry wt)$^{-1}$	181.7	106.6	18.9	28.7	T × L
Total carbohydrates (nonstructural), mg (plant)$^{-1}$	1539.0	956.2	89.8	121.8	T × L
Root tissue					
Dry weight, g (plant)$^{-1}$	2.1	2.6	1.6	1.6	L
Sugar, mg (g dry wt)$^{-1}$	70.6	45.0	42.2	32.4	T, L
Starch, mg (g dry wt)$^{-1}$	28.6	21.0	21.3	17.4	NS
Total carbohydrates (nonstructural), mg (plant)$^{-1}$	206.8	172.2	99.9	78.9	L
Nodules					
Dry weight, g (plant)$^{-1}$	0.2	0.3	0.1	0.1	L
Sugar, mg (g dry wt)$^{-1}$	56.6	67.8	54.4	44.2	NS
Starch, mg (g dry wt)$^{-1}$	90.2	83.8	84.1	97.4	NS
Total carbohydrates (nonstructural), mg (plant)$^{-1}$	28.1	32.1	19.6	17.2	L

[a] Treatment conditions were 10 h of dark at 18 or 27 C followed by 14 h of light at 27 C.
[b] Treatment conditions were 10 h of dark at 18 or 27 C followed by 14 h of dark at 27 C.
[c] NS: nonsignificant; L: light; T: temperature; T × L: temperature × light interaction.

variation in dark and light treatment. Root nodule activity did, however, vary with change in temperature from 18 to 27 C. In this range nodule activity was found to vary directly with change in shoot temperature.

In the short term (*i.e.* 24-h diurnal) absence of concurrent photosynthesis, nodule activity was effectively supported by the apparent temperature-mediated use of carbohydrate reserves. Temperature studies suggest that shoot tissue may serve as a primary source of carbohydrate reserves used for nodule activity. Analyses of the starch and sugar content of shoot, root, and nodule tissue harvested from these studies were consistent with this suggestion.

LITERATURE CITED

1. BERGERSEN FJ 1970 The quantitative relationship between nitrogen fixation and the acetylene reduction assay. Aust J Biol Sci 23: 1015–1025
2. CHING TM, S HEDTKE, SA RUSSELL, HJ EVANS 1975 Energy state and dinitrogen fixation in soybean nodules of dark grown plants. Plant Physiol 55: 796–798
3. DILWORTH MJ 1974 Dinitrogen fixation. Annu Rev Plant Physiol 25: 81–114
4. FISHBECK K, HJ EVANS, LL BOERSMA 1973 Measurement of nitrogenase activity of intact legume symbionts *in situ* using the acetylene reduction assay. Agron J 65: 429–433
5. HAM GE, RJ LAWN, WA BRUN 1976 Influence of inoculation, nitrogen fertilizers and photosynthetic source-sink manipulations on field grown soybeans. *In* PS Nutman, ed, Symbiotic Nitrogen Fixation in Plants, International Biology Program Series, Vol 7. Cambridge University Press, London, pp 239–253
6. HARDY RWF, RC BURNS, RR HEBERT, RD HOLSTEN, EK JACKSON 1971 Biological nitrogen fixation: a key to world protein. *In* TA Lie, EG Mulder, eds, Biological Nitrogen Fixation in Natural and Agricultural Habitats. Plant Soil Spec Vol, pp 561–590.
7. HARDY RWF, UD HAVELKA 1973 Symbiotic N_2-fixation: multi-fold enhancement by CO_2 enrichment of field grown soybean. Plant Physiol 51: S–35
8. HARDY RWF, UD HAVELKA 1976 Photosynthate as a major factor limiting nitrogen fixation by field-grown legumes with emphasis on soybeans. *In* PS Nutman, ed, Symbiotic Nitrogen Fixation in Plants, International Biology Program Series, Vol 7. Cambridge University Press, London, pp 421–439
9. HARDY RWF, RD HOLSTEN, EK JACKSON, RC BURNS 1968 The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. Plant Physiol 43: 1185–1207
10. HARPER JE 1976 Contribution of dinitrogen and soil or fertilizer nitrogen to soybean (*Glycine max* L. Merr) production. *In* LD Hill, ed, World Soybean Research. Interstate Printers and Publishers, Danville, ILL, pp 101–107
11. HARPER JE, JC NICHOLAS 1976 Control of nutrient solution pH with an ion exchange system: effect on soybean nodulation. Physiol Plant 38: 24–28
12. HART AL 1976 A root chamber for measuring the acetylene reducing activity of intact soya beans. NZ J Agric 19: 349–352
13. HOFSTRA G, JD HESKETH 1969 Effects of temperature on the gas exchange of leaves in the light and dark. Planta 85: 228–237
14. LAWRIE AC, CT WHEELER 1973 The supply of photosynthetic assimilates to nodules of *Pisum sativum* L. in relation to the fixation of nitrogen. New Phytol 72: 1341–1348
15. LAWN RJ, WA BRUN 1974 Symbiotic nitrogen fixation in soybeans. I. Effect of photosynthetic source-sink manipulations. Crop Sci 14: 11–16
16. MEDERSKI HJ, JG STREETER 1977 Continuous, automated acetylene reduction assays using intact plants. Plant Physiol 59: 1076–1081
17. SLOGER C 1969 Symbiotic effectiveness and N_2 fixation in nodulated soybean. Plant Physiol 44: 1666–1668
18. SLOGER C, D BEZDICEK, R MILBERG, N BOONKERD 1975 Seasonal and diurnal variations in $N_2(C_2H_2)$-fixing activity in field soybeans. *In* WDP Stewart, ed, Nitrogen Fixation by Free Living Organisms. International Biological Program Series, Vol. 6. Cambridge University Press, Cambridge, pp 271–284
19. STREETER JG 1972 Nitrogen nutrition of field grown soybean plants. I. Seasonal variations in soil nitrogen and nitrogen composition of stem exudate. Agron J 64: 311–314
20. UPMEYER DJ, HR KOLLER 1973 Diurnal trends in net photosynthetic rate and carbohydrate levels of soybean leaves. Plant Physiol 51: 871–874
21. VERNON LP, S ARONOFF 1952 Metabolism of soybean leaves. IV. Translocation from soybean leaves. Arch Biochem Biophys 36: 383–398
22. WARDLAW IF 1974 Temperature control of translocation. *In* RL Bieleski, AR Ferguson, MM Cresswell, eds, Mechanism of Regulation of Plant Growth. R Soc NZ Bull 12: 533–538
23. WHEELER CT 1971 The causation of the diurnal changes in nitrogen fixation in the nodules of *Alnus glutinosa*. New Phytol 70: 487–495