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Root and Nodule Respiration in Relation to Acetylene Reduction in Intact Nodulated Peas[1]

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ABSTRACT

Inoculated pea plants (*Pisum sativum* L.) were grown with N-free nutrients in a controlled environment room and rates of respiratory CO_2 evolution and C_2H_2 reduction by the intact nodulated roots were determined. Experiments followed changes related to diurnal cycles, light and dark treatments, partial defoliation, aging of plants and NH_4NO_3 addition. In all experiments, changes in C_2H_2 reduction were associated with parallel changes in the respiration rate, although in all but the defoliation experiment there was a basal level of respiration which was independent of the rate of C_2H_2 reduction. In conditions which affected growth or plant size as well as C_2H_2 reduction, respiration changed by an average of 0.42 mg CO_2 (μmol C_2H_2 reduced)$^{-1}$. However, some treatments decreased C_2H_2 reduction without greatly changing the growth and in these conditions respiration was decreased by an average of 0.27 mg CO_2 (μmol C_2H_2 reduced)$^{-1}$. While this value may also include some respiration associated with other processes, it is proposed that it more closely estimates respiration directly associated with energy utilization for acetylene reduction; whereas the higher value includes respiration related to maintenance and growth processes as well.

Biological N fixation requires energy to provide the ATP and reducing equivalents needed for the conversion of N_2 to NH_3 (9, 19). In the legume-*Rhizobium* symbiosis these are derived from the oxidation of the products of host plant photosynthesis, and considerable evidence now suggests that it is this supply of photosynthetic energy which often limits the N-fixing capability of symbiotic systems (5, 10, 12, 13, 24). Thus, significant increases in fixation may require either improved total photosynthetic productivity or more efficient use of available photosynthate (7, 10, 23). This second approach has been difficult because of the inability to determine the energy consumed by the N-fixing reactions. Because of the morphological and physiological complexities of symbiotic systems (6, 17), only indirect estimates are possible.

One approach has been to compare the rates of dry matter and N accumulation in legumes using N_2 and NO_3^- as N sources (3, 6). The limitations of this method have been fully discussed (1, 3), and even with sufficient controls (6), there are large space and time requirements and the results can at best only estimate the difference in energy requirements for N_2 and NO_2^- utilization.

Another approach is based on the assumption that the ATP and reducing power consumed by the nitrogenase system are derived from the complete oxidation of sugars and hence that measurements of respiration reflect this energy utilization (3, 17). This would be true only if all of the ATP and reducing equivalents in the root-nodule-bacteria system were used to support N fixation. However, in most plant systems total respiration can be considered as the sum of several additive components, the major two being those associated with providing energy for maintaining the tissues (maintenance respiration) and for growth (growth respiration) (15, 21, 25). In symbiotic systems, respiration associated with nitrogenase activity may be a third major component in which case the total respiration could be expressed as follows:

$$R = R_M W + R_G \, dW/dt + R_F(N_2\text{-ase}) \quad (1)$$

where R is the total respiration of roots and nodules, W is the root and nodule dry weight, N_2-ase is nitrogenase activity, and R_M, R_G and R_F are the maintenance, growth, and fixation coefficients, respectively. According to this proposal, if nitrogenase activity can be varied without changing the weight or growth rate, then the relationship between total respiration and nitrogenase should be linear and the slope of this relationship should represent the respiration required per unit of nitrogenase activity.

Except for some limited comparisons of respiration with N fixation (3, 17) or with C_2H_2 reduction (18), there is little information on the responses of both respiration and nitrogenase activity to environmental treatments. In this study these processes were examined under several conditions in order to find a method of estimating the respiration associated with N fixation.

MATERIALS AND METHODS

Plant Culture. Two seeds of a commercial field pea (*Pisum sativum* L. cv. Trapper) were sown with commercial inoculant (Hansen Inoculator Co., Milwaukee, Wis.) 2 cm deep into 10-cm plastic pots of Turface (Wyandotte Chemicals, Wyandotte, Mich.) and the pots were irrigated daily with water until seedling emergence. After emergence, the seedlings were selected for uniformity and reduced to one/pot. Thereafter plants were irrigated daily with 100 ml/pot of either water or thrice weekly with nutrient solution. The N-free nutrient solution was a modified Wilson's formulation (27) with iron supplied at 2 μg ml^{-1} as sodium ferric diethylenetriamine pentaacetate (Sequestrene, CIBA-GEIGY) and the +N nutrient was similar but with 15 mM NH_4NO_3.

Plants were grown in a controlled environment chamber (Enconaire, Winnipeg, Canada) with a photoperiod of 14 hr and day/night temperatures of 20 C/15 C. Illumination was provided by mixed fluorescent and incandescent lamps with a photon flux density (400-700 nm) at pot level of between 20 and 30 nE cm^{-2} sec^{-1} (13-20 klux). Under these conditions flowering began 6 to 7 weeks after planting.

Measuring System. Two plants were removed from the growth

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chamber to the laboratory gas exchange system (Fig. 1) where they were sealed around their lower stems with Terostat (Teroson, Heidelberg, FRG) into separate acrylic root chambers. In addition to the inlet and outlet ports for the measuring gas stream, each chamber had a secondary circulating system for air from the chamber through a pump (D) a copper heat exchanger (E) and back to the root chamber. The return flow in this circulation system was split and the ratio of flow into the chamber directly or through the pot was adjustable (V_1).

For measurements of CO_2 exchange, air from the laboratory compressed air supply was humidified to a dew point of 8 to 12 C and passed either directly to the measuring instruments or through rotameters (Matheson of Canada Ltd., Whitby, Canada) followed by the two root and two shoot chambers. When the exit gas stream from any chamber was switched (V_2) into the measuring system, the gas passed through the humidity sensor (H-Hygroline, Beckman Instruments Inc., Toronto, Canada) a magnesium perchlorate drying column (J) and the sample tube of the IR CO_2 analyzer (K-model 864, Beckman Instruments Inc.).

For acetylene reduction determinations, the inlet and outlet ports (V_3, V_4) were closed to produce a closed system with rapid air circulation. Acetylene was injected through a serum stopper (M) and at intervals 1-ml samples were withdrawn for gas chromatographic analysis of ethylene and acetylene. Because of the risk of leaks when the system was pressurized by injecting acetylene, a low concentration of acetylene (0.02 atm) was used. Rate of acetylene reduction was measured by increase in ethylene, and acetylene concentration was recorded as a check for leaks and to ensure that the rates were not affected by differences in acetylene concentration which varied from 0.019 to 0.021 atm.

The acrylic leaf chambers for photosynthetic CO_2 exchange measurements were similar to those described by Mahon *et al.* (14) with a secondary circulation system to control temperature and increase the linear air velocity without decreasing CO_2 differential. The internal area and volume were 22 cm^2 and 13 cm^3, respectively, and the air velocity was about 50 cm sec^{-1}.

In the measuring system light was provided by 400 w Sylvania Metalarc lamps which were vertically adjustable to vary irradiance levels. For dark treatments, plants were surrounded by heavy black cloth and the lamps turned off. Temperatures of leaf surfaces and rooting medium were monitored with copper-constantan thermocouples and were maintained between 23 and 27 C.

Ethylene and CO_2 determinations were calibrated against standard gases produced by precision gas proportioning pumps (Wösthoff, Bokum, FRG). The reference cell of the IR gas analyzer was flushed each day with a mixture of 320 $\mu l\ l^{-1}$ of CO_2 in air and sealed. For each CO_2 exchange determination, the CO_2 in the inlet air and the chamber air were measured and the CO_2 flux was calculated from the concentration difference and the rate of air flow. In all experiments the CO_2 concentration of the inlet air was between 300 and 340 $\mu l\ l^{-1}$ and the flow rates for measurements were 30 or 60 l hr^{-1} for leaves and 60 or 120 liters hr^{-1} for roots.

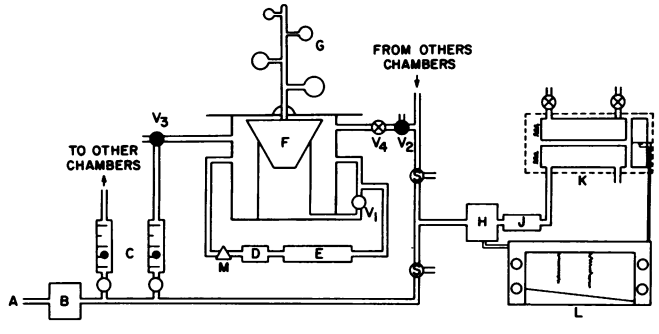

FIG. 1. Diagram of root chamber portion of the gas exchange system. A: compressed air supply; B: humidifier; C: rotameters; D: pump; E: heat exchanger; F: pot; G: plant shoot; H: humidity sensor; J: water absorber; K: IR CO_2 analyzer; L: recorder; M: injection port; O: needle valve; ●: three-way ball valve; ⊗: toggle shut-off valve; Ⓢ: three-way solenoid.

Experimental Procedures. To examine diurnal changes, two 5-week-old plants were sealed into the chambers. The irradiance at the shoot tips was 25 nE cm^{-2} sec^{-1} and the photoperiod was 14 hr. Rates of C_2H_2 reduction and root + nodule respiration were determined for both plants at two hourly intervals from 1100 hr to 1100 hr the following day.

The responses of C_2H_2 reduction and root + nodule respiration to changing light conditions were examined by pretreating duplicate plants (5-week-old) for 6 hr with an irradiance at the shoot tip of either 100 (high light) or 25 (low light) nE cm^{-2} sec^{-1} (400–700 nm). After pretreatment, C_2H_2 reduction and respiration were measured, one plant was darkened, and further measurements were recorded after 2, 4, and 8 hr of light or darkness. For comparison, all measurements of C_2H_2 reduction were repeated after flushing the root chambers for 30 min with 0.8:0.2 atm Ar/O_2 gas mixture. On subsequent days the procedure was repeated until four replicates of each treatment were completed.

Leaf removal was also used to vary C_2H_2 reduction. Duplicate plants for each treatment were measured immediately before and 1 and 2 days after defoliation. The three treatments were control, removal of all but the three youngest fully expanded leaves and removal of only these top three leaves. Measurements were at 25 nE cm^{-2} sec^{-1} and between measurements the plants were returned to the growth chamber.

To determine the relationships during growth, each week, 6 of 10 plants were randomly selected and measured with an irradiance of 60 nE cm^{-2} sec^{-1}. Acetylene reduction was measured both in air and in Ar/O_2 (0.8:0.2 atm).

For the nutrient experiment plants were grown for 4 weeks in normal N-free nutrient conditions. At this time rates of C_2H_2 reduction, root + nodule respiration, and photosynthetic CO_2 exchange in the third and fifth exposed leaves from the top were measured in eight replicate plants at an irradiance of 100 nE cm^{-2} sec^{-1}. Thereafter plants were irrigated daily—the treatment plants with +N and the control plants with N-free nutrient solutions. Two and 8 days later, the measurements were repeated using one treatment and one control plant in the two chambers in order to equalize the diurnal effects.

RESULTS

The volumes of the two root chambers containing filled pots were determined by dilution of acetylene as 1.97 and 2.06 liters. Because different levels of pot filling could affect the volume, 2 liters were used for calculation of results from both. When the secondary circulation of the root chambers had 60 liters hr^{-1} of air flow through the pot and the remainder (about 700 liters hr^{-1}) directly into the chamber, the rate of ethylene production was linear from 5 min after injection for at least 85 min. In all experiments this flow pattern was used, ethylene content of the chambers was measured after 10, 20, and 30 min, and the rates of acetylene reduction were calculated from the mean increase in ethylene (10–20 min and 20–30 min). Rates measured in this way were not affected by watering immediately before measurement or by the process of sealing plants into the chambers.

The rates of C_2H_2 reduction and root + nodule respiration varied similarly throughout the 24-hr light/dark cycle (Fig. 2a). There was a linear relationship between them but the regression line did not pass through the origin (Fig. 2b). The differences

between the duplicate plants averaged 16% of the mean for C_2H_2 reduction and 9% of the mean for respiration.

After 6 hr of high light exposure, the rates of both C_2H_2 reduction and root + nodule respiration were significantly ($P \leq .05$) higher than after 6 hr of low light (Fig. 3). However, both increased more rapidly under low light conditions and approached those of the high light-treated plants by the end of the measuring period. In darkness, plants which had been pretreated with high light decreased in C_2H_2 reduction only slightly during the first 4 hr and then markedly. The rate of C_2H_2 reduction decreased continuously during darkness in plants which had been pretreated with low light. In no treatment was there a significant ($P \leq .05$) difference between the rates of C_2H_2 reduction measured in air and in Ar/O_2.

In this experiment, there was also a linear relationship between the rates of respiration and C_2H_2 reduction and a significant ($P \leq .01$) positive intercept with the respiration axis (Fig. 4). There was no significant difference in linear regression coefficient between high or low light treatments or between light and dark treatments when analyzed separately.

The removal of the lower leaves had only a transient effect on both C_2H_2 reduction and root + nodule respiration, and the rates were similar to control values after 2 days (Fig. 5a).

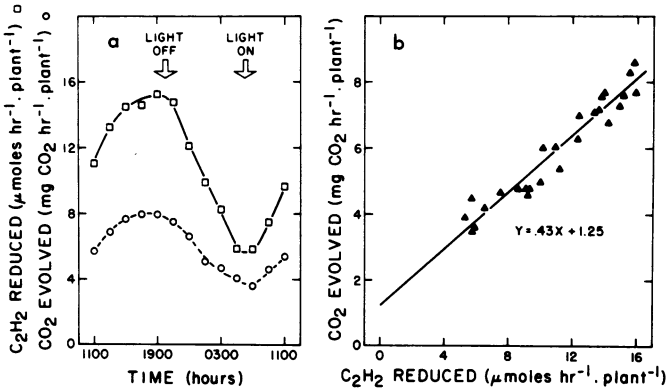

FIG. 2. a: Rates of C_2H_2 reduction (□) and root + nodule respiration (○) during a 24-hr period. Values are means from two plants grown for 5 weeks in N-free nutrient. b: Respiration (CO_2 evolved) as a function of C_2H_2 reduction (▲) for all data points of both plants. Irradiance during the photoperiod was 25 nE cm^{-2} sec^{-1} at shoot tip and day/night root temperatures averaged 25.4 C and 24 C, respectively.

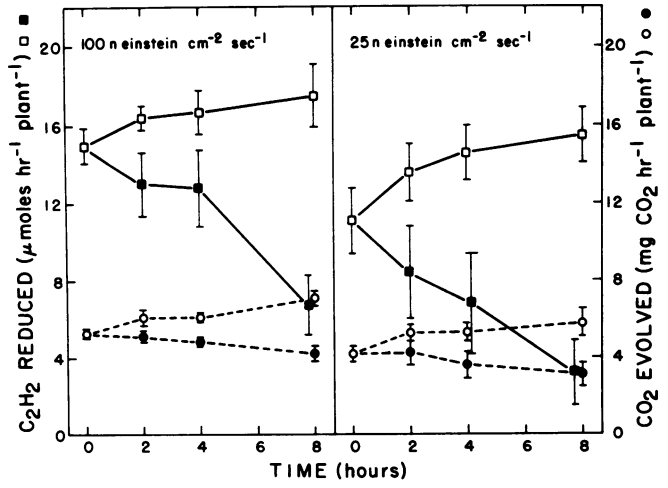

FIG. 3. Rates of C_2H_2 reduction (□■) and root + nodule respiration (○●) during light (○) and darkness (●) after 6-hr pretreatment at two levels of irradiance. Mean root temperatures were 25.3 C (high light) and 24.6 C (low light). Vertical bars indicate 2 × SE ($N = 4$).

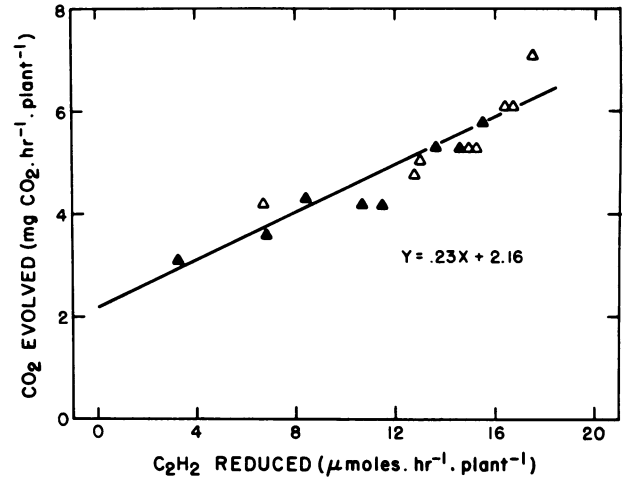

FIG. 4. Root + nodule respiration as a function of C_2H_2 reduction. Results are means ($N = 4$) of values from plants pretreated for 6 hr with high (△) and low (▲) light.

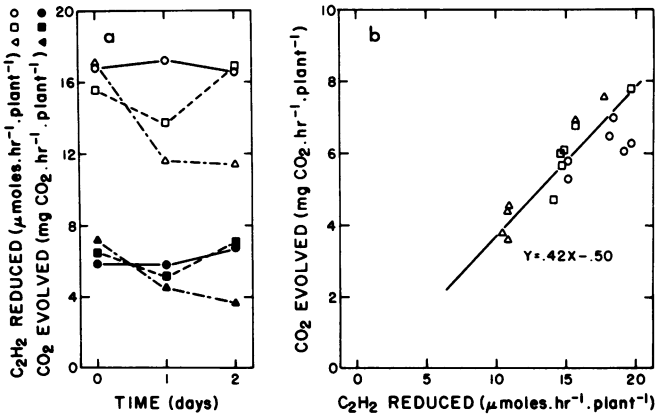

FIG. 5. a: Rates of C_2H_2 reduction (○□△) and root + nodule respiration (●■▲) during 2 days in control plants (○●), plants with lower leaves removed (□■), and with upper leaves removed (△▲). Values are means from duplicate plants. b: Respiration as a function of C_2H_2 reduction for results from all plants.

Removal of the top leaves, however, reduced both rates for at least 2 days. The differences between duplicates averaged 12 and 11% of the means for C_2H_2 reduction and respiration, respectively. C_2H_2 reduction and respiration were linearly related and the estimated regression line passed close to the origin (Fig. 5b).

During growth, the rates of both C_2H_2 reduction and root + nodule respiration increased continuously for up to 7 weeks from planting (Fig. 6a) and no significant ($P \leq .05$) difference was found between rates of C_2H_2 reduction measured in air and those measured in Ar/O_2. The relationship between respiration and C_2H_2 reduction was linear with a positive respiration intercept (Fig. 6b). With the large variability in older plants, significant ($P \leq .01$) correlations also existed between respiration and C_2H_2 reduction when rates in replicate plants were compared at each sampling time. Thus, the linear regression coefficients for the 5-, 6-, and 7-week measurements were 0.30, 0.25 and 0.27 mg CO_2 (μmol C_2H_2 reduced)$^{-1}$, respectively, all significantly ($P \leq .01$) less than the 0.40 mg CO_2 (μmol C_2H_2 reduced)$^{-1}$ obtained from the means of all measurements (Fig. 6b).

In the nutrient experiment, control plants growing on N-free nutrient increased in both C_2H_2-reducing and respiratory activities from day 0 to day 8 (Fig. 7a). With the addition of 15 mM

NH_4NO_3, the rate of C_2H_2 reduction decreased continuously; however, the rate of root + nodule respiration decreased only for 2 days and increased again by day 8. When root + nodule respiration was plotted as a function of C_2H_2 reduction (Fig. 7b), the data from days 2 and 8 showed similar slopes and the older plants had a greater respiration intercept. The rates of photosynthetic CO_2 uptake by leaves did not change significantly during the 8-day period with either nutrient regime (Table I), and the mean plant dry weights (± SE) after 8 days of treatment were 2.63 ± .17 (+N) and 2.85 ± .07 (N-free).

The relationship between root + nodule respiration and C_2H_2 reduction appeared linear in all experiments. The regression coefficients (Table II) were similar during changes related to the normal diurnal cycle, defoliation and plant age while short light/dark changes and comparison of N-treated and control plants of equivalent age indicated a smaller respiration rate/unit of C_2H_2 reduced.

DISCUSSION

The rates of both respiration and acetylene reduction were similar to previously reported values (11, 18, 20, 22). In all experiments the rate of root + nodule respiration was linearly related to the rate of C_2H_2 reduction, although the slope of the relationship was variable (Table II). With the single exception of the defoliation experiment (Fig. 5), the intercept with the respiration axis was significantly ($P \leq .01$) greater than zero and varied among experiments (Figs. 2, 4, 6, 7) and even between different aged plants in the same experiment (Fig. 7). This suggests that the total root + nodule respiration was not directly associated with the nitrogenase system. However, with the respiration model which is proposed in equation 1, linearity of the total respiration versus C_2H_2 reduction relationship can still exist when maintenance and/or growth respiration is constant (represented by the respiration intercept) or proportional to the rate of C_2H_2 reduction (included in the slope term).

During a 24-hr period, the rate of maintenance respiration is unlikely to have changed greatly (16); however, with a low irradiance level during the photoperiod and a 10-hr dark period, the energy supply to the roots and nodules may be depleted (18) sufficiently to decrease the growth as well as the nitrogenase activity and the slope would be influenced by parallel changes in respiration associated with both growth and C_2H_2 reduction.

A similar argument can be applied to the defoliation experiment (Fig. 5), *i.e.* that such drastic treatment is likely to disrupt the general supply of photosynthate to the root region and thus affect the respiration associated with both growth and C_2H_2 reduction.

Since rates of C_2H_2 reduction were still increasing after 6 weeks of growth (Fig. 6) and the plants were only just beginning to flower, the plants were probably in the exponential growth phase for most of the experiment. During this growth phase plant size, growth rate, and C_2H_2 reduction rate can be expected to increase similarly. Thus, the regression of total respiration on C_2H_2 reduction again is likely to overestimate the respiration directly associated with C_2H_2 reduction. When the relationship was estimated from replicate plants of the same age, which would be more similar in size and growth rate, the regression coefficients averaged 0.27 mg CO_2 (μmol C_2H_2 reduced)$^{-1}$ as compared to 0.40 mg CO_2 (μmol C_2H_2 reduced)$^{-1}$ estimated from the means of different aged plants. This may more closely represent the respiration associated with nitrogenase activity, although more extensive measurements over the entire life cycle (unpublished results) indicated some age effect on the slope even when replicates of the same age were considered.

Two experiments yielded slope values which were significantly lower than those from the above experiments (Table II). In the light/dark experiment, the total measuring time was 8 hr and half of the values were obtained during light treatment. It is unlikely that large changes in maintenance or growth respiration occurred. Although this slope of 0.23 mg CO_2 (μmol C_2H_2 reduced)$^{-1}$ is probably representative of the respiration required

Table I. Mean rates of photosynthetic CO_2 uptake by leaves 3 and 5 of pea plants grown for 4 weeks without added nitrogen followed by 2 or 8 days of +N or N-free nutrient treatment

Values are means ± standard errors from 2 leaves of 4 plants. The photon flux density during measurement was 100 n einsteins cm^{-2} s^{-1} and the mean leaf temperature was 28C.

Day	Net Photosynthetic CO_2 Uptake mg dm^{-2} hr^{-1}	
	-N	+N
0	29.3 ± 1.3	26.7 ± 1.0
2	27.8 ± 0.8	25.9 ± 0.6
8	29.1 ± 1.2	25.4 ± 1.0

Table II. Linear regression coefficients of root + nodule respiration on C_2H_2 reduction

Values are from all experiments described and are presented with standard errors (SE) and number of determinations (n). The same letter indicates no significant differences ($P \leq .05$).

Experiment	Regression (Coefficient) mg CO_2 (μmole C_2H_2 reduced)$^{-1}$	SE	n	
Diurnal	0.43	±.02	26	a
Light/Dark	0.23	±.02	16	b
Defoliation	0.42	±.05	18	ac
Age	0.40	±.02	6	a
Nitrogen day 2	0.30	±.03	8	bc
day 8	0.27	±.02	8	b

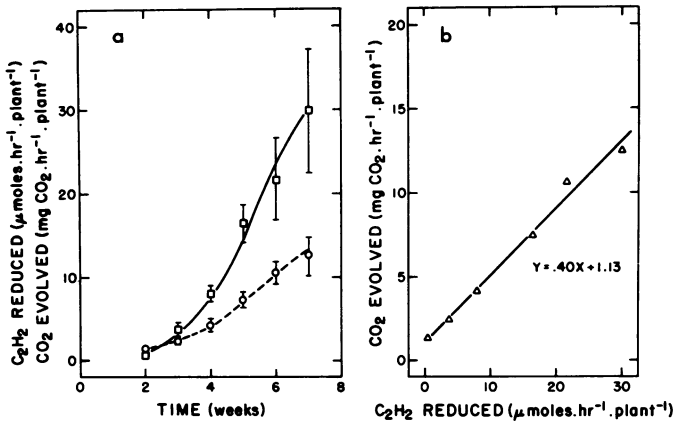

FIG. 6. a: Rates of C_2H_2 reduction (□) and root + nodule respiration (○) during growth. Values are means from six replicate plants and vertical bars indicate 2 × SE. b: Respiration as a function of C_2H_2 reduction.

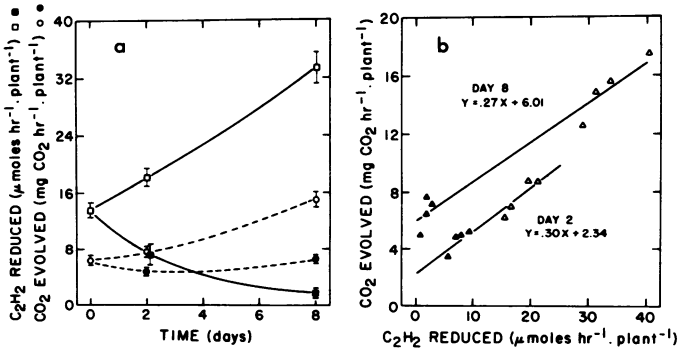

FIG. 7. a: Rates of C_2H_2 reduction (□■) and root + nodule respiration (○●) in plants grown for 4 weeks without soil N followed by growth on 15 mM NH_4NO_3 (●■) or N-free (○□) solution. Values are means from four replicate plants and vertical bars indicate 2 × SE. b: Respiration as a function of C_2H_2 reduction after 2 days and 8 days on +N (▲) and N-free (△) nutrient solution.

for C_2H_2 reduction, the method is time consuming and complicated by diurnal patterns.

In the nutrient experiment, the slopes of the respiration *versus* C_2H_2 reduction relationships based on N-free and +N-treated plants of similar age were the same (Fig. 7 and Table II). Previous studies have shown little difference in C economy of legumes using soil N as compared to those fixing N_2, and have related this to the theoretically similar energy requirements of N_2 fixation and NO_3^- reduction (6, 17). In the results reported here the rate of root + nodule respiration decreased after addition of NH_4NO_3 (Fig. 7). The similar photosynthetic rates (Table I) suggest that the decreased respiration was not related to a decrease in assimilate supply caused by nitrogen addition. Tentatively, it must be concluded that under the conditions which were used, little NO_3^- or NO_2^- reduction occurred in the roots or nodules. Other results with peas (4, 20, 26) support the idea that with this concentration of NH_4NO_3 applied to already nodulated plants, high levels of nitrate reduction would be expected in the shoot relative to that in the roots and nodules.

Since nutrient regime had no effect on the final dry weights, it is probable that it had little influence on the maintenance and growth respiration components. Thus, the major energy requirements for nitrate assimilation would not be associated with root and nodule respiration, although some energy might be required for uptake and/or transport of NO_3^- ions.

Nutrient regime had little effect on the final plant dry weights, and, while differences in root to shoot ratios or tissue composition may have been altered, it is likely that differences in growth and maintenance components of respiration were small relative to that associated with nitrogenase. If so, then the sum of maintenance and growth respiration would be indicated by the respiration intercept and the slope term would represent the respiration directly associated with C_2H_2 reduction.

In these studies C_2H_2 reduction was used as an indirect assay for N-fixing activity and it would be inappropriate to make any conversions of these values to rates of N fixation without an empirically determined conversion factor (2, 8). Although a low concentration of C_2H_2 in air was used (0.02 atm), there was no significant difference in rate of C_2H_2 reduction when C_2H_2 in Ar/O_2 was used, and it is reasonable to assume that the relative slope values are representative of the more general respiration-nitrogenase activity relationships.

The over-all results showed a consistent linear relationship between total root + nodule respiration and C_2H_2 reduction in all treatment conditions. This supports the use of respiration measurements as indicators of energy flow to the nitrogenase system. The positive respiration intercept, however, suggests that not all respiration was directly associated with C_2H_2 reduction. Thus, a simple ratio of respiration to N fixation will only indicate the true relationship when the other respiration components are negligible in relation to that associated with the N-fixing system.

In the present experiments, the slope of this respiration-C_2H_2 reduction relationship was variable and two groups of significantly different values were obtained. In the three experiments which would be expected to have variable growth and/or maintenance respiration, high values averaging 0.42 mg CO_2 (μmol C_2H_2 reduced)$^{-1}$ were found, but for the two experiments in which changes in growth and maintenance would be minimal, the slope term averaged 0.27 mg CO_2 (μmol C_2H_2 reduced)$^{-1}$. Similar low values were found during growth if only plants of the same age were considered. Although small parallel differences in growth or maintenance components could lead to some overestimation, it seems likely that these latter values do estimate the respiration directly associated with C_2H_2 reduction.

If this relationship can be verified, then these treatments can be used to estimate the energy consumed by the N-fixing reactions. Although similar results were obtained when C_2H_2 reduction and respiration were decreased by both short dark treatments and by application of NH_4NO_3, the simultaneous measurement of control and N-treated plants appears to be a better method since it requires less measuring time and has less possibility of being affected by changes in other respiration components.

LITERATURE CITED

1. ALLISON FE 1935 Carbohydrate as a primary factor in legume symbiosis. Soil Sci 39: 123-143
2. BERGERSEN FJ (1970) The quantitative relationship between nitrogen fixation and the acetylene-reduction assay. Aust J Biol Sci 23: 1015-1025
3. BOND G 1941 Symbiosis of leguminous plants and nodule bacteria. I. Observations on respiration and the extent of utilization of host carbohydrates by the nodule bacteria. Ann Bot NS 5: 313-337
4. CHEN P, DA PHILLIPS 1977 Induction of root nodule senescence by combined nitrogen in *Pisum sativum* L. Plant Physiol 59: 440-442
5. 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
6. GIBSON AH 1966 The carbohydrate requirements for symbiotic nitrogen fixation: a "whole-plant" growth analysis approach. Aust J Biol Sci 19: 499-515
7. GIBSON AH 1976 Limitation to dinitrogen fixation by legumes. *In* WE Newton, CJ Nyman, eds, Proc 1st Internat Symp of Nitrogen Fixation, Vol 2. Washington State University Press, Pullman, pp 400-428
8. HARDY RWF, RC BURNS, RD HOLSTEN 1973 Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biol Biochem 5: 47-81
9. HARDY RWF, AJ D'EUSTACHIO 1964 The dual role of pyruvate and the energy requirement in nitrogen fixation. Biochem Biophys Res Commun 15: 314-318
10. HARDY RWF, UD HAVELKA 1975 Nitrogen fixation research: a key to world food? Science 188: 633-643
11. LARUE TAG, WGW KURZ 1973 Estimation of nitrogenase in intact legumes. Can J Microbiol 19: 304-305
12. LAWN RJ, WA BRUN 1974 Symbiotic nitrogen fixation in soybeans. I. Effect of photosynthetic source-sink manipulations. Crop Sci 14: 11-16
13. LAWRIE AC, CJ 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
14. MAHON JD, SB LOWE, LA HUNT, M THIAGARAJAH 1977 Environmental effects on photosynthesis and transpiration in attached leaves of cassava (*Manihot esculenta* Crantz). Photosynthetica 11: 121-130
15. MCCREE KJ 1970 An equation for the rate of respiration of white clover plants grown under controlled environments. *In* Prediction and Measurement of Photosynthetic Productivity (Proc 1BP/PP Technical Meeting, Trebon). Centre for Agricultural Publishing and Documentation, Wageningen, pp. 221-229
16. MCCREE KJ 1974 Equations for the rate of dark respiration of white clover and grain sorghum as functions of dry weight photosynthetic rate and temperature. Crop Sci 14: 509-514
17. MINCHIN FR, JS PATE 1973 The carbon balance of a legume and the functional economy of its root and nodules. J Exp Bot 24: 259-271
18. MINCHIN FR, JS PATE 1974 Diurnal functioning of the legume root nodule. J Exp Bot 25: 295-308
19. MORTENSON LE 1964 Ferredoxin and ATP, requirement for nitrogen fixation in cell-free extracts of *Clostridium pasteurianum*. Proc Nat Acad Sci USA 52: 272-279
20. OGHOGHORIE CGO, JS PATE 1971 The nitrate stress syndrome of the nodulated field pea (*Pisum arvense* L.). *In* TA Lie, EG Mulder, eds, Biological Nitrogen Fixation in Natural and Agricultural Habitats. Plant Soil, Special Volume. Martinus Nyhoff, The Hague, pp 185-202
21. PENNING DE VRIES FWT 1972 Respiration and growth. AR Rees, KE Cockshull, DH Hand, RG Hurd, eds, Crop Processes in Controlled Environments. Academic Press, London, pp 327-347
22. PHILLIPS DA, KD NEWELL, SA HASSELL, CE FELLING 1976 The effect of CO_2 enrichment on root nodule development and symbiotic N_2 reduction in *Pisum sativum* L. Am J Bot 63: 356-362
23. SCHUBERT KR, HJ EVANS 1976 Hydrogen evolution: a major factor affecting the efficiency of nitrogen fixation in nodulated symbionts. Proc Nat Acad Sci USA 73: 1207-1211
24. STREETER JG 1974 Growth of two soybean shoots on a single root. J Exp Bot 25: 189-198
25. THORNLEY JHM 1970 Respiration growth and maintenance in plants. Nature 227: 304-305
26. WALLACE W, JS PATE 1965 Nitrate reductase in the field pea (*Pisum arvense* L.) Ann Bot NS 29: 655-671
27. WILSON DO, HM REISENAUER 1963 Cobalt requirement of symbiotically grown alfalfa. Plant Soil 19: 364-373