Plant Physiol. (1979) 64, 888–891
0032-0889/79/64/0888/04/$00.50/0

Economy of Photosynthate Use in Nitrogen-fixing Legume Nodules

OBSERVATIONS ON TWO CONTRASTING SYMBIOSES[1]

Received for publication December 29, 1978 and in revised form June 21, 1979

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ABSTRACT

The economy of C use by root nodules was examined in two symbioses, *Vigna unguiculata* (L.) Walp. (cv. Caloona):*Rhizobium* CB756 and *Lupinus albus* L. (cv. Ultra):*Rhizobium* WU425 over a 2-week period in early vegetative growth. Plants were grown in minus N water culture with cuvettes attached to the nodulated zone of their primary roots for collection of evolved CO_2 and H_2. Increments in total plant N and in C and N of nodules, and C:N weight ratios of xylem and phloem exudates were studied by periodic sampling from the plant populations. Itemized budgets were constructed for the partitioning and utilization of C in the two species. For each milligram N fixed and assimilated by the cowpea association, 1.54 ± 0.26 (standard error) milligrams C as CO_2 and negligible H_2 were evolved and 3.11 milligrams of translocated C utilized by the nodules. Comparable values for nodules of the lupin association were 3.64 ± 0.28 milligrams C as CO_2, 0.22 ± 0.05 milligrams H_2, and 6.58 milligrams C. More efficient use of C by cowpea nodules was due to a lesser requirement of C for synthesis of exported N compounds, a smaller allocation of C to nodule dry matter, and a lower evolution of CO_2. The activity of phosphoenolpyruvate carboxylase in nodule extracts and the rate of $^{14}CO_2$ fixation by detached nodules were greater for the cowpea symbiosis (0.56 ± 0.06 and 0.22 milligrams C as CO_2 fixed per gram fresh weight per hour, respectively) than for the lupin 0.06 ± 0.02 and 0.01 milligrams C as CO_2 fixed per gram fresh weight per hour. The significance of the data was discussed in relation to current information on theoretical costs of nitrogenase functioning and associated nodule processes.

Several investigations have considered the energy relationships of N fixation by nodulated legumes. Some of these have concerned the thermodynamics of the reactions (3), the energetics of nitrogenase functioning (5, 6), and the effects of H_2 evolution by nitrogenase on the efficiency of N fixation in specific symbioses (7, 20–23). Other investigations have assessed nodule functioning in terms of carbohydrate intake from the parent plant (9, 12, 15), CO_2 loss by the below-ground organs of nodulated plants (9, 11, 14) or by a detailed C economy of nodules (1). This paper describes an experimentally based approach to determine the cost of symbiosis involving simultaneous measurements of N_2 fixation, and CO_2 and H_2 evolution by attached nodules of intact legumes. These data are used to construct itemized budgets for the utilization of photosynthetically fixed C in two contrasting legume: *Rhizobium* associations.

[1] Supported by funds from the Australian Research Grants Committee and the Wheat Industry Research Council. DBL acknowledges support from a NSERC (Canada) Scholarship, RMR a Commonwealth Post-Graduate Award (Australia).

MATERIALS AND METHODS

Plant Material and Culture. The experiments were conducted on nodulated plants of cowpea (*Vigna unguiculata* [L.] Walp. cv. Caloona) or white lupin (*Lupinus albus* L. cv. Ultra) over the period 30 to 44 days after sowing in lupin, 30 to 43 days in cowpea. Nodules commenced to fix N_2 at 21 days in lupin, 14 days in cowpea. Seeds were sown in sand and inoculated with a *Rhizobium* strain known to develop effective crown nodulation on the relevant host species (WU425 for lupin and CB756 for cowpea). Seedlings were transferred to minus N water culture 10 days after sowing. Lateral roots developing within the top 6 cm of primary root were excised. Once nodules were visible on a plant the level of the culture solution was lowered below the nodulation zone, and a

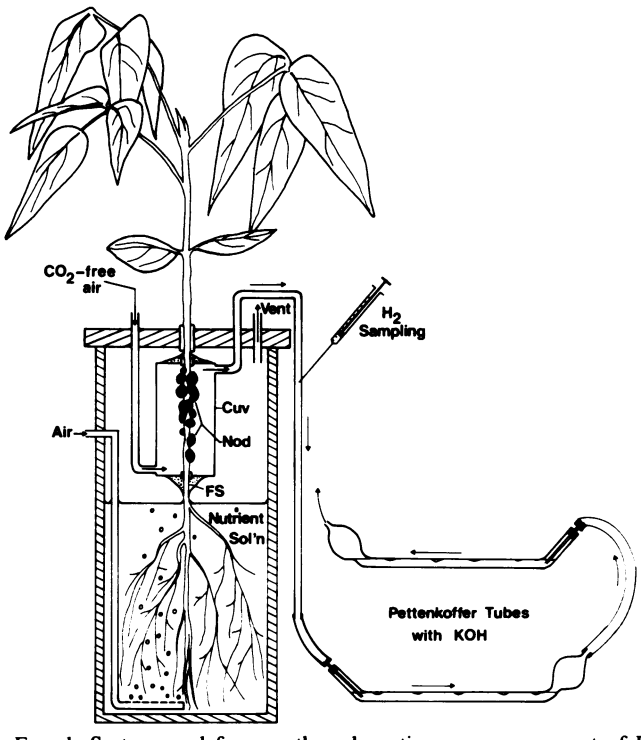

FIG. 1. System used for growth and continuous measurement of H_2 and CO_2 evolution from the nodulated regions (Nod) of roots of intact legumes. CO_2-free air was passed through a cuvette (Cuv) enclosing all of the nodules on the root and respired CO_2 collected in KOH in Pettenkoffer assemblies. Samples (0.5 ml) of gas were taken periodically for measurements of H_2 evolution. The flexible sealant (FS) used to seal the cuvette to the root was Terostat VII (Teroson GmbH, Heidelberg, FRG). The lower portion of the root system was immersed in minus N nutrient solution and aerated continuously.

plastic cuvette (volume 28 cm^3) sealed onto the nodulated zone of root (Fig. 1). All nodules on a plant were enclosed within the cuvette. CO_2-free air was passed continuously through the cuvettes at 30 to 70 cm^3/min, achieving an average CO_2 level (600–1,200 μl/l) in the cuvettes, similar to that found in the rhizosphere of comparable nodulated plants in sand culture (9, 14). Cowpea was maintained in a growth cabinet (12-h days of 35 C, 800–1,000 $\mu E/m^2 \cdot s$, 12-h nights of 18 C), lupin in a naturally lit glasshouse (11-h days, daily temperature range 8–25 C, July–August, Perth, Western Australia).

Growth and C and N Accumulation in Nodules and Plant Parts. Dry weight and C and N content were determined by periodic sampling. The techniques used were as described previously (15, 16).

Measurements of CO_2 and H_2 Evolution of Nodules. Individual Pettenkoffer assemblies (Fig. 1) were used to collect respired CO_2 from the cuvettes of five plants for each interval of the study periods (12). Measurements of respiration of freshly detached nodules and root segments were as described previously (9).

Samples (0.5 ml) of gas were taken at intervals on a diurnal basis from the cuvette effluent streams (Fig. 1) and analyzed for H_2 on a GLC equipped with a thermal conductivity detector and a column of Porapak N (4 mm × 2 m, 100–120 mesh). In addition, at three intervals in the study periods of each species, H_2 evolution was sampled from attached nodule clusters in cuvettes flushed with CO_2-free argon:O_2 (80:20, v/v, 30–70 cm^3/min). Relative efficiency of nodule fixation was then computed as described by Schubert and Evans (21).

Analyses of Xylem and Phloem Sap. Root (xylem) bleeding sap was collected from lupin and cowpea, stem base phloem sap from lupin. Analyses of sap for solutes were as described elsewhere (2, 8, 17, 18).

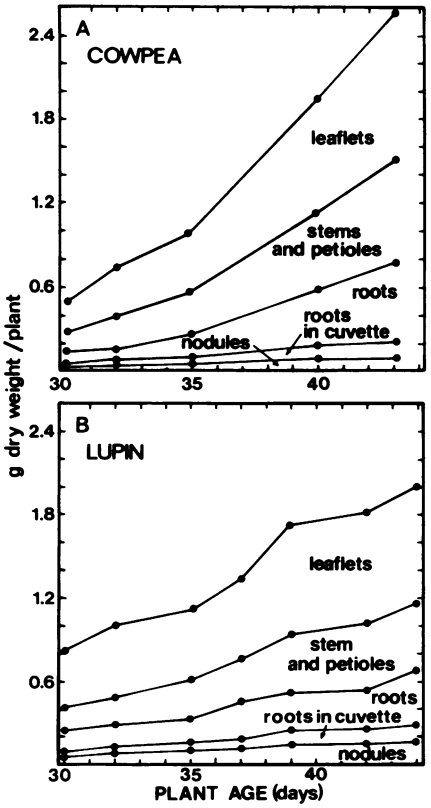

FIG. 2. Dry matter accumulation in plant parts of (A) cowpea inoculated with *Rhizobium* CB756, (B) lupin inoculated with *Rhizobium* WU425. Plots are cumulative with vertical distances between successive pairs of lines representing quantities of dry matter present in specific plant parts at a particular time in the study period. Portions of root inside and outside the cuvette are shown separately. Plants grown in minus N water culture with cuvettes attached (see Fig. 1).

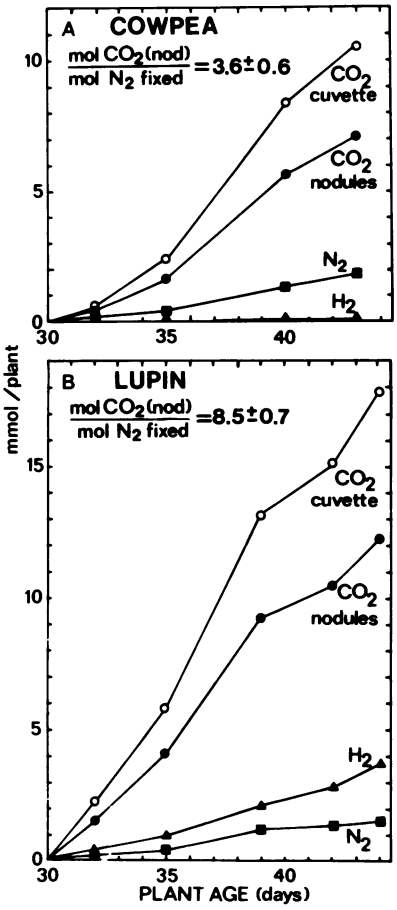

FIG. 3. Time courses of H_2 and CO_2 evolution and N_2 fixation (increase in total plant N) in (A) cowpea inoculated with *Rhizobium* CB756 and (B) white lupin inoculated with *Rhizobium* WU425. Estimates of CO_2 evolution due to nodules (CO_2 nodules) were made as described in the text and are plotted separately from the total CO_2 evolution of the enclosed zone of nodulated root (CO_2 cuvette). Plants grown in minus N culture with cuvettes attached (see Fig. 1).

CO_2 Fixation of Nodule Extracts and Detached Nodules. Cell-free extracts from nodules were assayed for PEP[2] carboxylase (EC 4.1.1.31) by measuring PEP-dependent fixation of $^{14}CO_2$ at 30 C (19). Ability of freshly detached nodules to fix $^{14}CO_2$ was compared in the two species by enclosing samples (2 g fresh weight) of nodules in a closed 250-cm^3 vessel. The nodules were incubated with $^{14}CO_2$ for 30 min at 25 C and then extracted with hot 80% ethanol. After acidification to remove dissolved CO_2 the extracts were analyzed for ^{14}C by liquid scintillation.

RESULTS AND DISCUSSION

Growth. Changes in dry weight of plant parts over the study period were as shown in Figure 2, A and B. All parts increased in weight, including those enclosed in cuvettes. Mean growth rates in water culture with cuvettes attached were 84 ± 7 (SE) mg dry weight/plant·day for lupin, 161 ± 19 for cowpea. Plants of comparable age in sand culture recorded rates of 93 ± 9 and 154 ± 23 mg dry weight/plant·day, respectively.

N_2 Fixation and CO_2 and H_2 Evolution from Nodules. Fixation of N_2, determined from increments in plant N, increased in both

[2] Abbreviation: PEP: phosphoenolpyruvate.

species over the study periods. The total CO_2 evolved into the cuvette (Fig. 3) was due to respiration of both nodules and the segment of supporting root which was enclosed. The separate contributions of nodules and the root segment to the CO_2 released to the cuvette were estimated from measured respiration rates of freshly detached organs (14). For example, in lupin from 32 to 35 days 3.56 mmol CO_2/plant was collected from 0.43 g fresh weight of nodules and a supporting root segment of 0.30 g. For 30 min following detachment the nodules respired 43.5 μmol CO_2/g fresh weight·h, the supporting root segment 25.8 μmol/g fresh weight·h. These data indicated that 73% of the total CO_2 released to the cuvette was due to nodules, 27% to the supporting root. Using this technique the CO_2 evolution due to nodules alone was estimated at each sampling time during the study period (Fig. 3). For both species this was 65 to 85% of the total CO_2 efflux to the cuvette.

Table I. *Economy of C and N in Nodules of Two Legume:Rhizobium Associations*

	Cowpea: CB756	Lupin WU425
	mg/plant·day (±SE[a])	
1. C incorporated into nodule mass	2.50 ± 1.15	3.35 ± 0.78
2. C loss as CO_2 in respiration	5.72 ± 1.46	10.20 ± 1.68
3. C exported with fixed N in xylem[b]	3.36	4.86
4. Total C imported in phloem (1+2+3)	11.58	18.41
5. N imported as translocate in phloem[c]	0.13	0.27
6. N incorporated into nodule mass	0.29 ± 0.15	0.60 ± 0.17
7. N incorporated from current fixation (6-5)	0.17	0.34
8. Total N fixed	3.72 ± 0.73	2.80 ± 0.69
9. N exported (8-7)	3.56	2.46
	mg/mg	
10. C imported/N fixed (4÷8)	3.11	6.58
11. C (as CO_2) evolved/N fixed (2+8)	1.54 ± 0.26	3.64 ± 0.28

[a] SE for experimentally derived data only.

[b] C:N (weight basis) ratio of xylem sap 0.94 in cowpea, 1.97 in lupin.

[c] C:N (weight basis) ratio of stem base phloem sap 69 in lupin. Assumed that proportion of nodules N increment supplied by phloem was similar to that in lupin.

The data showed substantial differences between the species in CO_2 output per unit of N_2 fixed. The mean value for lupin was 8.5 ± 0.7 (SE) mol CO_2/mol N_2 fixed, for cowpea 3.6 ± 0.6 mol CO_2/mol N_2 fixed.

H_2 Evolution and the Relative Efficiency of N_2 Fixation. Considerably more H_2 was evolved from nodules of the lupin symbiosis than from nodules of the cowpea association (Fig. 3). The mean rates were 3.06 ± 0.68 mol H_2/mol N_2 fixed in lupin and 0.02 ± 0.01 mol H_2/mol N_2 fixed in cowpea, giving relative efficiency values (4, 21) for these two host cultivar:*Rhizobium* strain symbioses of 0.58 to 0.61 and 0.95 to 0.98, respectively. In cowpea the low rate of H_2 evolution could be due to efficient coupling of electron flow to N_2 reduction (21, 23) or to the activity of a unidirectional uptake hydrogenase (7, 22).

Composition of Transport Fluids. Asparagine was the major solute of lupin xylem sap, ureides (allantoin + allantoic acid) the principal compounds exporting fixed N in cowpea. The C:N ratio (weight basis) of xylem sap was lower in cowpea (0.94:1) than in lupin (1.97:1), due to the high N content of ureides. Sucrose, Asn, Gln, Val, Ser, Asp, Ile, Lys, and Phe were the major organic solutes of stem base phloem sap of lupin. The average C:N ratio of the phloem sap was 69:1.

C and N Economy of Nodules. As in previous studies (12, 15), C and N budgets for nodules were constructed using data for N_2

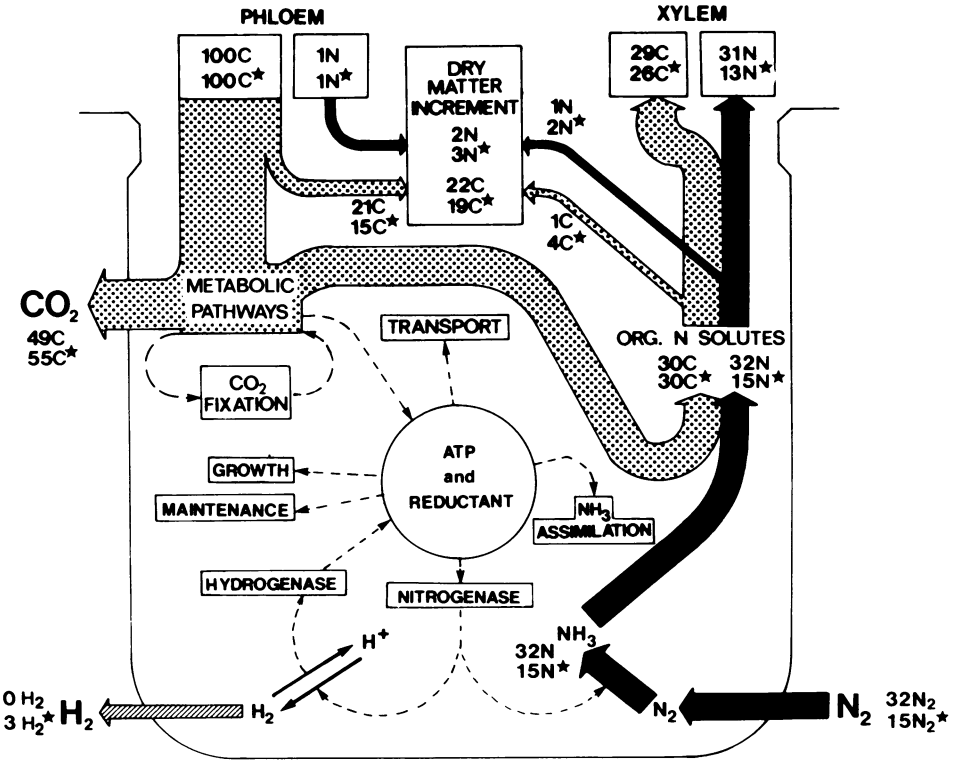

FIG. 4. Partitioning and utilization of translocated C by N-fixing nodules of cowpea:*Rhizobium* CB756 and white lupin:*Rhizobium* WU425. All budget items are expressed on a weight basis and in terms of a net intake by the nodule of 100 mg of C as translocated carbohydrate. Experimental data on C and N economy are given as weights (mg) of C and N transported or consumed by the nodule. Values for the lupin association are marked by a star and are given below those for the cowpea association. The scheme includes items of the nodule's budget (---), which, on theoretical grounds, would have consumed or produced ATP and reductant during nodule functioning and would therefore have had bearing on the observed CO_2 output of the nodule. The possibility is suggested of fixation of respired CO_2 by the carboxylase systems of the nodules.

fixation/plant, C and N increments in nodule dry matter, net loss of C as CO_2 from the nodules, C:N ratios of xylem sap (cowpea and lupin) and stem base phloem sap (lupin) (Table I). In the absence of information for the composition of phloem sap of cowpea we assumed that nodules of this species received the same proportion (around 50%) of their N increment from phloem as lupin.

Cowpea was the more efficient in utilizing C for N_2 fixation, requiring 3.1 mg C in fixing 1 mg of N *versus* 6.6 mg C/mg N in lupin. The poorer efficiency of C use in lupin nodules was due to a larger requirement in all items of C expenditure (Table I).

The utilization of C by the two associations was further compared (Fig. 4) on the basis of a budgeted expenditure of 100 mg of C (as translocated carbohydrate) from the host plants. Data were included for CO_2 and H_2 evolution, N_2 fixation, C and N increments in nodule dry matter, and C export with fixation products via the xylem. The scheme, also indicated items of expenditure (Fig. 4 ---), for which experimental data were not available, but which on theoretical grounds would have consumed or produced ATP and reductant during nodule functioning. A recent publication (1) provided theoretically based assessments of the costs for reduction of N_2 to NH_3 by nitrogenase, for the subsequent assimilation of NH_3 to form the nitrogenous groups of organic solutes, and for the respiration associated with growth and maintenance of nodule tissues. These were together equivalent to 2.0 mg C as CO_2 mg N assimilated in *Vigna* and 2.1 (same units) in *Lupinus*, suggesting little difference between the species in these respects. The item marked "transport" in Fig. 4, involving membrane transfers in bacteroidal tissue and in the subsequent release of exported products to the xylem from the nodule symplast (13), was similarly regarded to be unlikely to be very different in the two species, with a possible minor saving in *Vigna* in view of the 4 N atoms exported per ureide molecule, *versus* 2N per amide in *Lupinus*. Assuming transport to involve ATPase activity (10) in at least three membrane transfers, a cost of 3 mol ATP (equivalent to 0.5 mol CO_2)/mol NH_3 fixed would appear reasonable, and, in view of the relatively small size of this item, differences in the form of solute transported would be unlikely to have a major effect on the over-all C budget of the nodule.

Viewed in these terms we concluded that the more likely causes of the substantial difference in C economy of nodules of the two associations were differing costs relating to H_2 evolution and associated unidirectional hydrogenase activity, basic differences in cost of metabolic pathways ancillary to ureide and amide production, and differing degrees of CO_2 conservation through carboxylase activity.

The possibility was also considered of differences between the nodules in ATP requirements for *in vivo* functioning of nitrogenase. Judging from *in vitro* studies (5, 6), this probably represented a large item of expenditure.

Evidence was obtained of substantial differences between the associations in CO_2-fixing capacity. The average PEP carboxylase activities were 0.56 ± 0.06 (SE) mg C as CO_2 fixed/g fresh weight for cowpea nodules, and for lupin, 0.06 ± 0.02 mg C as CO_2 fixed/g fresh weight·h. Rates of CO_2 fixation based on the uptake of $^{14}CO_2$ by detached nodules were also very greatly different, 0.22 mg C as CO_2 fixed/g fresh weight·h for cowpea nodules *versus* 0.01 mg C as CO_2 fixed/g fresh weight·h for lupin nodules. Differences of this magnitude were likely to have had considerable impact on the C economy of nodules of the two associations.

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