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CARBON FLOW, PHOTOSYNTHESIS, AND N_2 FIXATION IN MYCORRHIZAL AND NODULATED FABA BEANS (*VICIA FABA* L.)

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Summary—Carbon flows to vesicular–arbuscular mycorrhizal fungi and rhizobial symbionts of 4- to 5-week-old faba beans, *Vicia faba* L., were measured by determining the distribution of $^{14}CO_2$-C fixed by above-ground plant parts. Mycorrhizal fungi of both nodulated and non-nodulated hosts utilized ca. 4% of the C fixed by their hosts. Nodules utilized 6% of the C fixed by non-mycorrhizal hosts and 12% of the C fixed by mycorrhizal hosts. Measured rates of CO_2 fixation for symbiotic beans were higher than for non-symbiotic beans. Nodulated root systems of mycorrhizal beans fixed more $^{15}N_2$ than nodulated root systems of non-mycorrhizal plants. An increase in nodule biomass for plants infected with both rhizobia and mycorrhizal fungi was concluded to be the major factor increasing N_2 fixation rates.

INTRODUCTION

Most plant species can benefit from symbiotic associations with various types of mycorrhizal fungi. Legumes are also able to benefit from symbiotic associations with N_2-fixing bacteria. The benefits derived by the host from rhizobial infections, and the energy cost of this symbiosis have been quantified by several authors (Minchin and Pate, 1973; Mahon, 1977; Atkins *et al.*, 1978; Haystead *et al.*, 1979; Ryle *et al.*, 1979a).

Energy cost estimates for mycorrhizal symbioses have been more limited. Ectomycorrhizae of temperate trees have been calculated to consume as much as 33% of the total gross yearly production of forests (Newman, 1978). Harley (1971) cites a study claiming that two-thirds of the photosynthate of *Pinus cembra* may be translocated to ectomycorrhizae. Studies on the C requirements of vesicular–arbuscular mycorrhizal fungi have been limited. Ho and Trappe (1973) established that C does move from hosts to fungal symbionts and Pang and Paul (1980) found the mycorrhizal root systems evolved more CO_2 than non-mycorrhizal root systems. To date, however, the actual quantities of C involved in maintaining a mycorrhizal symbiosis have not been determined.

The question of carbon cost of vesicular–arbuscular mycorrhizal fungi is important to an overall understanding of the mycorrhizal symbioses. The literature has many reports of increased plant growth due to mycorrhizal infection, but also contains reports of negative growth responses. Many of the negative responses are coupled with increased plant concentrations of P, K, Ca, Fe, Zn, Cu, and S (Gerdemann, 1964; Rhodes and Gerdemann 1978; Lambert *et al.*, 1979). Stribley *et al.* (1980) originally attempted to explain the higher concentrations of elements in the host tissues as a result of carbon utilization of mycorrhizal fungi. However, they have found that no simple relation between mycorrhizal infection and carbon drain could explain the elevated elemental concentrations (D. P. Stribley and P. B. Tinker, personal communication, 1981). Estimates of mycorrhizal fungal biomass of up to 17% of the host root mass have been reported (Hepper, 1977). The large biomass of fungus plus the concentrations of lipid within the fungal structures suggest that the quantities of C translocated to the fungus could be substantial (Cooper and Lösel, 1978).

The interaction of legume hosts and VA mycorrhizal fungi is further complicated by the presence of a second symbiont, the N_2 fixing rhizobia. Previous studies (Smith and Daft, 1977; Daft and El Giahmi, 1974; Crush, 1974) have shown that, while the total nitrogenase activity of a mycorrhizal nodulated root increases, the nitrogenase activity per unit weight of root does not. These studies measured N_2 fixation by acetylene reduction. $^{15}N_2$ tracer techniques have the advantage of more accurate quantification of N_2 fixation under more normal conditions.

We have assessed the C flow to mycorrhizal symbionts and nodules of faba beans, *Vicia faba* L., using mycorrhizal biomass measurements and ^{14}C labelling techniques. Fixation studies with $^{15}N_2$ were also performed on mycorrhizal and non-mycorrhizal nodulated faba beans.

MATERIALS AND METHODS

Surface-sterilized seeds of *Vicia faba* cv. Diana, were planted in 3200 g of 1:1 (v/v) autoclaved sand:sterilized soil (15% ethylene oxide for 48 h) in 4-l plastic pots. The soil (Ap of an Oxbow loam, Black Chernozemic) was obtained from a summerfallow field previously planted to wheat. The final sand–soil mixture contained 16 μg NO_3-N and 4 μg

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$NaHCO_3$-extractable P g^{-1} soil. Mycorrhizal fungal inoculum, consisting of dried roots and adhering soil from faba beans previously infected with *Glomus mosseae*, was placed in a layer 5 cm below the seed. Dried roots and adhering soil from non-mycorrhizal plants were added to pots of non-mycorrhizal plants. In addition, 10 $\mu g\, g^{-1}$ P as KH_2PO_4 was added to non-mycorrhizal pots to produce plants of equal size to mycorrhizal plants. A commercial rhizobium inoculum (*R. leguminosarum*, Legume Aid Co., Agricultural Laboratories, Columbus, Ohio), where used, was sprinkled over the seed at the time of planting. Rhizobially inoculated plants received 20 $\mu g\, g^{-1}$ N as $Ca(NO_3)_2$ while uninoculated plants received 100 $\mu g\, g^{-1}$ N. Faba beans were grown under light conditions of 19 klx with 16 h light and 8 h dark with corresponding temperatures of 24 and 20°C. Soils were watered up to 90% of field capacity on the basis of daily gravimetric measurements. For most experiments, the plants were 4–5 weeks old but, in a separate experiment, they were 5–6 weeks old. Plants were transferred to the labelling chamber 48 h before the onset of the labelling period.

Mycorrhizal fungal biomass was estimated by microscopically measuring fungal structures associated with the root systems of mycorrhizal faba beans grown under the same conditions as plants used for carbon flow experiments. Six soil cores (2.5 cm dia) were removed from intact pots and gently washed to remove soil particles. Cores contained an average of 20 cm of root. Three of the cores were used to determine external hyphal weights, vesicle weights, and percent infection. External mycorrhizal hyphae were picked from the root segments, blended in 100 ml of H_2O and aliquots mounted onto glass slides. The mounted pieces of hyphae were measured microscopically. Root segments were then cleared in 10% KOH and stained with acid fuschin in lactophenol. Percent infection was determined by a line intercept method (Ambler and Young, 1977). Internal vesicles in the stained root segments were measured microscopically. Roots in the remaining three cores were cleaned of external hyphae, cleared in 10% KOH, dried, blended and an aliquot filtered onto a millipore filter (0.4 μm). The material on the filter was stained with acid fuschin in lactophenol. Internal hyphal segments were measured. The biovolume measurements were converted to biomass using a factor of 0.35 g cm^{-3} (Van Veen and Paul, 1979). The relation between external hyphal biomass and total mycorrhizal biomass was found to be significant ($r^2 = 0.98$). This relation was used to convert external hyphal weights to total mycorrhizal fungal weights in the C flow experiments.

Carbon flow measurements were obtained from plants exposed to $^{14}CO_2$ in a closed chamber similar to that used by Pang and Paul (1980) with below- and above-ground atmospheres kept separate. $^{14}CO_2$ was added to the above-ground atmosphere and maintained at levels between 0.03 and 0.05% during the labelling period. Below-ground air was scrubbed clean of atmospheric CO_2 before passage through the below-ground compartment. Respired $^{14}CO_2$ from the root systems was collected in 0.2 N NaOH bubble traps that were replaced and sampled at timed intervals. Total CO_2 evolution from the root systems was determined by titration of the excess NaOH with 0.1 N HCl (phenolphthaline indicator) following precipitation of CO_3^{2-} with $BaCl_2$.

C flow was calculated from data obtained from plants labelled with $^{14}CO_2$ for 48 h of continuous light, then grown in $^{12}CO_2$ for an additional 96 h of continuous light. At the end of the experiments, 2.5-cm cores were removed from the soil for fungal biomass ^{14}C determination. The remaining roots were washed from the soil, the nodules were picked from the roots, and the plant material and soil were dried, weighed, and ground. For comparison, a similar experiment was performed using 5- to 6-week-old plants that were labelled for 8 h, then grown for 116 h of continuous light.

CO_2 fixation rates of symbiotic and non-symbiotic faba beans were determined using plants labelled for 8 h, and maintained for an additional 16 h of continuous light in $^{12}CO_2$. Plants were harvested and ^{14}C in biomass and respired ^{14}C added to determine total ^{14}C fixed. CO_2 fixation rates were calculated by dividing the total ^{14}C fixed during the labelling period by the total shoot weight at the end of the experiment. N_2 fixation rates were determined after circulating $^{15}N_2$ (atom % abund. = 0.701 − 0.820) in the below-ground atmosphere of plants for two 16-h-light:8-h-dark periods. Below-ground systems in this case were modified so that the atmosphere was circulated in a closed system. Below-ground O_2 concentrations were monitored and maintained at 20% O_2 (v/v) by additions of O_2.

C contents and specific activities of C in plant material and soil were determined after combustion in a Lindeburgh dry combustion furnace. ^{14}C liberated by sample combustion was collected in NaOH and measured by liquid scintillation. Fungal ^{14}C contents were determined by dissolving the external VA mycorrhizal hyphae, picked from the soil cores, in 4 ml of Biosolve tissue solubilizer (72 h, 30°C) and counting the samples by liquid scintillation. The ^{14}C content of the external hyphae was multiplied by the ratio of VA biomass:external VA biomass previously determined to calculate the ^{14}C contained in the mycorrhizal fungal biomass.

Shoot N contents were determined after semi-micro-Kjeldahl digestion (Bremner, 1965). ^{15}N was measured using an Atlas GD 150 mass spectrometer with a double-collector system.

Calculation of C flow to symbionts

C flow to symbionts was calculated as the sum of the ^{14}C incorporated into symbiont biomass and the $^{14}CO_2$ respired by the symbionts. ^{14}C in symbiont biomass was measured directly. Respired ^{14}C was calculated by assuming that any increase in $^{14}CO_2$ evolution from root systems was due to symbiont metabolism. Mahon's (1977) respiration equation for a legume root system was modified to include terms for mycorrhizal fungi (equation 1):

$$R = R_m P + R_g(dP/dt) + R_m M + R_g(dM/dt) + R_m N + R_g(dN/dt) + F + E \quad (1)$$

where R = total root respiration; P = plant root biomass; N = nodule biomass; M = mycorrhizal fungal biomass; R_m = maintenance respiration for each biomass component; R_g = growth respiration for each

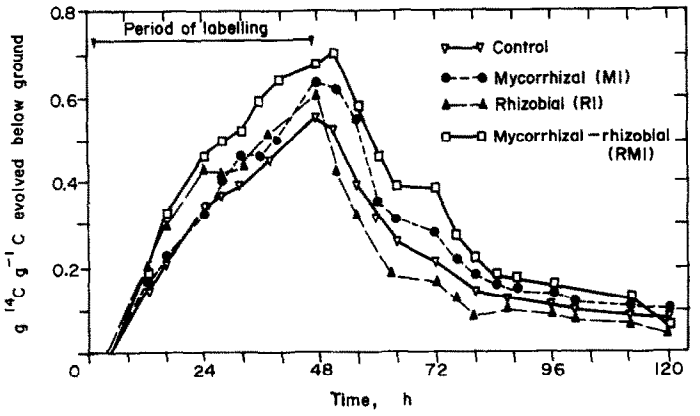

Fig. 1. Evolution of $^{14}CO_2$ from roots of beans (4- to 5-week-old) following a 48-h exposure of shoots to above-ground $^{14}CO_2$.

biomass component; dP/dt, dN/dt, dM/dt = change in biomass component with time; F = respiration associated with nitrogen fixation; and E = respiration from root exudates.

The plant root component, $R_mP + R_g(dP/dt)$ and the E values were the same for all treatments, therefore differences in respiration between treatments were assumed to be due to the symbionts presence.

Fungal and rhizobial portions of the C flow in doubly infected hosts were determined by assuming that the symbionts of doubly infected plants incorporated ^{14}C into their biomass in the same proportion as the symbionts of singly infected plants.

RESULTS

Carbon flow to symbiotic organisms

The distribution of ^{14}C fixed by beans was used to determine C flow to symbionts. Summation of the label in shoots, roots, soil, symbiotic material, and respired CO_2 after 120 h of continuous light yielded 95% recovery of the added label. Curves of below-ground ^{14}C evolution showed that, at the time of harvest, ^{14}C was no longer being respired (Fig. 1). This indicated that the balance of the ^{14}C label, was incorporated in compounds that were not likely to be metabolized further in the near future.

The distributions of ^{14}C (Table 1) in the control and singly infected treatments were very similar. *ca.* 66% of the label in 4- and 5-week-old beans was recovered as plant and symbiont biomass (46% as shoot biomass, 20% as root biomass). The other 34% was respired, mainly from the root. Shoot respiration accounted for very little of the total because of continual light conditions, i.e. no dark respiration. Subsequent labelling experiments using 16 h light and 8 h dark showed that while ^{14}C loss by shoot respiration increased, the C flow to the roots was not affected. A

Table 1. ^{14}C distribution in 4- and 5-week-old (48 h) and 5- to 6-week old (8 h) symbiotic and non-symbiotic beans

	4- to 5-week-old (48 h)				5- to 6-week old (8 h)			
	Control	Mycorr. (MI)	Rhiz. (RI)	Mycorr.-Rhiz. (RMI)	Control	Mycorr. (MI)	Rhiz (RI)	Mycorr.-Rhiz. (RMI)
				Plant data				
CO_2 fixation rate*	7.02 *c*	7.60 *b*	7.92 *b*	8.23 *a*	6.79 *c*	6.96 *b*	7.32*b*	9.24 *a*
Shoot weight (g)	2.04 *a*	2.47 *a*	1.98 *a*	1.84 *a*	4.31 *a*	4.40 *a*	3.64 *a*	3.59 *a*
Root weight (g)	1.45 *a*	1.73 *a*	1.32 *a*	1.18 *a*	2.03 *a*	1.65 *a*	1.75 *a*	1.64 *a*
Nodule weight (g)	—	—	0.10	0.10	—	—	0.11	0.15
Mycorrhizal infection (%)	—	52.8	—	47.2	—	58.6	—	54.8
				^{14}C *distribution* (%)				
Shoot biomass	42.5	47.4	44.9	41.7	54.6	52.0	46.8	42.0
Shoot respiration	2.5	2.0	1.7	2.3	1.7	1.0	2.0	1.1
Root biomass	22.1	19.5	18.3	15.0	20.7	20.2	25.0	16.8
Root respiration	32.6	27.3	28.9	22.1†	23.0‡	26.8‡	24.6‡	37.9‡
Mycorrhizal biomass	—	0.75	—	0.88	—	ND	—	ND
Mycorrhizal respiration	—	2.75	—	3.32	—	ND	—	ND
Nodule biomass	—	—	1.28	2.41	—	—	1.61	2.24
Nodule respiration	—	—	4.55	9.13	—	—	ND	ND

* (mg C g^{-1} shoot C h^{-1}) calculated using shoot weights as measured at the end of the experiment.
†3.2% of ^{14}C unaccounted for.
‡Root + symbiont respiration.
a–c Means followed by the same letter do not differ ($P < 0.5$).

Table 2. N_2 fixation by mycorrhizal (RMI) and non-mycorrhizal (RI) nodulated beans (4- to 5-week-old)

	Nodule wt/ root wt ($mg\ g^{-1}$)	% N in shoot	N fixed (mg)	N fixed per unit nodule wt ($mg\ g^{-1}$)
Rhizobial	87.7	3.81	0.78	16.2
Mycorrhizal-rhizobial	104.0**	4.34*	1.06*	15.8

* Significantly different ($P < 0.1\%$) from rhizobial treatment.
** Significantly different ($P < 0.01\%$) from rhizobial treatment.

smaller proportion of the label was recovered as root respiration from control and singly infected 5- to 6-week-old plants (Table 1). $^{14}CO_2$ evolution from doubly infected root systems tended to increase slightly between 4- to 5- and 5- to 6-weeks. Most of the growth of the faba beans during the experimental period occurred in the shoot as evidenced by the increase in shoot:root ratio.

CO_2 fixation rates were higher for each symbiotic treatment than for non-symbiotic plants (Table 1). Values calculated are lower than actual CO_2 fixation rates because of plant growth after the labelling period. In a subsequent experiment, CO_2 fixation rates were calculated for plants harvested soon after the end of the labelling period. Non-symbiotic plants had CO_2 fixation rates of 17.4 mg C g^{-1} shoot C h^{-1} and doubly infected plants fixed 20.2 mg C g^{-1} shoot C h^{-1}.

The host root tissue of non-symbiotic plants incorporated the translocated ^{14}C with an efficiency of 0.4 mg C incorporated in root biomass per mg C translocated to the root. Doubly infected plants maintained less ^{14}C in their root biomass and respired a higher proportion from the root system (efficiency = 0.3 mg mg^{-1} C). Singly infected treatments were intermediate in incorporation efficiency. The symbionts in all cases contained 1–2% of the added label.

Mycorrhizal fungi of both singly (MI) and doubly infected (RMI) 4- to 5-week-old plants respired or incorporated *ca.* 4% of the C fixed by their hosts. The fungal symbionts incorporated this C into fungal biomass with an efficiency of 0.21 mg C incorporated mg^{-1} C supplied.

Nodules of 4- to 5-week-old faba beans utilized 6% of the C fixed by singly infected (RI) plants and 12% of the C fixed by doubly infected (RMI) plants (Table 1). The supplied C was incorporated into nodule biomass with an efficiency of 0.22 mg C incorporated mg^{-1} C supplied.

Since calculation of C flow to nodules of RMI plants showed that they utilized more C than nodules of RI plants, a study was performed to determine if the increased C use affected symbiotic N_2 fixation. N_2 fixation rates were higher for RMI hosts, but this appeared to be a function of a larger mass of nodules per unit weight of root, not increased efficiency (Table 2).

DISCUSSION

Carbon flow to mycorrhizal and rhizobial symbionts was calculated as the sum of respired carbon and carbon incorporated into symbiont biomass. The CO_2-C evolved by the symbionts had to be mathematically separated from the CO_2-C evolved by the host. The CO_2 evolved by the symbionts was calculated by comparison of the respiration values for the different treatments, i.e. symbiont respiration equals the difference in root respiration between symbiotic and control treatments. Two assumptions were made in this calculation: first, that the respiration rate of the host root tissue was not changed by the presence of the symbiont and, secondly, that each symbiont of a doubly infected host incorporated C into its biomass with the same efficiency as when it was present as the sole symbiont. Symbiotic respiration as calculated for RMI plants did not account for all of the $^{14}CO_2$ respired from the below-ground system. One or both of the symbionts of RMI hosts may have respired a higher proportion of the C supplied to it, thereby decreasing the value for efficiency of C incorporation into biomass for that symbiont. Since the root systems of RMI plants fixed more N_2 when exposed to $^{15}N_2$ than the root systems of RI plants, it is likely that the nodules are responsible for the additional ^{14}C respiration in this experiment. However, this cannot be stated unequivocally.

Another possibility is that host tissue of symbiotic roots increased its CO_2 evolution. Pate *et al.* (1979) found that host tissue subtending nodules had higher rates of CO_2 evolution than host root tissue not associated with nodules. As well, cells containing arbuscules have been found to contain 22 times more cytoplasm than neighboring cells lacking fungal structures (Cox and Tinker, 1976). Thus, not all the increase in CO_2 evolution may be due to metabolism of microbial symbionts. Nonetheless, the increased respiration is still associated with symbiotic systems and, therefore, can be considered part of the carbon cost.

Summation of symbiont respired plus biomass ^{14}C yielded a value for the C flow to the symbionts. This C can be considered to be lost to the host and so would lead to reduced host growth were it not for the plants' ability to increase, in part, its CO_2 fixation rate to compensate for the symbiont C use. The mycorrhizal fungi of 4- to 5-week-old plants used 3.5% of the C fixed by MI hosts and 4.2% of the C fixed by RMI hosts. Compared to the control plants, CO_2 fixation rates were 8% higher in MI plants and 17% higher in RMI plants. Nodule tissue used 6% of the C fixed by RI plants and 12% of the C fixed by RMI plants while the hosts increased their CO_2 fixation rates by 13 and 17%. The C flow calculations did not consider the C returned by the symbiont to the host as C used by the symbiont. Because of increased CO_2 fixation rates by symbiotic hosts, these plants

can essentially receive the benefits of the symbioses without major losses of C needed for their own tissues. Shoot weight comparisons for all of the plants used for ^{14}C labelling experiments indicated that, in some cases, RMI hosts tended to produce less dry matter than RI or MI or uninfected plants, but the differences were not significant at the 95% level.

The basis of increased CO_2 fixation is not understood. Levy and Krikun (1980) found that mycorrhizal lemon seedlings recovered from water stress more quickly than non-mycorrhizal seedlings, and therefore had higher photosynthetic rates. This, they assumed, was due to mycorrhizal effects on water balance of the plant. C. R. Johnson, J. A. Menge and S. Schwab (personal communication, 1981) also found elevated CO_2 fixation rates for mycorrhizal orange seedlings over non-infected controls and concluded that increased P levels in the leaf tissue was responsible for the increase. In the present study, P was added to non-mycorrhizal plants, however, increased uptake of P, or some other nutrient, by mycorrhizal roots may still explain the increased levels of CO_2 fixation. Geiger (1976) has also suggested that increases and decreases in CO_2 fixation rates are probably due to indirect mechanisms, possibly involving hormonal control, rather than direct mechanisms of feedback control by product inhibition. His hypothesis may explain why growth of infected hosts has been increased in some studies, but not in others. Perhaps symbioses that do not result in increased growth are influenced by some incompatibility of host and fungus.

Legumes growing in poor soils show increased growth in response to mycorrhizal or rhizobial inoculation. Plants infected with both symbionts often show growth responses larger than those expected if the individual symbiotic effects were added. The same result often can be obtained by using fertilizer P instead of mycorrhizal inoculum. Thus, it appears that VA mycorrhizae assist in plant uptake of P, but do not interact directly with the nodules (Carling *et al.*, 1978). This theory is supported by Smith and Daft (1977) who found that on a dry weight basis, the P percentage and nitrogenase activity of hosts did not increase, even though overall growth was greater for dually infected plants.

Formation of larger and more numerous nodules on dually infected root systems has the effect of increasing the total nitrogenase activity of a root even though the activity per unit weight does not change (Daft and El Giahmi, 1974; Crush, 1974; Smith and Daft, 1977). Mosse *et al.* (1976) found that non-mycorrhizal roots of three legumes (clover, *Stylosanthes* and *Centrosema*) did not nodulate in a P deficient soil whereas mycorrhizal plants of the same species did. In this case, the control plants were too P deficient to support nodules.

Our $^{15}N_2$ fixation studies support the findings of previous studies using acetylene reduction techniques. C flow calculations also show that more assimilate is supplied to nodules of RMI plants. Whether the host supplies more C because it has more C to spare due to increased CO_2 fixation, or whether the nodules create a large C sink, so that the plant increases CO_2 fixation to supply the necessary C is not known. The increased N_2 fixation by RMI plants may also be due to differences in the host nutritional balance. P was added to control and RI plants in the ^{14}C labelling experiments at levels to compensate for the lack of fungus. However, since RMI plants still had higher N_2 fixation rates, some other factor, possibly a microelement that aids bacterial N_2 fixation may be supplied by the fungus. Since no direct contact between fungus and bacteria has been reported, it appears that the necessary factor would have to be supplied to the bacteria through the host cells.

Other authors working with nodulated legumes have reported C consumption values for nodules ranging from 3 to 25% of the C fixed by the host (Atkins *et al.*, 1978; Haystead *et al.*, 1979; Ryle *et al.*, 1979b). These measurements were performed on plants of unknown mycorrhizal state. In this study, mycorrhizal infection appeared to stimulate N_2 fixation by the modules. The calculated values for C flow to nodules of RI hosts are lower than most values reported in the literature; however, the values for C flow to the nodules of RMI plants are comparable to those reported by Atkins *et al.* (1978) for plants in a reproductive state.

Minchin and Pate (1973) and Ryle *et al.* (1979a) report values of 0.14–0.18 mg N fixed mg^{-1} C respired by nodulated roots. When the total N in the plant is considered, comparable values of 0.15–0.17 mg N fixed mg^{-1} C respired from the root systems of faba beans are obtained. Mycorrhizal infection did not affect this ratio since N_2 fixation was higher in the presence of mycorrhizal fungi.

Quantitative measurements of C flow to mycorrhizal symbionts are presented. The fungi constituted less than 2% of the plant mass (5% of the root mass) and utilized *ca.* 4 of the C fixed by the host. From an energy point of view, the plant was supporting an expensive symbiont. Since the host compensated for the C used by the symbiont to some extent, the cost of the symbioses was not great.

REFERENCES

Ambler J. R. and Young J. L. (1977) Techniques for determining root length infected by vesicular-arbuscular mycorrhizae. *Journal. Soil Science Society of America* **41,** 551–556.

Atkins C. A., Herridge D. F. and Pate J. S. (1978) The economy of carbon and nitrogen in nitrogen-fixing annual legumes: Experimental observations and theoretical considerations. In *Isotopes in Biological Dinitrogen Fixation*, pp. 211–240. IAEA, Vienna.

Bremner J. M. (1965) Organic forms of nitrogen. In *Methods of Soil Analyses, Part 2. Chemical and Microbial Properties* (C. A. Black, Ed.), pp. 1238–1255. American Society for Agronomy, Madison, Wisconsin.

Carling D. E., Riehle W. O., Brown M. F. and Johnson D. R. (1978) Effects of a vesicular-arbuscular mycorrhizal fungus on nitrate reductase and nitrogenase activities in nodulating and non-nodulating soybeans. *Phytopathology* **68,** 1590–1596.

Cooper K. M. and Lösel D. M. (1978) Lipid physiology of vesicular-arbuscular mycorrhizae. I. Composition of lipids in roots of onion, clover and ryegrass infected with *Glomus mossae*. *New Phytologist* **80,** 143–151.

Cox G. and Tinker P. B. (1976) Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizae. I. The arbuscle and phosphorus transfer: a quantitative ultrastructural study. *New Phytologist* **77,** 371–378.

CRUSH J. R. (1974) Plant growth responses to vesicular-arbuscular mycorrhiza. VII. Growth and nodulation of some herbage legumes. *New Phytologist* **77**, 743–749.

DAFT M. J. and EL GIAHMI A. A. (1974) Effect of Endogone mycorrhizae on plant growth. VII. Influences of infection on the growth and nodulation in French bean (*Phaseolus vulgaris*). *New Phytologist* **73**, 1139–1147.

GEIGER D. R. (1976) Effects of translocation and assimilate demand on photosynthesis. *Canadian Journal of Botany* **54**, 2337–2345.

GERDEMANN J. W. (1964) The effect of mycorrhizae on the growth of maize. *Mycologia* **56**, 342–349.

HARLEY J. L. (1971) Fungi in ecosystems. *Journal of Ecology* **59**, 653–668.

HAYSTEAD A., KING J. and LAMB W. I. C. (1979) Photosynthesis, respiration, and nitrogen fixation in white clover. *Grass and Forage Science* **34**, 125–130.

HEPPER C. M. (1977) A colorimetric method for estimating vesicular-arbuscular mycorrhizal infection in roots. *Soil Biology & Biochemistry* **9**, 15–18.

HO I. and TRAPPE J. M. (1973) Translocation of ^{14}C from *Festuca* plants to their endomycorrhizal fungi. *Nature: New Biology* **244**, 30–31.

LAMBERT D. H., BAKER D. E. and COLE H. JR (1979) The role of mycorrhizae in the interactions of phosphorus with zinc, copper, and other elements. *Journal. Soil Science Society of America* **43**, 976–980.

LEVY Y. and KRIKUN J. (1980) Effect of vesicular-arbuscular mycorrhizae on *Citrus jambhiri* water relations. *New Phytologist* **85**, 25–31.

MAHON J. D. (1977) Respiration and the energy requirement for nitrogen fixation in nodulated pea roots. *Plant Physiology* **60**, 817–821.

MINCHIN F. R. and PATE J. S. (1973) The carbon balance of a legume and the functional economy of its root nodules. *Journal of Experimental Botany* **24**, 259–271.

MOSSE B., POWELL C. L. and HAYMAN D. S. (1976) Plant growth responses to vesicular-arbuscular mycorrhizae. IX. Interactions between VA mycorrhizae, rock phosphate and symbiotic nitrogen fixation. *New Phytologist* **76**, 331–342.

NEWMAN E. I. (1978) Root microorganisms: their significance in the ecosystem. *Biological Reviews* **53**, 511–554.

PANG P. C. and PAUL E. A. (1980) Effects of vesicular-arbuscular mycorrhizae on ^{14}C and ^{15}N distribution in nodulated faba beans. *Canadian Journal of Soil Science* **60**, 241–250.

PATE J. S., LAYZELL D. B. and ATKINS C. A. (1979) Economy of carbon and nitrogen in a nodulated and non-nodulated (NO_3 grown) legume. *Plant Physiologist* **64**, 1083–1088.

RHODES L. H. and GERDEMANN J. W. (1978) Hyphal translocation and uptake of sulfur by vesicular-arbuscular mycorrhizae of onion. *Soil Biology & Biochemistry* **10**, 355–360.

RYLE G. J. A., POWELL C. E. and GORDON A. J. (1979a) The respiratory costs of nitrogen fixation in soyabeans, cowpea, and white clover. I. Nitrogen fixation and the respiration of the nodulated root. *Journal of Experimental Botany* **30**, 135–144.

RYLE G. J. A., POWELL C. E. and GORDON A. J. (1979b) The respiratory costs of nitrogen fixation in soyabeans, cowpea, and white clover. II. Comparisons of the cost of nitrogen fixation and the utilization of combined nitrogen. *Journal of Experimental Botany* **30**, 145–153.

SMITH S. E. and DAFT M. J. (1977) Interactions between growth, phosphate content and nitrogen fixation in mycorrhizal and non-mycorrhizal *Medicago sativa*. *Australian Journal of Plant Physiology* **4**, 403–413.

STRIBLEY D. P., TINKER P. B. and RAYNER J. H. (1980) Internal phosphorus concentrations and carbon loss in plants infected by vesicular-arbuscular mycorrhiza. *New Phytologist* **86**, 261–267.

VAN VEEN J. A. and PAUL E. A. (1979) Conversion of biovolume measurements of soil organisms, grown under various moisture tensions, to biomass and their nutrient content. *Applied and Environmental Microbiology* **37**, 686–692.