EFFECTS OF VESICULAR-ARBUSCULAR MYCORRHIZA ON ^{14}C AND ^{15}N DISTRIBUTION IN NODULATED FABABEANS

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A two-compartment growth chamber in which the aboveground plant materials were exposed to $^{14}CO_2$ and the belowground portion was exposed to $^{15}N_2$ under normal atmospheric pressure was designed for carbon and nitrogen transfer studies. *Vicia faba* infected with vesicular-arbuscular fungus *Glomus mossae* and non-mycorrhizal plants fixed similar quantities of N_2 at an age of $6^{1}/_{2}$ wk. Approximately 0.10 mg N was fixed · g^{-1} dry plant materials · day^{-1} and 40 mg C · g^{-1} dry matter · day^{-1} were synthesized by mycorrhizal and non-mycorrhizal fababeans during 48 h exposure to $^{14}CO_2$ at $6^{1}/_{2}$ wk with no apparent difference in yield of dry matter. The non-mycorrhizal plants transferred 37% of the fixed ^{14}C beneath ground. The mycorrhizal ones transferred 47% of the fixed ^{14}C beneath ground. Most of the difference could be accounted for in the belowground respiration. The $^{14}CO_2$ produced by root-microbial systems of the mycorrhizal fababeans was twice as great as that of the nonmycorrhizal; both contained active rhizobium.

Les auteurs ont construit pour étudier les échanges de carbone et d'azote une enceinte de végétation à deux compartiments. Les parties aériennes des plantes (*Vicia faba*) étaient exposées à un flux de $^{14}CO_2$ et les parties souterraines à un courant de $^{15}N_2$ à la pression atmosphérique normale. Des plantes de féverole infectés de mycorhizes à arbuscules et vésicules, *Glomus mossae* et les plants non mycorrhizés ont fixé les mêmes quantités de N_2 à l'âge de $6^{1}/_{2}$ semaines. Les deux types de plantes ont fixé environ 0.10 mg N et produit 40 mg C · j^{-1} · g^{-1} de matière végétale sèche au cours d'une période d'exposition de 48 h à $^{14}CO_2$ sans qu'on ait pu constater de différences apparente dans le rendement de matière sèche. Les plantes non mycorrhizées ont transféré 37% du ^{14}C fixé aux organes souterrains, les plantes mycorrhizées 47%. Le gros de la différence se justifie par la respiration plus intense des organes souterrains. Le $^{14}CO_2$ produit par le complexe racine-micro-organismes des plants mycorrhizés a été le double de celui produit par les plantes sans mycorrhizes. Les deux types de plantes étaient en outre colonisées par une microflore rhizobienne active.

Plant-microbial associations can consist of a close interaction between a legume plant, a fairly specific bacterial genus (*Rhizobium*) and the non-specific vesicular-arbuscular (V-A) mycorrhiza (Smith 1974). Research has confirmed the advantage of V-A mycorrhiza in the transfer of phosphorus from soil to mycorrhizal plants growing in P-deficient soils (Ross 1971; Jackson et al. 1972; Powell 1975; Hayman et al. 1975; Rhodes and Gerdemann 1975, 1978; Azcon et al. 1976; Mosse et al. 1976; Barrow et al. 1977). V-A mycorrhizal legumes also have been shown to have increased nodulation and acetylene reduction (N_2 fixation) compared to non-mycorrhizal legumes (Crush 1974, 1976; Daft and El-Giahmi 1974, 1976; Mosse et al. 1976).

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Both the rhizobia and the mycorrhiza have carbon requirements that must be supplied by the plant. The degree to which the relationship is symbiotic or parasitic depends on the plant's photosynthetic requirements relative to the supply of nitrogen by the bacteria and phosphorus by the fungal partner. The higher requirements for photosynthate during nitrogen fixation have been demonstrated (Hardy and Havelka 1975; Lawrie and Wheeler 1975; Pate 1975; Minchin and Pate 1973). Few studies have recognized and investigated the three components of the system: the plant, the fungus, and the bacterium. This report shows that experimental design and initial results of the use of ^{14}C and ^{15}N labelling to measure the effect of vesicular-arbuscular mycorrhiza on belowground respiration and nitrogen fixation. It is part of a long-range study to gain a better understanding of how these partners react under natural conditions.

MATERIALS AND METHODS
Isolation of V-A Mycorrhizal Spores from Saskatchewan Soils
Soil from Saskatchewan cropped with fababeans (*Vicia faba* 'Ackerperle') was collected for isolation of V-A mycorrhizal spores by wet sieving and decanting (Gerdemann and Nicolson 1963). Materials collected on a 100-μm sieve were examined under the stereomicroscope for spores. The most abundant type had a yellowish-translucent color and was 120–140 μm in diameter (Fig. 1). These were propagated by inoculation of fababeans growing in sterilized soil. Months later, large numbers of spores were harvested. After staining the root segments of fababeans with 0.01% acid fuchsin (Furlan and Fortin 1973), the presence of appressoria, vesicles and arbuscules (Fig. 2) confirmed that the spores were the vesicular-arbuscular type. The V-A mycorrhizal spores were identified to be those of *Glomus mossae* by Dr. V. Furlan (Department of Forest Ecology, Laval University, Quebec City, Quebec).

Preparation of Plants for Isotope Labelling
Fababean seeds washed in dilute borax (1:1 ratio of borax to water) were pregerminated on nutrient agar before transplanting to autoclaved Bradwell

sandy loam soil (pH 6.8, 3.7% organic matter). The Bradwell soil was autoclaved at 121°C and 103.5 kPa for 15 min, and was stored at 4°C for 1–2 wk before planting. During seedling transplant, roots were inoculated with commercial fababean inoculum (Nitragin Co., Milwaukee, Wis.) and 30–40 *Glomus mossae* spores. The control plants were inoculated with rhizobium inoculum only. Plants were grown under a light intensity of 9500–12 000 lux at 20°C for 18 h and at 16°C during a 6-h dark period.

Labelling of Fababean Plants by ^{14}C and ^{15}N
A two-compartment labelling chamber similar to that of Warembourg and Paul (1973) was built to facilitate the labelling of plants with ^{15}N during nitrogen fixation by belowground plant parts and with ^{14}C during photosynthesis by aboveground plant materials. The aboveground and belowground compartments were separated and sealed with Terostat (Teroson, Germany) in such a way that there was no cross contamination of the gaseous atmosphere between the two compartments. The ^{14}CO$_2$ generated from Na$_2$ ^{14}CO$_3$ (specific activity = 50 μCi/g) and lactic acid was circulating in the aboveground compartment; ^{15}N-labelled molecular nitrogen (3.2526 atom % ^{15}N) was transferred to the belowground compartment using a molecular sieve cooled with liquid N$_2$. A flow diagram of the labelling system is shown in Fig. 3.

Plants were labelled for 48 h, after which ^{15}N$_2$ and ^{14}CO$_2$ were replaced by normal, i.e. non-labelled, N$_2$ and CO$_2$. The light intensity within the labelling chamber was 8000–10 500 lx during the 18-h daylight period. The plants were harvested after a further 4$^{1}/_{2}$ days growth in the chamber. During the period that the plants were in the chamber, CO$_2$ was continuously trapped in 0.2 N NaOH from belowground compartments for quantitiative carbon and radioisotope analysis. The radioactivity of ^{14}CO$_2$ in the photosynthesis chamber was checked by removing 10 cm^3 of gaseous volume with disposable syringes, transferring this to prevacuumed scintillation vials containing 1 mL of 0.2 N NaOH, and counting after addition of scintillant (Warembourg and Paul 1973). Carbon dioxide concentrations in the aboveground atmosphere were determined by gas chromatography (de Jong and Schappert 1972). The atom % ^{15}N of N$_2$ in the N$_2$-fixation chamber was analyzed mass spectrometrically before and after the labelling period.
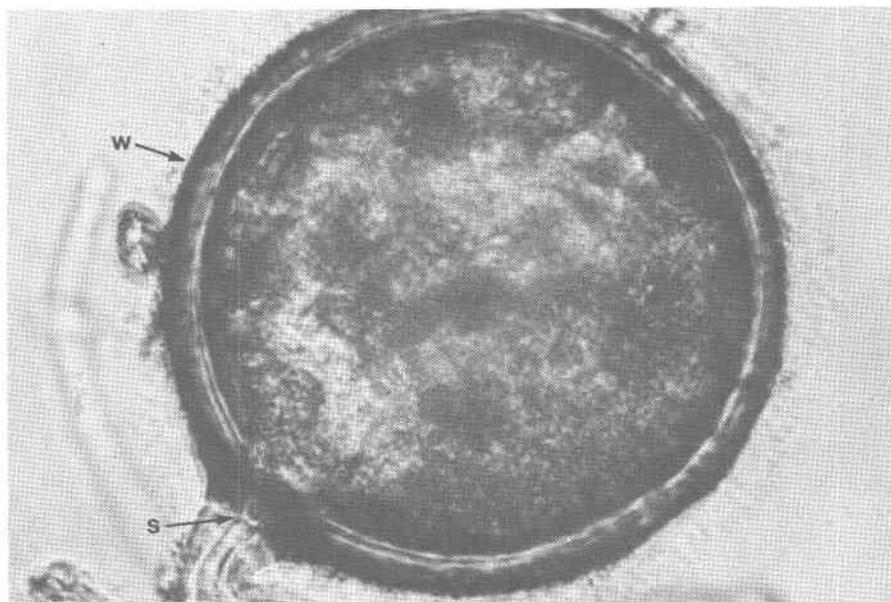
Fig. 1. Spore of *Glomus mossae* ($\times 5$, 140 μm diam.) isolated from a Saskatchewan soil cultivated with fababean. Note the thick multilayer spore wall (W) and the hyphal attachment closed by a thin septum (S) at point of hypha and spore attachment.

Analysis of Plant Materials

At harvest, the aboveground plant materials were separated into leaves and stems. Roots washed free of soil were cut into 1- to 2-cm sections, stained with 0.01% acid fuchsin and examined for root infection (Furlan and Fortin 1973) {% V-A infection = [(Number of infected root segments)/(Total number of segments examined)] $\times$ 100)}. The oven-dried plant materials were ground to pass a 2-mm sieve. Total carbon was determined by wet combustion (Thorn and Shu 1953) and total nitrogen by the Kjeldahl technique (Bremner 1965). Carbon-14 in the plant materials was determined by scintillation counting (Warembourg and Paul 1973) and ^{15}N in the samples was determined by the mass spectrometer (Rennie and Paul 1971). The results were reported along with the standard error and the average of six replicates.

RESULTS

Fixation of Molecular Nitrogen

Nitrogen-15 was recovered in the aboveground plant parts as well as the root systems of $6^{1}/_{2}$-wk-old fababeans after the below-ground growth compartment was supplied with ^{15}N$_2$ for 48 h followed by 96 h in normal N$_2$. The quantities of ^{15}N in the leaves, stems and root systems did not differ ($P \leqslant 0.05$) between mycorrhizal and non-mycorrhizal plants (Tables 1 and 2). The amounts of nitrogen fixed by plants, i.e. 0.48 mg N in the presence of mycorrhiza and 0.63 mg N in the absence of mycorrhiza were not significantly different at the 0.05% level but were significantly different at the 0.10% level. The rate of N$_2$ fixation during the 48-h labelling period was 10 μg N $\cdot$ h^{-1} for the mycorrhizal and 13 μg N $\cdot$ h^{-1} for the non-mycorrhizal fababeans. The rate of N$_2$ fixation by the mycorrhizal and non-mycorrhizal fababeans was approximately the same (0.1 mg N $\cdot$ g^{-1} dry plant materials $\cdot$ day^{-1}) on the basis of fixation per unit weight {Rate of dinitrogen fixation by fababean (mg N $\cdot$ g^{-1} dry matter $\cdot$ day^{-1}) = mg ^{15}N of plant parts/[(g dry weight of plant parts) $\times$ (Number of days plants supplied with ^{15}N$_2$)]}. This suggested that the ability
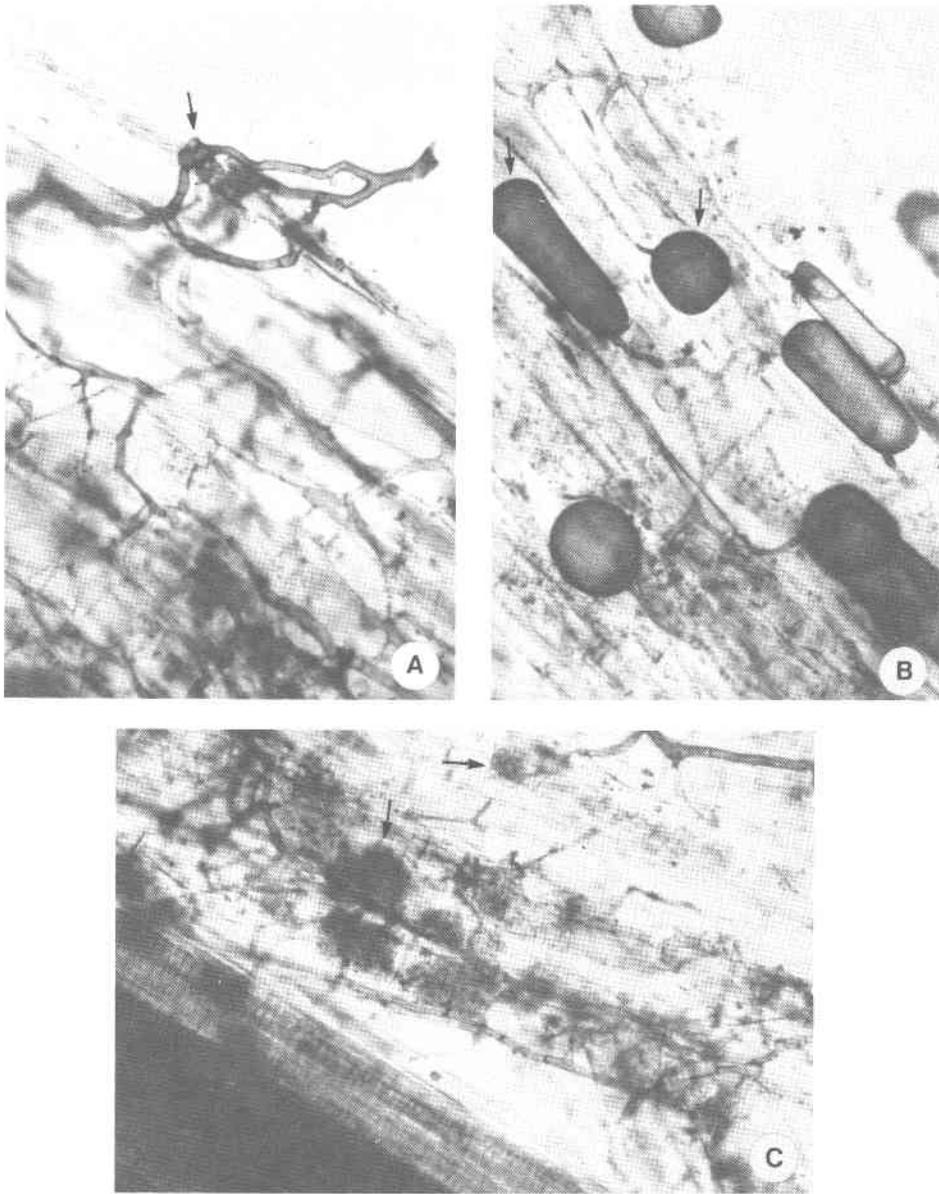
Fig. 2.   Root of *Vicia faba* 'Ackerperle' infected by *Glomus mossae*: (A) appressorium ($\times$ 10); (B) vesicles ($\times$ 10; spherical, 80 μm; rectangular-shaped, 148 μm $\times$ 16 μm); (C) arbuscules ($\times$ 10).
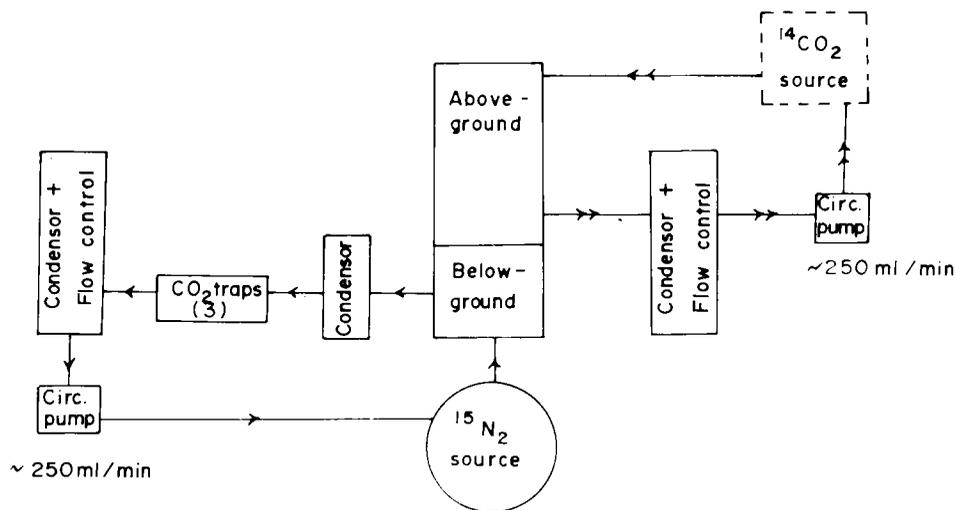
Fig. 3.   Flow diagram of the two-compartment labelling growth chamber.

Table 1.   Yield of dry matter, quantities of ^{15}N and percent distribution of fixed-N in 6½-wk mycorrhizal fababeans after 48 h ^{14}C-^{15}N labelling followed by 96 h in normal atmosphere

Plant parts	Yield dry matter (g)	^{15}N (mg)	Distribution fixed-N (%)†
Leaves	0.73±0.04	0.25±0.10	52±10
Stems	0.56±0.07	0.10±0.01	21±5
Roots and nodules	1.11±0.07	0.13±0.02	27±4
Total	2.40±0.07	0.48±0.10	100

†Distribution of fixed-N (%) = [(^{15}N in plant parts)/(Total ^{15}N in whole plants)] × 100.

Table 2.   Yield of dry matter, quantities of ^{15}N and percent distribution of fixed-N in 6½-wk non-mycorrhizal fababeans after 48 h ^{14}C-^{15}N labelling followed by 96 h in normal atmosphere

Plant parts	Yield dry matter (g)	^{15}N (mg)	Distribution fixed-N (%)†
Leaves	0.82±0.07	0.25±0.04	40±6
Stems	0.66±0.04	0.15±0.02	24±3
Roots and nodules	1.22±0.10	0.23±0.03	36±4
Total	2.70±0.19	0.63±0.03	100

†Distribution of fixed-N (%) = [(^{15}N in plant parts)/(Total ^{15}N in whole plant)] × 100.

of the fababean-rhizobium association to fix N_2 was not altered by the presence of mycorrhizal fungi. The plants that were inoculated with mycorrhiza spores had an average of 10–35% root infection. During the 144-h growth period, 27% and 36% of the N_2 fixed in 48 h remained in the roots of mycorrhizal and non-mycorrhizal plants, respectively. The balance was translocated to the aboveground materials during active photosynthesis and cell development (Tables 1 and 2).

Fixation of Carbon Dioxide
The mycorrhizal and non-mycorrhizal plants fixed, respectively, 44 mg $C \cdot g^{-1}$ dry matter $\cdot$ day^{-1} and 36 mg $C \cdot g^{-1}$ dry matter $\cdot$ day^{-1} over the 48-h labelling period. {Rate of carbon fixation by fababean (mg $C \cdot g^{-1}$ dry matter $\cdot$ day^{-1} = (mg ^{14}C of plant parts + mg ^{14}C respired from belowground)/[(g dry weight of plant parts) $\times$ (number of days plant supplied with $^{14}CO_2$)]}. A large percentage of the carbon, i.e. 26% for mycorrhizal and 15% for non-mycorrhizal (Tables 3 and 4), was lost in the atmosphere via belowground CO_2 evolution.

Total ^{14}C utilized by the mycorrhizal (209 mg C) and non-mycorrhizal (194 mg C) plants was not significantly different at the 0.05% level (Tables 3 and 4). Of the 230–240 mg ^{14}C generated from $^{14}Na_2CO_3$ by addition of lactic acid over a 48-h

labelling period, 88 and 83% were recovered from mycorrhizal and non-mycorrhizal plants, respectively, as plant carbon and belowground respiration. Approximately 0.03% of the C remained in the atmosphere and the balance of approximately 13% by difference was assumed to remain in the soil. Unfortunately, analyses of ^{14}C in soils were not conducted. Also no attempt was made to measure losses of carbon by photorespiration after the 48-h ^{14}C-^{15}N labelling period. The distribution of ^{14}C in plant parts of mycorrhizal and non-mycorrhizal fababeans was similar (Tables 3 and 4).

Carbon Dioxide Respired from Belowground
The CO_2 collected from belowground includes both root respiration and microbial respiration since the soil was not maintained under sterile conditions after seedlings transplant and inoculation with mycorrhizal spores and rhizobia. The relative specific activity of CO_2 respired from the belowground compartment by the non-mycorrhizal fababean-soil systems was only slightly lower than the mycorrhizal one (Fig. 4). However, the absolute quantity of ^{14}C released by the myrorrhizal plants during the period in question was significantly higher than that of the non-mycorrhizal fababeans (Fig. 5). Differences became distinct 36 h after initiation of the ^{14}C labelling and gradually increased as time progressed. The

Table 3.   Quantities of ^{14}C, recovery of added ^{14}C and percent distribution of photosynthate-^{14}C of 6½-wk mycorrhizal fababean after 48 h ^{14}C-^{15}N labelling followed by 96 h in normal atmosphere

	^{14}C (mg)	Recovery of added ^{14}C† (%)	Distribution of photosynthate-^{14}C (%)‡
Leaves	65±14	27±5	30±4
Stems	47±5	20±3	23±4
Roots and nodules	36±7	15±3	17±2
Respired CO_2	61±4	26±1	30±2
Total	209	88	100

†% recovery of added ^{14}C (%) = [(Plant ^{14}C or ^{14}C respired from belowground)/(^{14}C generated from Na_2 $^{14}CO_3$ and lactic acid)] $\times$ 100 where total $^{14}CO_2$ generated = 240 mg ^{14}C.
‡Distribution of photosynthate -^{14}C (%) = [(Plant ^{14}C or ^{14}C respired from belowground)/(Plant ^{14}C + ^{14}C respired from belowground)] $\times$ 100.

Table 4. Quantities of ^{14}C, recovery of added ^{14}C and percent distribution of photosynthate-^{14}C of 6½-wk non-mycorrhizal fababean after 48 h [14]C-^{15}N labelling followed by 96 h in normal atmosphere

	[14]C (mg)	Recovery of added [14]C† (%)	Distribution of photosynthate-[14]C (%)‡
Leaves	70±12	30±4	36±3
Stems	56±8	24±3	28±1
Roots and nodules	33±4	14±1	18±2
Respired CO_2	35±3	15±1	18±2
Total	194	83	100

†Recovery of added [14]C (%) = [(Plant ^{14}C or ^{14}C respired from belowground)/(^{14}C generated from Na_2 [14]CO_3 and lactic acid)] × 100 where total [14]CO_2 generated = 230 mg [14]C.
‡Distribution of photosynthat-[14]C (%) = [(Plant ^{14}C or ^{14}C respired from belowground)/(Plant [14]C + [14]C respired from belowground)] × 100.

61 mg ^{14}C respired from belowground portions of mycorrhizal plants accounted for 30% of the total ^{14}C acquired by plants during the 96-h labelling period. In the non-mycorrhizal plants the 35 mg accounted for only 18% of the total net photosynthesis.

Carbon-Nitrogen Turnover
In a legume system where nitrogen fixation is occurring, the plant must be actively photosynthesizing in order to satisfy the energy-consuming process of N_2 fixation. The mycorrhizal plant was able to fix 2.3 μg N · mg^{-1} C photosynthesized during the 48-h [14]C-^{15}N labelling period (Table 5). The non-mycorrhizal fababean fixed 3.2 μg N_2-N for every mg C fixed. Because of the low number of replicates, these values were not significantly different at the 0.05 or the 0.10% level. The ratio of N:C remained
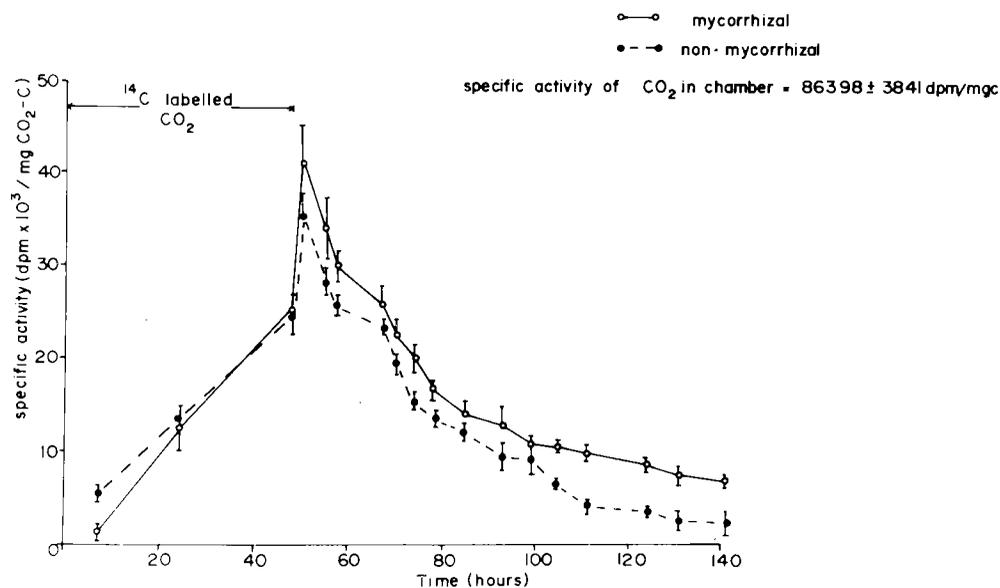
Fig. 4. Relative specific activity of belowground respiration of 6½-wk mycorrhizal and non-mycorrhizal fababeans.
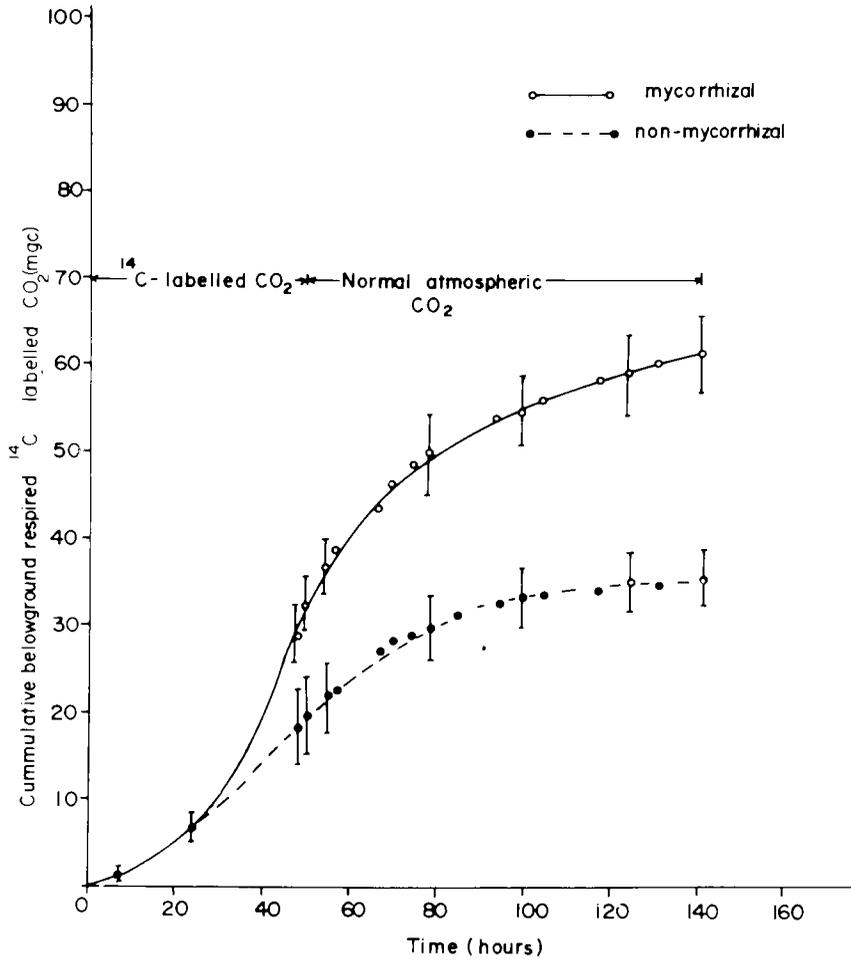
Fig. 5.   Cumulative belowground ^{14}C labelled CO_2 of 6^{1}/$_2$-wk mycorrhizal and non-mycorrhizal fababeans.

Table 5.   Nitrogen-carbon ratios of 6.5-wk mycorrhizal and non-mycorrhizal fababeans after 48 h ^{14}C-^{15}N labelling followed by 96 h in normal atmosphere

Parameters[†]		N fixed/C metabolized (μg N · mg^{-1} C · 48 h^{-1})	
Nitrogen	Carbon	Mycorrhizal	Non-mycorrhizal
L	L	3.84	3.55
S	S	2.12	2.68
R+D	(R+D)+CO$_2$	2.37	3.36
L+S+(R+D)	L+S+(R+D)	3.24	3.95
L+S+(R+D)	L+S+(R+D)+CO$_2$	2.30	3.23

[†]Leaves (L), stems (S), R+D (roots + nodules) and CO_2 (carbon dioxide respired from belowground).

relatively constant among the plant parts of the infected and non-infected plants (Table 5).

DISCUSSION

Under the conditions of this experiment, the inoculation of V-A mycorrhizal fungi (*Glomus mossae*) into a growth system of fababean and rhizobia did not result in enhanced plant growth, root nodulation or nitrogen fixation. Therefore our data failed to confirm experiments reported by Azcon-G de Aguilar and Barea (1978), Daft and El-Giahmi (1974) and Mosse et al. (1976) that significant improved nodulation and N_2 fixation were obtained by legumes after V-A mycorrhizal infection.

It has been documented that V-A mycorrhiza enhance phosphorus uptake of infected plants (Rhodes and Gerdemann 1978). The Bradwell soil used in this experiment is not high in available phosphorus (10 ppm); however, the autoclaving probably released adequate phosphorus for plant growth. Under these conditions, the mycorrhiza could not be expected to increase plant growth.

The quantity and distribution of the fixed N_2 and photosynthate in fababean are comparable with results obtained by Lawrie and Wheeler (1975) with *Vicia faba* and Oghoghorie and Pate (1972) with *Pisum sativum*. This experiment did not differentiate between the ^{14}C or ^{15}N going into mycorrhiza or into the rhizobium symbiont. However, the higher $^{14}CO_2$ respiration by the mycorrhizal fababeans with relatively no changes in total dry matter (see Tables 1 and 2) indicates that the plant could compensate for the needs of the mycorrhiza.

The $6^{1}/_{2}$-wk period was chosen for labelling because at this point in time both the rhizobium and the mycorrhiza were growing actively as indicated by nodule-formation and percent V-A mycorrhizal infection of roots. Staining of fababean root systems with acid fuchsin at different stages of plant growth showed that mycorrhiza fungi infected fababeans later than did the rhizobia. It is clear that much additional work will be required before a complete understanding of the relative carbon and nitrogen requirements of the three partners (legumes-V-A mycorrhizae-rhizobia) in this symbiosis are understood. The data obtained from our experimental technique indicate the feasibility of the approach which should lead us to a better understanding of the nutrition of plants which are really an association of three different organisms. This association may range from symbiosis to parasitism depending on the relative contribution of each of the partners. In this experiment the mycorrhiza appeared to be parasites in that no beneficial effects occurred to the plant. The roots of the mycorrhizal plant showed 30% infection and evolved considerably more CO_2 without a deleterious effect on plant growth, indicating that the plant may be able to compensate for the increased needs of its microbial partners.

ACKNOWLEDGMENT

AZCON, R., BAREA, J. M. and HAYMAN, D. S. 1976. Utilization of rock phosphate in alkaline soils by plants inoculated with mycorrhizal fungi and phosphate-solubilizing bacteria. Soil Biol. Biochem. **8**: 135–138.

AZCON-G. DE AGUILAR, C. and BAREA, J. M. 1978. Effects of interactions between different culture fractions of 'phosphobacteria' and *Rhizobium* on mycorrhizal infection, growth and nodulation of *Medicago sativa*. Can. J. Microbiol. **24**: 520–524.

BARROW, N. J., MALAJCZUK, N. and SHAW, T. C. 1977. A direct test of the ability of vesicular-arbuscular mycorrhiza to help plants take up fixed soil phosphate. New Phytol. **78**: 269–276.

BREMNER, J. M. 1965. Total nitrogen. Pages 1149–1178 *in* Methods of soil analysis, Part 2. Amer. Soc. Agron. Monogr. 9. Madison, Wis.

CRUSH, J. R. 1974. Plant growth responses to vesicular-arbuscular mycorrhiza. VII. Growth and nodulation of some herbage legumes. New Phytol. **73**: 643–749.

CRUSH, J. R. 1976. Endomycorrhizas and

legume growth in some soils of the Mackenzie Basin, Caterbury, New Zealand. N.Z. J. Agric. Res. **19**: 473–476.

DAFT, M. M. and EL-GIAHMI, A. A. 1974. Effect of endogone mycorrhiza on plant growth. VII. Influence of infection on the growth and nodulation in french bean (*Phaseolus vulgaris*). New Phytol. **73**: 1139–1147.

DAFT, M. J. and EL-GIAHMI, A. A. 1976. Studies on nodulated and mycorrhizal peanuts. Ann. Appl. Biol. **83**: 273–276.

DE JONG, E. and SCHAPPERT, H. J. V. 1972. Calculation of soil respiration and activity from CO_2 profiles in soil. Soil Sci. **113**: 328–333.

FURLAN, V. and FORTIN, J. A. 1973. Formation of endomycorrhizae by *Endogone calospora* on *Allium cepa* under three temperature regimes. Natur. Can. **100**: 467–477.

GERDEMANN, J. W. and NICOLSON, T. H. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc. **46**: 235–244.

HARDY, R. W. F. and HAVELKA, V. D. 1975. Nitrogen fixation research: a key to world food. Science **188**: 633–643.

HAYMAN, D. S., JOHNSON, A. M. and RUDDLESDIN, I. 1975. The influence of phosphate and crop species on *Endogone* spores and vesicular-arbuscular mycorrhiza under field conditions. Plant Soil **43**: 489–495.

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

JACKSON, N. E., FRANKLIN, R. E. and MILLER, R. H. 1972. Effects of vesicular-arbuscular mycorrhizae on growth and phosphorus content of three agronomic crops. Soil Sci. Soc. Amer. Proc. **36**: 64–67.

LAWRIE, ANN C. and WHEELER, C. T. 1975. Nitrogen fixation in the root nodules of *Vicia faba* L. in relation to the assimilation of carbon. I. Plant growth and metabolism of photosynthetic assimilates. New Phytol. **74**: 429–436.

MINCHIN, R. F. and PATE, J. S. 1973. The carbon balance of a legume and the functional economy of its root nodules. J. Exp. Bot. **24**: 259–271.

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

OGHOGHORIE, C. G. O. and PATE, J. S. 1972. Exploration of the nitrogen transport system of a nodulated legume using ^{15}N. Planta (Berl.) **104**: 35–49.

PATE, J. S. 1975. Physiology of the reaction of nodulated legumes to environment. Pages 335–360 in P. S. Nutman, ed. Symbiotic nitrogen fixation in plants. International Biological Programme, Vol. 7. Cambridge University Press, Great Britain.

POWELL, C. L. 1975. Plant growth responses to vesicular-arbuscular mycorrhiza. VII. Uptake of P by onion and clover infected with different *Endogone* spore types in P labelled soils. New Phytol. **75**: 563–566.

RENNIE, D. A. and PAUL, E. A. 1971. Isotope methodology and techniques in soil-plant nutrition and plant physiology. University of Saskatchewan Press. 142 pp.

RHODES, L. H. and GERDEMANN, J. W. 1978. Translocation of calcium and phosphate by external hyphae of vesicular-arbuscular mycorrhizae. Soil Sci. **126**: 125–126.

RHODES, L. H. and GERDEMANN, J. W. 1975. Phosphate uptake zones of mycorrhizal and non-mycorrhizal onions. New Phytol. **75**: 555–561.

ROSS, J. P. 1971. Effect of phosphate fertilization on yield of mycorrhizal and non-mycorrhizal soybeans. Phytopathology **61**: 1400–1403.

SMITH, S. E. 1974. Mycorrhizal fungi. Pages 275–313 in A. E. Laskin and H. Lechevalier, eds. CRC Crit. Rev. Microbiol.

THORN, J. A. and SHU, P. 1953. A new apparatus for rapid carbon determination by wet combustion. Can. J. Chem. **29**: 558–562.

WAREMBOURG, F. R. and PAUL, E. A. 1973. The use of $^{14}CO_2$ canopy techniques for measuring carbon transfer through the plant-soil system. Plant Soil **38**: 331–345.