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 6½ wk. Approximately 0.10 mg N was fixed g$^{-1}$ day$^{-1}$ and 40 mg C g$^{-1}$ dry matter day$^{-1}$ were synthesized by mycorrhizal and non-mycorrhizal fababees during 48 h exposure to $^{14}$CO$_2$ at 6½ 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 fababees 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 fèverole infectées de mycorhizes à arbusules et vésicules, *Glomus mossae* et les plantes non mycorrhizées ont fixé les mêmes quantités de N$_2$ à l’âge de 6½ semaines. Les deux types de plantes ont fixé environ 0.10 mg N et produit 40 mg C 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 apparentes 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 photosynthesis 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 fababean (Vicia faba ‘Ackerperle’) was collected for isolation of V-A mycorrhizal spores by wet sieving and decanting (Gerdemann and Nicoson 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 fababean growing in sterilized soil. Months later, large numbers of spores were harvested. After staining the root segments of fababean 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$_2$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 4½ 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 quantitative 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 evacuated scintillation vials containing 1 mL of 0.2 N NaOH, and counting after addition of scintillant (Warembourgh 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 mosseae* (×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)] × 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 and nitrogen-15 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 6½-wk-old fababean after the belowground 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 ≤ 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·h$^{-1}$ for the mycorrhizal and 13 μg N·h$^{-1}$ for the non-mycorrhizal fababean. The rate of N$_2$ fixation by mycorrhizal and non-mycorrhizal fababean was approximately the same (0.1 mg N·g$^{-1}$ dry plant materials·day$^{-1}$) on the basis of fixation per unit weight (Rate of dinitrogen fixation by fababean (mg N·g$^{-1}$ dry matter·day$^{-1}$) = mg $^{15}$N of plant parts/[g dry weight of plant parts] × (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 \(\mu\)m; rectangular-shaped, 148 \(\mu\)m \(\times\) 16 \(\mu\)m; (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

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Yield dry matter (g)</th>
<th>$^{15}$N (mg)</th>
<th>Distribution fixed-N (%)$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>0.73±0.04</td>
<td>0.25±0.10</td>
<td>52±10</td>
</tr>
<tr>
<td>Stems</td>
<td>0.56±0.07</td>
<td>0.10±0.01</td>
<td>21±5</td>
</tr>
<tr>
<td>Roots and nodules</td>
<td>1.11±0.07</td>
<td>0.13±0.02</td>
<td>27±4</td>
</tr>
<tr>
<td>Total</td>
<td>2.40±0.07</td>
<td>0.48±0.10</td>
<td>100</td>
</tr>
</tbody>
</table>

$^+$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

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Yield dry matter (g)</th>
<th>$^{15}$N (mg)</th>
<th>Distribution fixed-N (%)$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>0.82±0.07</td>
<td>0.25±0.04</td>
<td>40±6</td>
</tr>
<tr>
<td>Stems</td>
<td>0.66±0.04</td>
<td>0.15±0.02</td>
<td>24±3</td>
</tr>
<tr>
<td>Roots and nodules</td>
<td>1.22±0.10</td>
<td>0.23±0.03</td>
<td>36±4</td>
</tr>
<tr>
<td>Total</td>
<td>2.70±0.19</td>
<td>0.63±0.03</td>
<td>100</td>
</tr>
</tbody>
</table>

$^+$Distribution of fixed-N (%) = [($^{15}$N in plant parts)/(Total $^{15}$N in whole plant)] × 100.
of the fababean-rhizobium association to fix N₂ 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 N₂ 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·g⁻¹ dry matter·day⁻¹ and 36 mg C·g⁻¹ dry matter·day⁻¹ over the 48-h labelling period. The rate of carbon fixation by fababean (mg C·g⁻¹ dry matter·day⁻¹ = (mg ¹⁴C of plant parts + mg ¹⁴C respired from belowground)/(g dry weight of plant parts) × (number of days plant supplied with ¹⁴CO₂)). 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 CO₂ evolution.

Total ¹⁴C 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 ¹⁴C generated from ¹⁴Na₂CO₃ 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 ¹⁴C in soils were not conducted. Also no attempt was made to measure losses of carbon by photorespiration after the 48-h ¹⁴C-¹⁵N labelling period. The distribution of ¹⁴C in plant parts of mycorrhizal and non-mycorrhizal fababeans was similar (Tables 3 and 4).

**Carbon Dioxide Respired from Belowground**

The CO₂ 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 CO₂ 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 ¹⁴C released by the mycorrhizal 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 ¹⁴C labelling and gradually increased as time progressed. The

| Table 3. Quantities of ¹⁴C, recovery of added ¹⁴C and percent distribution of photosynthate-¹⁴C of 6½-wk mycorrhizal fababean after 48 h ¹⁴C-¹⁵N labelling followed by 96 h in normal atmosphere |
|-----------------|-----------------|-----------------|
|                 | ¹⁴C (mg)         | Recovery of added ¹⁴C (%) | Distribution of photosynthate-¹⁴C (%) |
| Leaves          | 65±14           | 27±5            | 30±4              |
| Stems           | 47±5            | 20±3            | 23±4              |
| Roots and nodules | 36±7           | 15±3            | 17±2              |
| Respired CO₂    | 61±4            | 26±1            | 30±2              |
| Total           | 209             | 88              | 100               |

¹% recovery of added ¹⁴C (%) = [(Plant ¹⁴C or ¹⁴C respired from belowground)/(¹⁴C generated from Na₂¹⁴CO₃ and lactic acid)] × 100 where total ¹⁴CO₂ generated = 240 mg ¹⁴C.

distribution of photosynthate-¹⁴C (%) = [(Plant ¹⁴C or ¹⁴C respired from belowground)/(Plant ¹⁴C + ¹⁴C respired from belowground)] × 100.
Table 4. Quantities of 14C, recovery of added 14C and percent distribution of photosynthate-14C of 61/2-wk non-mycorrhizal fababean after 48 h 14C-15N labelling followed by 96 h in normal atmosphere

<table>
<thead>
<tr>
<th></th>
<th>14C (mg)</th>
<th>Recovery of added 14C (%)</th>
<th>Distribution of photosynthate-14C (%)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>70±12</td>
<td>30±4</td>
<td>36±3</td>
</tr>
<tr>
<td>Stems</td>
<td>56±8</td>
<td>24±3</td>
<td>28±1</td>
</tr>
<tr>
<td>Roots and nodules</td>
<td>33±4</td>
<td>14±1</td>
<td>18±2</td>
</tr>
<tr>
<td>Respired CO₂</td>
<td>35±3</td>
<td>15±1</td>
<td>18±2</td>
</tr>
<tr>
<td>Total</td>
<td>194</td>
<td>83</td>
<td>100</td>
</tr>
</tbody>
</table>

†Recovery of added 14C (%) = [(Plant 14C or 14C respired from belowground)/(14C generated from Na₂14CO₃ and lactic acid)] × 100 where total 14CO₂ generated = 230 mg 14C.
‡Distribution of photosynthate-14C (%) = [(Plant 14C or 14C respired from belowground)/(Plant 14C + 14C respired from belowground)] × 100.

61 mg 14C respired from belowground portions of mycorrhizal plants accounted for 30% of the total 14C 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 N₂ fixation. The mycorrhizal plant was able to fix 2.3 μg N·mg⁻¹C photosynthesized during the 48-h 14C-15N labelling period (Table 5). The non-mycorrhizal fababean fixed 3.2 μg N₂-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

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![Fig. 4. Relative specific activity of belowground respiration of 61/2-wk mycorrhizal and non-mycorrhizal fababean.](image-url)
Fig. 5. Cumulative belowground $^{14}$C labelled CO$_2$ of 6$rac{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-15N labelling followed by 96 h in normal atmosphere

<table>
<thead>
<tr>
<th>Parameters$^\dagger$</th>
<th>N fixed/C metabolized ($\mu$g N : mg$^{-1}$ C : 48 h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mycorrhizal</td>
</tr>
<tr>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>R+D</td>
<td>(R+D)+CO$_2$</td>
</tr>
<tr>
<td>L+S+(R+D)</td>
<td>L+S+(R+D)</td>
</tr>
<tr>
<td>L+S+(R+D)+CO$_2$</td>
<td>L+S+(R+D)+CO$_2$</td>
</tr>
</tbody>
</table>

$^\dagger$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 N₂ 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 N₂ and photosynthate in fababean are comparable with results obtained by Lawrie and Wheeler (1975) with *Vicia faba* and Oghoghoerie and Pate (1972) with *Pisum sativum*. This experiment did not differentiate between the ¹⁴C or ¹⁵N going into mycorrhiza or into the rhizobium symbiont. However, the higher ¹⁴CO₂ respiration by the mycorrhizal fababean 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 6½wk 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 fababean 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 is 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 CO₂ 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**

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CRUSH, J. R. 1976. Endomycorrhizas and


