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Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants

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SUMMARY

Cucumber (*Cucumis sativus* L.) plants grown in PVC tubes with a partially sterilized soil-sand mixture were inoculated with the vesicular-arbuscular mycorrhizal fungus *Glomus fasciculatum* (Thaxter) Gerdemann & Trappe emend. Walker & Koske or left uninoculated. The soil column of each PVC tube was divided into a root and a hyphal compartment by a mesh bag (60 μm), which retained the roots but allowed external hyphae to pass. Inoculated plants rapidly became infected and an extensive mycelium developed. Three weeks after seedling emergence plants were labelled with $^{14}CO_2$ for 16 h. The distribution of ^{14}C within the plants and the ^{14}C flow into external hyphae and soil were measured during an 80 h chase period. Below-ground respiration in mycorrhizal plants accounted for 27 % of the photoassimilated ^{14}C. Organic ^{14}C in the soil represented 3·1 % of the fixed ^{14}C, and 26 % of this was located in external hyphae.

Based on conservative assumptions concerning dry weight of internal mycorrhizal infection and growth yield of the fungus, it was estimated that mycorrhizal events consumed 20 % of photoassimilated ^{14}C. The specific incorporation of C by the external mycelium in the hyphal compartment was 41 μg C mg^{-1} dry wt. d^{-1}.

The importance of external VA mycorrhizal hyphae for the distribution of plant-derived C in the soil volume and as a substrate source for the soil biota is discussed.

Key words: Carbon-14, rhizodeposition, VA mycorrhizas, external hyphae, *Cucumis sativus*.

INTRODUCTION

Organic C lost from living roots constitutes an important source of substrate for heterotrophic soil microorganisms in the rhizosphere. This C flow or rhizodeposition has been quantified by means of $^{14}CO_2$ labelling of the plants and published values vary between 2 and 30 % of C assimilated by photosynthesis (Whipps, 1987; Van Veen, Merckx & Van De Geijn, 1989). Plant age (Whipps, 1987; Merckx *et al.*, 1986), plant species (Whipps, 1987), and presence of microorganisms (Barber & Martin, 1976) influence the values obtained. Patterns of exudation are also influenced by the nutrient status of the plant (Graham, Leonard & Menge, 1981; Schwab, Menge & Leonard, 1983) and by environmental conditions (Whipps & Lynch, 1986).

Nearly all plant species hitherto included in studies of rhizodeposition form VA mycorrhizas under field conditions. Intraradical VA mycorrhizal fungi may constitute up to 16 % of the root dry weight (Hepper, 1977; Bethlenfalvay *et al.*, 1982) and hyphae outside the root may form a dense network extending several cm from the root surface (Abbott & Robson, 1985; Rhodes & Gerdemann, 1975). Consequently, VA mycorrhizas may influence the flow rate and composition of root exudates and a substantial proportion of root-derived C in the soil will be located in the external hyphae. In spite of this, studies on rhizodeposition have not yet included VA mycorrhizas as a main factor.

The objective of this work was to study C flow into the rhizosphere of mycorrhizal and non-mycorrhizal cucumber plants. The experiment involved pulse labelling with $^{14}CO_2$, and compartmentation of roots and mycorrhizal hyphae facilitated a detailed study of hyphal C incorporation.

MATERIALS AND METHODS

Plants and mycorrhizal inoculum

Cucumber (*Cucumis sativus* L.) cv. Aminex (F1 hybrid) was used as test plant because it rapidly becomes heavily infected after seedling emergence. Seeds were surface sterilized and germinated between moist Kleenex tissues before planting. One cm root pieces from a maize pot culture infected by an isolate of *Glomus fasciculatum* (Thaxter) Gerdemann & Trappe emend. Walker & Koske obtained from Dr G. Safir, Michigan State University, were

used as VA mycorrhizal inoculum. Autoclaved root pieces were supplied to the control plants.

Growth conditions

The growth medium was a 1:2 (w/w) mixture of sieved (2 mm) clay soil and sand. The mixture had a pH of 7·0, contained 15 mg P kg^{-1} extractable with 0·5 mol dm^{-3} $NaHCO_3$ (Olsen *et al.*, 1954), and was supplied with basal nutrients at the following rates [μmol (kg growth medium)$^{-1}$]: K_2SO_4 (2008), $MgSO_4.7H_2O$ (2029), $MnSO_4.H_2O$ (118), $CuSO_4.5H_2O$(100), $ZnSO_4.7H_2O$(35), $CoCl_2.6H_2O$ (21), H_3BO_3 (81), and $Na_2MoO_4.2H_2O$ (21). Indigenous propagules of VA mycorrhizal fungi in the growth medium were eliminated by irradiation (10 kGy, 10 MeV electron beam). The growth medium was placed in open PVC tubes (180 mm length, 36 mm inner diameter) with a piece of nylon netting fastened to the bottom end by rubber bands. A bag of stainless steel mesh (170 mm length, 30 mm diameter) was positioned in the centre of each tube. The mesh size was 60 μm and allowed hyphae but not roots to pass. Growth medium (100 g) was packed below and around each bag.

Fifteen mycorrhizal and 15 non-mycorrhizal tubes were prepared by filling the bags with 150 g growth medium carefully mixed with either 2 g fresh or 2 g autoclaved inoculum, respectively. All tubes received 25 ml washings ($\leqslant$ 38 μm) of fresh inoculum. One germinated cucumber seed was planted inside each bag, and the tubes were watered daily as needed. Each plant received 10 mg N in a $Ca(NO_3)_2$ solution at 5, 11, 15, 18, 20, and 22 days after seedling emergence. Plants were maintained in a growth chamber under a 16/8 h light/dark cycle at 21/16 °C, 80–90 % RH. Osram daylight lamps (HQI-T250 W/D) provided a photosynthetically active radiation (PAR) of 500–550 μmol m^{-2} s^{-1} (400–700 nm) at the level of seedling emergence. The position of the individual PVC tubes was changed at intervals.

Labelling with $^{14}CO_2$

Five mycorrhizal and five control plants were visually selected for uniform size and transferred to the ^{14}C labelling unit 21 days after seedling emergence. The next day plants were labelled with $^{14}CO_2$ for one photoperiod (16 h) and were then maintained for a further 80 h to allow for equilibration of the assimilated ^{14}C in the plant-soil system.

The labelling unit (Fig. 1) consisted of a 690 × 320 × 400 mm perspex canopy with air inlet and outlet ports. The canopy was placed against an airtight seal on an aluminium plate. The ten PVC tubes, with plants, were inserted in this plate through apertures which were hermetically sealed with 0-rings. Ordinary PVC closing sockets with a 5 mm copper tube inserted were used to seal the soil-root system of each plant. An 0-ring was used for airtightening the lower socket while Terostat IX (Teroson GmbH, Heidelberg, FRG) was used for the upper socket which had a gap for the plant stem. A flow of CO_2-free moist air (50 cm^3 min^{-1}) was passed through the soil-root system of each plant (Fig. 1). The temperature control of the growth chamber was used to maintain 21 °C inside the perspex canopy during the light period. PAR inside the box was 450 μmol m^{-2} s^{-1} and RH 85 %. The perspex canopy was purged with CO_2-free air during the dark period prior to $^{14}CO_2$ labelling.

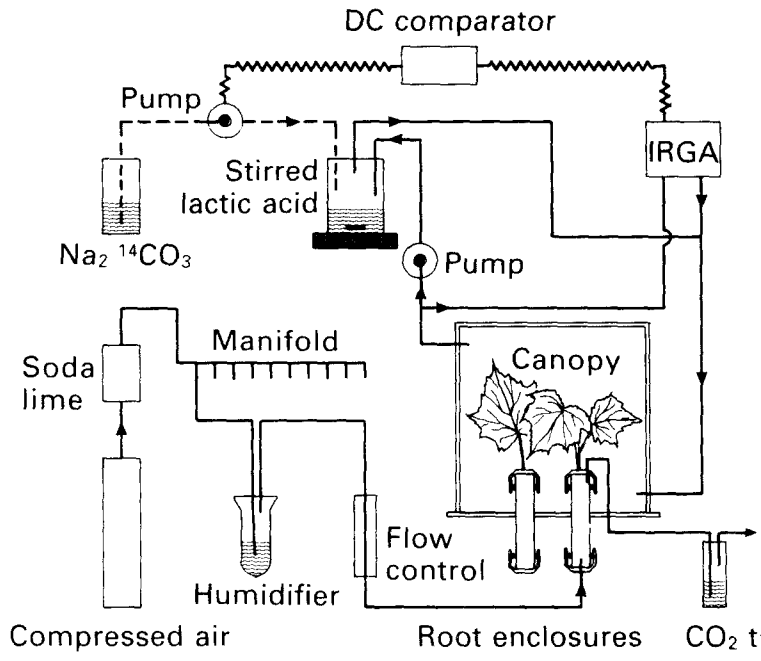

Figure 1. Schematic diagram of equipment used for ^{14}C labelling and measurement of ^{14}C flow from roots of *Cucumis sativus*. ———, – – – and —∿— represent gas flow, liquid flow, and electric circuit, respectively.

During the 16 h labelling period, air with $^{14}CO_2$ (specific activity 7·4 MBq g^{-1} C) was pumped from a CO_2 generating unit to the perspex box and back in a closed circuit at 20 dm^3 min^{-1} (Fig. 1). The $^{14}CO_2$ was generated by gradually adding a 833 mol m^{-3} $Na_2\,^{14}CO_3$ solution to a surplus of 50 % lactic acid via a peristaltic pump (LKB, Varioperpex 12000). The CO_2 concentration in the gas circuit was continuously monitored by infrared gas analysis (ADC model 225Mk3). The peristaltic pump was activated by a DC comparator connected to the analog output of the IRGA (Fig. 1). When the mV output from the IRGA dropped below a prefixed voltage, the DC comparator started the pump and this system maintained the CO_2 concentration between 370 and 415 mm^3 dm^{-3}. After the labelling period the perspex box was purged of $^{14}CO_2$ and during each of four subsequent dark periods the $^{14}CO_2$ produced by dark respiration of the shoots was collected in traps containing 30 % ethanolamine in methanol. The perspex box was removed during light periods. The CO_2 traps, which were developed by Dr H. Sørensen (see Macfadyen, 1970), are 100 % efficient and were used also to collect $^{14}CO_2$ developed by the individual soil-root systems (Fig. 1).

The CO_2 traps for below-ground $^{14}CO_2$ devel-

opment were changed at the start and at the end of each photoperiod. Plants were watered at the start of each photoperiod.

Harvest

Plants were harvested 96 h after initiation of labelling. Shoots were separated into leaves and stems + petioles and fresh weight was recorded. Weighed photocopies of the leaves were transformed to leaf areas by means of the specific area of the copy paper. Dry weights of above-ground plant parts were obtained after drying at 80 °C for 24 h. The intact soil-root cylinders were taken out of the PVC tubes and the soil from the hyphal compartment (HC) could easily be separated from the mesh bag. Total weight of the HC soil was recorded, duplicate 2 g samples were blended with 250 cm^3 water, and 3·5 cm^3 aliquots of this were filtered and stained with trypan blue on 25 mm membrane filters (1·2 μm) (Abbott, Robson & De Boer, 1984). Subsequently, the remaining HC soil was suspended in water in a beaker and soil organic matter with external mycorrhizal hyphae was decanted onto a 38 μm sieve. This was repeated until only sand particles were left in the beaker. The sieve fraction was retained on a filter, dried at 80 °C and weighed. Washings and sand residues were collected separately for each plant. Roots with soil were taken out of the mesh bag and the soil was gently washed from the roots. Hyphae left in the soil suspension from the root compartment (RC) were collected on a 38 μm sieve as already described, and washings and sand residues were retained.

Each soil washing mix was diluted to 3 dm^3 with water and 10 cm^3 chloroform was added to stop microbial activity. After 24 h of sedimentation, duplicate 3 cm^3 samples were taken from the aqueous phase and frozen. The whole mixture was then filtered and the material retained by the filter dried at 80 °C and weighed. The root system was cut into 1 cm pieces and a weighed subsample was taken for measurement of root length and VA mycorrhizal infection. The remaining roots were dried at 80 °C and weighed.

Analyses

Radioactivity of all samples was measured by liquid scintillation counting (LSC). All CO_2 traps were made up to equal volumes with ethanolamine and 3 cm^3 aliquots from these and from the soil-root washings were counted on a Nuclear-Chicago MkII liquid scintillation counter. Weighed subsamples of all plant parts and filters with the 38 μm and sand fractions were burned in a Packard Tricarb B306 sample oxidizer and the evolved CO_2 trapped in Carbosorb. These samples were counted on a Beckman LS1801 liquid scintillation counter. The total radioactivity of roots and external hyphae in the RC of mycorrhizal plants was obtained by adding counts of roots with adhering hyphae and counts of the RC 38 μm sieve fraction.

Phosphorus concentrations in shoots and roots were measured after wet digestion using the molybdate-blue method (Murphy & Riley, 1962) and N concentrations were assayed by a Kjeldahl method. The subsamples of fresh roots were cleared and stained by standard methods (Kormanik & McGraw, 1982) and total and mycorrhizal root length was measured by a line-intersect technique (Tennant, 1975). A similar method was used to measure the length of hyphae from the HC collected on membrane filters. In 20 fields of view all intersections between hyphae and a grid in a 12·5 × ocular were counted using a 16 × objective. The average diameter of hyphae was obtained from measurements of 200 randomly selected hyphae at 1000 × magnification.

RESULTS

Comparisons between mycorrhizal and non-mycorrhizal plants

Plants inoculated with *G. fasciculatum* had 95 % of their root length infected 26 days after seedling emergence and infection was dense with well-developed arbuscules and many vesicles. The uninoculated control plants remained non-mycorrhizal.

Shoot dry weights of mycorrhizal and non-mycorrhizal cucumber plants differed only slightly, whereas root dry weights of mycorrhizal plants were twice those of control plants (Table 1). Phosphorus concentrations (mg g^{-1} DW) in leaves and roots, respectively, were 1·7 and 2·4 in mycorrhizal plants and 1·3 and 1·1 in controls. Nitrogen concentrations were similar in the two treatments. Total radioactivity in shoots was 45 % higher in mycorrhizal than in control plants. In contrast, the activity in roots and in below-ground respiration of mycorrhizal plants were about 5-fold the corresponding values in control plants (Table 1). The ^{14}C activity of soluble and insoluble extraradical fractions of organic C in mycorrhizal plants were approximately twice those of the controls. The radioactivity in the 38 μm sieve fraction from the HC of mycorrhizal plants was 15-fold that of control plants. This confirms that external mycelium of the VA mycorrhizas was the major factor contributing to the activity of the 38 μm fraction. Root hairs penetrating the mesh were probably responsible for most of the activity detected in the HC of non-mycorrhizal plants (Table 1).

Mycorrhizal plants retained a healthy appearance throughout the experimental period, whereas stress symptoms were observed in control plants about 24 h after transfer to the labelling unit. The stress was observed in the form of wilting symptoms spreading from the xylem vessels of the oldest leaf.

Table 1. *Plant dry weights and radioactivity in shoots, roots, below-ground CO_2 output, and three organic soil C fractions of 26-day-old non-mycorrhizal (NM) and mycorrhizal (M) plants of* Cucumis sativus *80 h after labelling of shoots with $^{14}CO_2$ for 16 h*

	NM	M
Dry weight (mg plant^{-1})		
Shoot	1066 ± 28*	1179 ± 20
Root	147 ± 19	287 ± 25
Radioactivity (Bq plant^{-1})		
Shoot	106010 ± 8447	155281 ± 2546
Root	7441 ± 1081	37474 ± 2231
Below-ground CO_2 output	15449 ± 1669	73847 ± 2944
38 μm sieve fraction		
HC†	62 ± 7	990 ± 107
RC†	ND‡	ND
Soluble organic C		
HC	270 ± 15	471 ± 25
RC	1259 ± 258	2789 ± 252
Insoluble organic C		
HC	561 ± 21	764 ± 36
RC	1250 ± 115	2400 ± 215
Total	132301 ± 11091	274014 ± 3397

* Means ± SE of five plants.
† HC, hyphal compartment; RC, root compartment.
‡ ND, not determined.

Table 2. *Uptake of C and distribution of ^{14}C in 26-day-old mycorrhizal* Cucumis sativus *80 h after labelling of shoots with $^{14}CO_2$ for 16 h*

C uptake	
Total (mg C d^{-1})	37·0 ± 0·5*
Specific (mg C dm^{-2} h^{-1})	1·36 ± 0·03
^{14}C distribution (%)	
Shoot	54·1 ± 0·6
Shoot respiration	2·5
Root	13·2 ± 0·8
External VA mycorrhizal hyphae†	0·8 ± 0·1
Soil organic C	2·3 ± 0·1
Below-ground respiration	27·0 ± 1·1
Ratio ^{14}C lost from roots : ^{14}C translocated to roots	0·70 ± 0·09

* Means ± SE of five plants.
† Hyphal densities assumed to be similar in HC and RC.

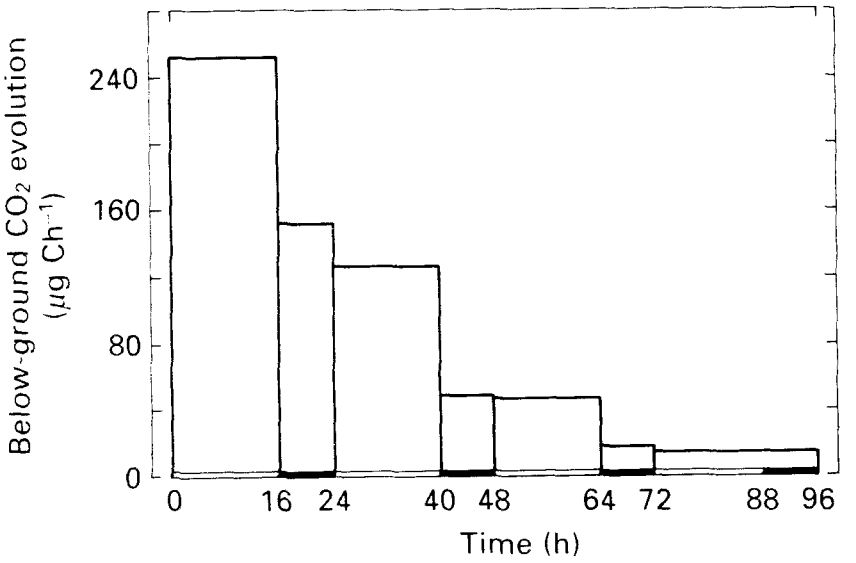

Figure 2. Below-ground $^{14}CO_2$ output from the root enclosures of VA mycorrhizal *Cucumis sativus* during the 16 h shoot labelling and the 80 h chase periods. Data are presented as total C being calculated from the measured radioactivities and the specific activity of the $^{14}CO_2$ used for labelling.

Further tests indicated that such stress symptoms may be caused by the stream of CO_2-free air passing through the soil-root system. Due to these stress symptoms, detailed comparisons between mycorrhizal and control plants are not valid and further results on incorporation and distribution of ^{14}C are presented for mycorrhizal plants only.

Uptake of C and distribution of ^{14}C in the mycorrhizal plant-soil system

Absolute values for C flow in the plant-soil system were calculated from the total radioactivity measured and the specific activity of the ^{14}C used in labelling. Total and specific C uptake is shown in Table 2.

At harvest, 80 h after labelling, the below-ground output of $^{14}CO_2$ was approaching a low constant rate (Fig. 2). This indicates that the assimilated $^{14}CO_2$ had equilibrated in the soil-root system. The pattern of decline in $^{14}CO_2$ output reveals that the rate of root respiration was higher in the light than in the dark periods; furthermore, it is consistent with a single pool of ^{14}C carbohydrates with a half-time of approximately 20 h being the source of respired ^{14}C (Fig. 2).

About 43 % of the assimilated ^{14}C was translocated

Table 3. *^{14}C loss from the roots of mycorrhizal* Cucumis sativus *as a proportion of ^{14}C incorporated in root tissue and specific amounts of extraradical C in hyphal and in root compartments (HC and RC).*

	mg ^{14}C ($g^{14}C$ in roots)$^{-1}$	μg C g^{-1} dry soil	
		HC	RC
External VA mycorrhizal hyphae	62·6 ± 6·2*	1·39 ± 0·15	1·39 ± 0·15†
Soluble C	90·0 ± 5·9	0·71 ± 0·04	2·89 ± 0·41
Insoluble C	87·4 ± 10·1	1·15 ± 0·05	2·49 ± 0·22
CO_2	2040 ± 192	—	—
Sol. C: Insol. C	1·03 ± 0·13	0·62 ± 0·05	1·16 ± 0·15

* Means ± SE of five plants.
† Hyphal densities assumed to be similar in HC and RC.

Table 4. *Length, dry weight, and C incorporation of hyphae in hyphal compartment (HC) and length, VA mycorrhizal infection and C incorporation of roots in root compartment of mycorrhizal* Cucumis sativus *80 h after labelling of shoots with $^{14}CO_2$ for 16 h.*

Hyphae in HC	
Length (cm g^{-1} dry soil)*	2708 ± 206†
Diameter (μm)	2·6 ± 0·1
Dry weight (μg g^{-1} dry soil)‡	34 ± 3
C incorporation	
Total (μg C $plant^{-1}$ d^{-1})	125 ± 14
Specific (μg C mg^{-1} dry wt. d^{-1})	41 ± 3
Roots	
Total length (cm g^{-1} dry soil)	24 ± 1
VA mycorrhizal length (cm g^{-1} dry soil)	23 ± 1
C incorporation	
Total (μg C $plant^{-1}$ d^{-1})	4965 ± 301
Specific (μg C mg^{-1} dry wt. d^{-1})	17 ± 1

* Data corrected for hyphal counts in HC of NM plants.
† Means ± SE of five plants.
‡ Dry wt. = Biovolume × 0·23 (Bakken and Olsen, 1983).

to the root system and 70 % of this was lost as CO_2 and organic C (Table 2). For calculation of ^{14}C content of hyphae in the RC it was assumed that hyphal density was unaffected by the presence of roots. This assumption was confirmed by more thorough measurements of hyphal lengths in hyphal and root compartments in a later experiment with mycorrhizal cucumber plants of a similar age (Jakobsen, unpublished). Total radioactivity in external hyphae thus calculated constituted 0·8 % of total ^{14}C assimilated while 2·3 % of the ^{14}C label was detected in organic soil C (Table 2). Root tissue as well as fungal tissue contributed to the radioactivity in the roots. Likewise, below-ground respiration arose from metabolic activity of both roots, hyphae and general microflora.

About 74 % of the extraradical organic C was equally distributed between water soluble and insoluble C. The remaining 26 % was contained in external hyphae (Table 3). Higher specific activities of soluble and insoluble C were detected in the RC than in the HC. This was most marked for soluble C resulting in a ratio of soluble : insoluble C which was nearly twice as high in the RC as in the HC (Table 3).

Incorporation of C by external hyphae

The dry weight of VA mycorrhizal hyphae in the HC was estimated from the measured volume of hyphae (Table 4). Assuming similar density and diameter of hyphae in HC and RC, total dry weight of external hyphae was 7·5 mg or 2·6 % of root dry weight. Total C incorporation in external hyphae was calculated to be 306 μg $plant^{-1}$ d^{-1} or 6 % of the C incorporation into roots. The specific C incorporation by hyphae (Table 4), which may also be expressed as a relative growth rate of 0·1 d^{-1}, was 2·4-fold that of roots (Table 4).

DISCUSSION

The use of separate hyphal compartments in this study of C flow from mycorrhizal roots made it possible, for the first time, to distinguish directly C in external mycorrhizal hyphae from soluble and

insoluble soil C. Hyphae contained 26 % of the extraradical organic ^{14}C from 22-day-old $^{14}CO_2$-labelled cucumber plants suggesting VA mycorrhizas should be considered in further studies of C loss from roots and its availability to soil microorganisms.

Below-ground allocation and respiration of ^{14}C in mycorrhizal cucumbers accounted for 43 and 27 %, respectively, of photoassimilated ^{14}C (Table 2). These results are similar to those obtained with mycorrhizal faba beans (Pang & Paul, 1980), soybeans (Harris, Pacovsky & Paul, 1985), and leeks (Snellgrove *et al.*, 1982), but higher than the corresponding values for non-mycorrhizal plants of similar size. In the present work below-ground allocation and respiration of ^{14}C in the apparently stressed control plants were only 20 and 12 %, respectively. Thus the C drain by the fungal tissues of VA mycorrhizas consistently appears to be large enough to significantly affect the C distribution within the plant and the below-ground CO_2 output.

Root external organic ^{14}C represented 3·1 % of ^{14}C fixed (Table 2) and this is in the same range as, or somewhat lower, than that found by other workers (cf. Whipps & Lynch, 1985; Van Veen *et al.*, 1989). This does not represent the true flow of organic C from root to soil as some of the C would have been lost by microbial respiration. Recent results indicate that microbial activity may account for as much as 80 % of the total below-ground respiration (Helal & Sauerbeck, 1989).

Root and microbial respiration could not be distinguished in the present study, but total allocation of ^{14}C to the fungal symbiont may be estimated if some assumptions are made. The overall fungal growth yield is typically in the range 0·2–0·4 mg hyphal C (mg substrate C)$^{-1}$ (Perlman, 1965) and estimates of 0·2 have been reported for VA mycorrhizal fungi (Kucey & Paul, 1982; Harris & Paul, 1987). If the growth yield was also 0·2 in the present work and if hyphal densities were similar in HC and RC, then C allocation to external hyphae was 1·5 mg ^{14}C d^{-1} or about 4 % of photoassimilated ^{14}C. Conservatively, the dry weight of *G. fasciculatum* inside the heavily infected roots may be set to 10 % of the root dry weight (Hepper, 1977; Bethlenfalvay *et al.*, 1982). Provided that the specific allocation of ^{14}C (mg ^{14}C g^{-1} dry wt.) was similar in internal and external hyphae and using a growth yield of 0·2, C allocation to internal hyphae could be estimated to be 5·9 mg ^{14}C d^{-1} or 16 % of the fixed ^{14}C. The fungal biomass of the VA mycorrhizas and its respiration thus consumed 20 % of the photoassimilated ^{14}C.

The lower ratio of soluble : insoluble organic ^{14}C in HC than in RC (Table 3) is in accordance with the results of Whipps (1984, 1987) who found that the ratio decreased consistently in the order rhizoplane, rhizosphere, and bulk soil. This may be explained by rapid microbial immobilization of soluble material released from the roots. In a similar study, Helal & Sauerbeck (1986) found that plant derived organic C was displaced 10–20 mm away from the roots, a zone which is usually not included in the rhizosphere (Rovira & McDougall, 1967). External hyphae of VA mycorrhizas may well contribute to a more even distribution of plant derived C in the soil volume. Although the HC in this work was only 3 mm wide, 43 % of its organic ^{14}C was located in the hyphae (Table 3) and ^{32}P studies have indicated that hyphae extend up to 8 cm from the roots (Rhodes & Gerdemann, 1975). Furthermore, hyphae from a well-infected cucumber root system spread more than 20 mm into an adjoining hyphal compartment within 14 days (Jakobsen, unpublished). Possibly some of the hyphal C is reabsorbed by the plant when the hyphae die, but otherwise carbon will be distributed to other components of the soil ecosystem as hyphae are grazed by soil animals (Finlay, 1985; Moore, St. John & Coleman, 1985; Rabatin & Stinner, 1985) and decomposed by other microorganisms.

Hyphal lengths in the HC of heavily infected 26-day-old cucumber plants were 27 m (g dry soil)$^{-1}$ (Table 4) which is similar to results obtained with 10-week-old ryegrass (Tisdall & Oades, 1979) and 5 to 7-week-old subterranean clover (Abbott & Robson, 1985). In contrast to the latter report, the hyphal background was low in the soil-sand mixture used in the present work [68 cm (g dry soil)$^{-1}$]. The specific incorporation of ^{14}C into hyphae in HC was 2·6-fold that indirectly determined for 6-week-old soybean by Harris *et al.* (1985). However, the ratio of hyphal to root-specific ^{14}C incorporation was similar in the two experiments. This may indicate a higher ratio of viable : total hyphae and roots in the younger plants in this work or a difference determined by host species.

This work has considered only young plants labelled with $^{14}CO_2$ for one photoperiod. A more thorough understanding of the importance of C in external hyphae of VA mycorrhizas in relation to non-hyphal C derived from roots will require further integration of measurements with respect to mycorrhizal ontogeny and insight into factors determining rates of turn-over of hyphae.

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