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The role of the external mycelial network of vesicular-arbuscular mycorrhizal fungi: a study of carbon transfer between plants interconnected by a common mycelium

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Abstract. The transfer of ^{14}C from *Lolium perenne* (the donor) to *Plantago lanceolata* (the receiver), mediated by vesicular-arbuscular (VA) mycorrhizal fungi, was examined when the two species were grown together or separately. The VA mycorrhizal infection led to a significant increase, relative to that in uninfected plants, in the ^{14}C transferred from donor to receiver plants, not only when the roots of the two plants were growing in intimate mixture, but also when they were separated by a root-free zone of 2.33 cm. The majority of isotope transfer between the two plant species was along the direct pathway via VA mycelium.

Key words: Vesicular-arbuscular mycorrhiza – ^{14}C transfer

Introduction

It has been documented by the use of isotope-tracer methods that colonization of plant roots by vesicular-arbuscular (VA) mycorrhizal fungi can provide channels for the transfer of carbon-containing substances between associated plants (Hirrel and Gerdemann 1979; Francis and Read 1984; Read et al. 1985). Two main mechanisms could be involved in this process: (1) direct transfer of carbon between plants through mycelium which connects individuals. This process could be enhanced by the generally low levels of host specificity shown by VA fungi; (2) indirect transfer involving leakage of carbon from roots of one plant, its absorption by VA mycorrhizal hyphae scavenging in the rhizosphere, and transfer to neighbouring plants. In addition to these two, there is a third possible mechanism which is not mediated by VA mycorrhizal fungi. This involves the leakage of carbon from roots of one plant and its subsequent absorption by the roots of neighbouring plants. However, the extent to which these mechanisms of carbon transfer between plants are involved is not very well understood. With the exception of the autoradiographic studies of Francis and Read (1984), the experiments designed to study ^{14}C transfer processes between plants have so far not provided the information necessary to discriminate between these processes.

The present work used $^{14}CO_2$ in an attempt to provide a better understanding of the mechanisms mediated by VA mycorrhizal fungi which may be involved in the transfer processes occurring between plants. Two separate experiments were carried out using chambers specifically designed to facilitate discrimination between direct and indirect methods of ^{14}C transfer. The influence of shade on the processes of transfer was also examined to determine the extent to which source-sink relationships are involved, as suggested by Francis and Read (1984), in determining the pattern of carbon transfer between plants.

Materials and methods

Experiment 1

Square pots (7 × 7 × 12 cm) were constructed using plastic drain pipe. Each pot was divided longitudinally into three identical sections (160 cm^3) by two pieces of nylon mesh screen (Nybolt PA-40/23; 39-μm mesh screen and 23% of free surface), which acted as a barrier to plant roots but allowed VA hyphae to pass freely. Four 10-cm vertical cuts were made into the walls of each pot starting at the base, leaving a 2-cm uncut section at the top. The nylon screens were then fed through these slits and glued to the outside of the pot. Plastic bases were then attached to the bottom of the pots. The final configuration of the pots is depicted in Fig. 1.

Each pot section was filled with twice autoclaved dune sand, and either two pre-infected mycorrhizal (M) or two nonmycorrhizal (NM) control plants of *Lolium perenne* were planted in one of the outer sections (A in Fig. 1). The mycorrhizal *Lolium* plants were produced by growing them for 3 weeks in small trays (21 × 16 × 5 cm) which contained approximately 30 g of root segments infected with VA mycorrhizal fungi collected in the field spread to form two layers of inoculum between two layers of autoclaved dune sand. In the case of the NM control plants, approxi-

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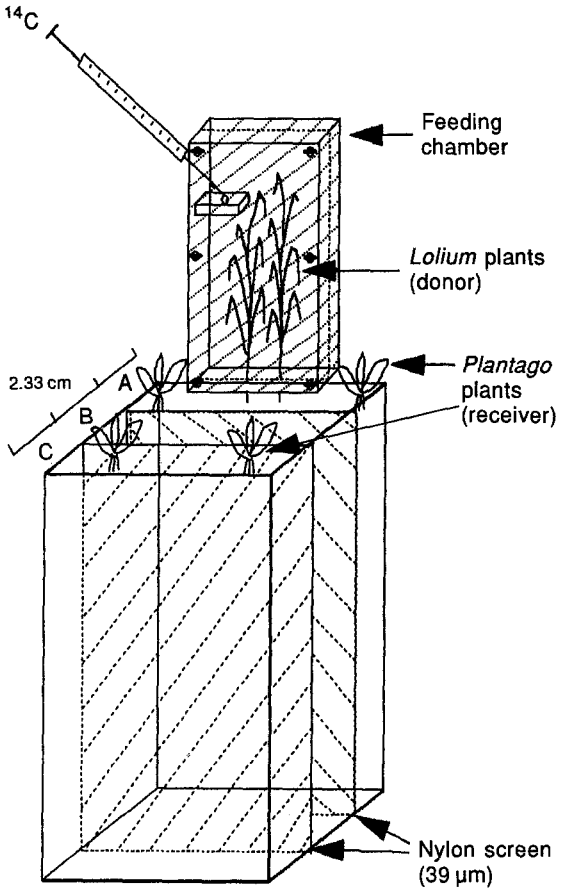

Fig. 1. Design of the pots, showing the feeding of ^{14}C

mately 25 ml of filtrate solution (filter paper Whatman No. 1) derived from root washings was added to the sand in order to ensure that the control plants received a bacterial population comparable with that present in the mycorrhizal medium, but lacking VA mycorrhizal propagules.

The pots were then placed in a controlled-environment growth room with an 18-h light period at 20–25° C and 6-h dark period at 15–18° C. The pots were watered every 2 days with distilled water. After 5 weeks, 5 ml of a nutrient solution containing: N 20 ppm; P 8 ppm; K 5 ppm; Ca 31 ppm; Mg 10 ppm; Fe 1 ppm; Mn 0.2 ppm; B 0.2 ppm; Mo 0.04 ppm; Zn 0.04 ppm and Cu 0.04 ppm was added to each section of the pots.

Twelve weeks after the *Lolium* had been planted, two pregerminated seeds of *Plantago lanceolata* were planted in the outer section (C) and another two were planted in the same compartment (A) as the *Lolium* donor plants of both M and NM categories. The *Plantago* plants in section C were thus isolated from those in A by the whole of section B – a distance of approximately 2.33 cm, while those in section A were growing in intimate mixture with the *Lolium*.

Four weeks after transferring the *Plantago*, the *Lolium* donor plants were fed with $^{14}CO_2$. Prior to feeding with $^{14}CO_2$, the soil surface was completely covered with paraffin wax in order to eliminate transfer of gaseous $^{14}CO_2$ from the soil to the plant shoots. Two further treatment conditions were also imposed:

1. Dark: The *Plantago* receiver plants in sections A and C were completely covered with aluminium foil to exclude light for a period of 48 h.

2. Full light: The *Plantago* receiver plants in sections A and C remained uncovered (exposed to the light for 48 h).

There were eight pots for each treatment condition, four with M and four with NM plants.

Immediately after commencement of the light and dark treatments, *Lolium* donor plants were fed with $^{14}CO_2$ by sealing their shoots into transparent plastic boxes (4 × 1 × 12 cm) which served as feeding chambers (Fig. 1). Each of these also contained a small dish into which isotope was injected. The isotope was supplied as a solution of sodium bicarbonate ($NaH_2C^{14}O_3$). A small volume of the bicarbonate solution containing 1.48 MBq of ^{14}C was injected into the feeding dish through a hole in the box wall using a Hamilton microsyringe. The ^{14}C was subsequently released as $^{14}CO_2$ by adding 1.0 ml of 10% lactic acid to the feeding dish through the same hole, which was then immediately sealed to prevent escape of ^{14}C.

The leaves of donor plants were exposed to the isotope for 48 h, after which time the *Plantago* receiver plants in each compartment were individually harvested and freed from sand particles. The shoots and roots of all plants were then separated and subsamples of the roots were taken to determine the percentage root length infected with VA mycorrhizal fungi (Giovannetti and Mosse 1980).

For determination of the levels of radioactivity, the plant material was oven-dried (48 h at 80° C), weighed and ground. Thereafter, subsamples of roots or shoots of known dry weight were placed in vials and digested by adding 10 ml of the one-step scintillant/digestant Fluorosol. The vials were incubated for 24 h at 50° C to ensure complete digestion of the plant material and subsequently kept in a cool, dark place for 24 h to permit subsidence of any chemiluminescence. The levels of radioactivity were the measured using a liquid scintillation counter (Packard, Tri-Carb 300C and 300CD). All counts were corrected for colour quenching and background.

Experiment 2

In this experiment, exactly the same procedures as described for experiment 1 were employed, except that:

1. The pots planted with *Lolium* (donor) plants were maintained for 8- instead of 12 weeks in the growth room before the receiver plants were planted.

2. In addition to *P. lanceolata*, a non-mycotrophic species, *Arabis hirsuta*, was also employed as a receiver plant. Thus, two pregerminated seeds of *A. hirsuta* as well as two of *P. lanceolata* were planted in each section A (root systems of donor and receiver plants intermingled) and in each section C (receiver roots separated from donor roots by two nylon mesh screens with a gap of 2.33 cm between them). After 5 weeks, instead of 4 weeks as in experiment 1, the shoots of *Lolium* plants were fed with $^{14}CO_2$, applying 0.74 MBq rather than 1.48 MBq.

3. The leaves of *Lolium* donor plants were exposed to the isotope for 4 and 8 days, instead of 2 days as in experiment 1, to provide a longer period of isotope transfer between plants and so to increase the possibility of detecting movement through the various pathways.

Results

Experiment 1

The detection of infection in the roots of *Plantago* receiver plants (Table 1) confirmed that transfer of infection had occurred from donor to receiver plants, forming VA links even when the plants were separated by two barriers and 2.33 cm of sand. There appeared to be a slightly lower infection in the latter condition, which is

Table 1. Percentage of root length infected by vesicular-arbuscular (VA) mycorrhizal fungi in the *Plantago lanceolata* receiver plants. Four replicate plants were examined. M, Mycorrhizal-infected plants; NM, non-infected controls; A, donor and receiver plants growing with their root systems intermingled (section A in Fig. 1); C, receiver roots separated from the donor roots by two nylon mesh screens (39 μm) with a gap of 2.33 cm between them (section C in Fig. 1)

VA status	Treatment			
	Full light		Dark	
	A	C	A	C
M	38.7	34.1	40.1	33.3
NM	0.0	0.0	0.0	0.0

Table 2. Radioactivity in *Plantago lanceolata* receiver plants after shoots of the *Lolium perenne* donor plants had been fed with $^{14}CO_2$ for 48 h. Four replicate plants were examined. dpm, Disintegrations per minute

Treatment	VA status	^{14}C Radioactivity (dpm/mg dry wt.)					
		Shoot		Root		Total[a]	
		A	C	A	C	A	C
Full light	M	21	5	75	53	96 a	58 a
	NM	18	6	38	27	56 b	33 b
Dark	M	21	6	134	85	155 c	91 c
	NM	44	7	25	31	69 bd	38 b

[a] Values followed by the same letter in the same column are not statistically different ($P=0.05$)

Table 3. Total radioactivity in the whole donor (*Lolium perenne*) and receiver (*Plantago lanceolata*) plants after $^{14}CO_2$ feeding of donor plants for 48 h. Four replicate plants were examined

Treatment	VA status	^{14}C radioactivity (dpm)			Radioactivity in receiver as % of that in donor plants	
		Lolium	*Plantago*			
			A	C	A	C
Full light	M	21737956	36557a	20453a	0.17	0.09
	NM	15667074	21332b	13989b	0.13	0.09
Dark	M	13649496	54756c	37235c	0.40	0.27
	NM	12388415	26224bd	15874b	0.21	0.13

probably to be expected bearing in mind the delay involved in infection reaching section C from A.

The levels of radioactivity in M *Plantago* receiver plants were significantly higher than those in their NM counterparts in both light and dark treatments and in both compartments A and C (Table 2). The effects of shading on carbon transfer to the receivers were evident, providing they were mycorrhizal, and independent of whether the infected plants were growing with their root systems together or separated from the donor plants. NM plants in both compartments A and C had consistently lower levels of radioactivity than M plants, these levels being unaffected by darkness (Table 2). The levels of radioactivity in whole M *Plantago* plants grown in the dark were 1.6 times greater than those in the light treatment in both compartments A and C (Table 2).

When the data were expressed as ^{14}C in the whole plant, the levels of radioactivity in M *Plantago* receiver plants were also significantly higher than in their NM counterparts (Table 3). The dark treatment led to high levels of activity in M receivers in both compartments A and C, while those plants in the NM conditions were unaffected.

The proportion of total ^{14}C radioactivity transferred to receiver from donor plants was as high as 0.4% in the M dark treatment in compartment A and 0.27% in the same treatment in compartment C (Table 3). The accumulation of radioactivity in the dark treatments was consistently higher than in the light, suggesting that a "sink" effect was in operation.

Table 4. Percentage of root length infected by VA mycorrhizal fungi in the *Plantago lanceolata* receiver plants. Four replicate plants were examined

Time (days)	VA status	Treatment			
		Full light		Dark	
		A	C	A	C
4	M	95.8	88.6	98.6	89.7
	NM	0.0	0.0	0.0	0.0
8	M	90.9	94.5	93.4	97.4
	NM	0.0	0.0	0.0	0.0

Experiment 2

The dark treatment did not affect the levels of VA colonization in the roots of receiver *Plantago* (Table 4), which were extremely high. In the case of *Arabis* plants grown in the presence of VA inoculum, some fungal structures were detected inside the roots but no functional infection in the form of arbuscule development was observed. Hirrel et al. (1978), Ocampo et al. (1980), and Ocampo (1986) have also reported that slight VA mycorrhizal infections develop in non-host plants when a mycorrhizal host plant is present. Cortical mycelium and vesicles (but not arbuscules) developed in the non-host plants and there were many clumps of endophyte mycelium on the root surfaces, usually attached to entry points (Ocampo et al. 1980).

At 4 days, M *Plantago* receiver plants in the dark regime contained significantly lower levels of radioactivity than those in the full light in both compartments A and C (Table 5), the values in the dark receivers being just less than half those in the light. Transfer to the NM receivers at 4 days was almost undetectable in both compartments and treatments.

By 8 days, the situation in the M plants had changed dramatically, particularly in compartment C, where

Table 5. Radioactivity in *Plantago lanceolata* receiver plants after the shoots of *Lolium perenne* donors had been fed with $^{14}CO_2$ for 4 or 8 days. Four replicate plants were examined

Time (days)	Treatment	VA status	^{14}C Radioactivity (dpm/mg dry wt.)					
			Shoot		Root		Total	
			A	C	A	C	A	C
4	Full light	M	1	1	555	97	556 a	98 a
		NM	0	0	2	1	2 b	1 b
	Dark	M	13	2	192	40	205 c	42 c
		NM	4	0	2	2	6 b	2 b
8	Full light	M	31	8	563	168	594 a	176 d
		NM	9	4	217	21	226 c	25 e
	Dark	M	36	22	560	468	596 a	490 f
		NM	88	22	411	35	499 a	57 c

Table 6. Radioactivity in *Arabis hirsuta* (non-mycotrophic species) receiver plants after the shoots of *Lolium perenne* donors had been fed with $^{14}CO_2$ for 4 days. Four replicate plants were examined

Treatment	VA status	^{14}C Radioactivity (dpm/mg dry wt.)					
		Shoot		Root		Total	
		A	C	A	C	A	C
Full light	M	7	0	1	0	8 ab	0 a
	NM	5	0	0	0	5 a	0 a
Dark	M	8	0	3	4	11 b	4 a
	NM	4	1	5	1	9 b	2 a

there was a doubling of activity in full light and a 10-fold increase in activity in the dark relative to that at 4 days (Table 5). The net effect of these changes over the 4 days between samplings was that dark-treated receivers in the M condition finally contained nearly 3 times more radioactivity than that seen in their light-grown counterparts. Over the same period, the amount of radioactivity transferred to the C compartment containing NM plants was very small.

Nearly all of the activity detected in whole receiver plants at 8 days in the dark treatment was located in the roots, and it is likely that it is being retained in the mycorrhizal fungus (Table 5).

The levels of ^{14}C radioactivity in the *Arabis* (non-mycotrophic) receiver plants at 4 days were not significantly influenced by the treatments or by the presence of inoculum (Table 6). No radioactivity was detected in the plants growing in compartment C in the light treatment and only a negligible amount was observed in the dark treatment. Unfortunately, all samples of *Arabis* at the 8 days exposure were lost as a result of a fault in the scintillation counter.

Discussion

The presence of infection in the receiver plants confirmed that the VA fungus had grown from donor to receiver roots, even when they were separated by a 2.33 cm zone of sand from which roots were excluded. Bethlenfalvay et al. (1991) and Camel et al. (1991) have shown that VA hyphae can spread from their associated roots into the soil over distances of 6 and 9 cm. Since the onset of sporulation of VA fungi requires 4–8 weeks and new spores have an endogenous dormancy cycle of 6 weeks to 6 months (Tommerup 1983), it seems that the colonization observed in receiver roots is attributable exclusively to growth of mycelium from roots of donor plants. Read et al. (1976) observed that most plants in seminatural grassland become heavily infected very soon after seed germination. They suggested that infection of the developing root system must arise from contact with mycelium spreading from plants with established infection, and that as a consequence of this pattern of infection many plants within the community must be interlinked by mycorrhizal hyphae. This has since been confirmed by studies of the development of infection of seedlings in the field (Read and Birch 1988).

The experiments described in this present work showed that mycorrhizal infection led to significant increases, relative to that seen in uninfected plants, in the quantities of ^{14}C transferred from donor to receiver plants, not only when the roots of the two plants were growing in intimate mixture, but also when they were separated by a root-free zone of 2.33 cm.

In the non-mycorrhizal condition, transfer of isotope occurred from donor to receiver plants growing with their roots intermingled but only very small quantities reached compartment C even after 8 days of feeding.

The view that the major pathway for transfer of isotope from donor to receiver plants involves direct plant-plant transfer through the fungal mycelium is strengthened by the observation that the non-host receiver plant *Arabis hirsuta* acquired very little isotope when grown in compartment C with infected *Plantago*.

Although the amounts of isotope transferred from NM donors to NM receivers in compartment A was generally small, it is evident that a pathway does exist. Such transfer has been observed previously in plants growing with roots intermixed (Hirrel and Gerdmann 1979; Francis and Read 1984; Read et al. 1985). It is known that organic C leaked from plant roots can be assimilated by microorganisms associated with the rhizosphere of neighbouring roots (Paul and Clark 1989). It is possible also that such roots have some heterotrophic ability directly to assimilate simple sugars.

It is more difficult to explain the albeit low level of labelling in roots of NM plants in the "C" compartment. There appear to be two possible explanations for such transfer. One is that a continuous film of water around sand particles between compartments A and C provides a pathway along which small quantities of labelled material can diffuse. Alternatively, since the NM plants were not grown aseptically, saprophytic hyphae may grow from section A to section C, forming channels for

transfer of material leaked into the rhizosphere of donors to the rhizospheres of receivers. In order to discriminate between these pathways, it would be necessary to repeat these experiments under aseptic conditions.

The exposure of the receiver plants to a dark regime led to a significant increase in transfer of ^{14}C relative to that observed in the full light regime at 2 and 8 days of exposure. Francis and Read (1984) showed that the magnitude of ^{14}C transfer is strongly influenced by shading of receiver plants, indicating that movement is governed by source-sink relationships. The failure to detect such an effect at 4 days is of interest. The very much higher levels of infection of the *Lolium* donor plants and the reduced amount of ^{14}C fed in this experiment relative to experiment 1 (0.74 vs 1.48 MBq) are factors which may have contributed to the delay observed in response to shading. Activity transferred from shoots to roots of donors would be expected to be retained more effectively in the fungal tissues under these circumstances.

The relatively high transfer of ^{14}C between donor and receiver plants in the M condition at 4 days in both full light and dark treatments when their roots were closely intermingled in section A may have occurred for the same reason. Analysis of transfer from donor to receiver in the NM condition does not suggest that indirect transfer by leakage of carbon from donor roots followed by its reabsorption from the soil pool by the receiver roots is of major importance. It is more likely that the greater amount of carbon detected in M receiver plants in section A is due to a combination of the following effects: (1) increase of the direct transfer pathway, facilitated by the occurrence of a greater number of hyphal bridges in the densely intermingled infected roots; (2) facilitation of greater flux of ^{14}C by the short path length between the two sets of root systems.

The results contained in this present work revealed that VA mycorrhizal fungi can play an extremely important role in plant communities by providing channels for direct transfer of carbon between individual plants. This would not only facilitate effective recycling of carbon but would also greatly restrict losses of this element to the general soil microbial communities.

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