RECIPROCAL TRANSFER OF NUTRIENTS BETWEEN ERICACEOUS PLANTS AND A *CLAVARIA* SP.*

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SUMMARY

A close physiological relationship between some ericaceous plants, *Rhododendron* sp. and *Pieris japonica*, and a member of the genus *Clavaria* is indicated by radioisotope translocation studies. [^{14}C]photosynthate from host leaves was detected in *Clavaria* fruiting bodies and ^{32}P applied to *Clavaria* fruiting bodies was detected in plant roots. Possible transfer mechanisms, including that of a mycorrhizal association, are discussed.

INTRODUCTION

Consistently each autumn, in field-grown nursery stock and garden plantings, we have found numerous simple, clavate fruiting bodies of *Clavaria argillacea* Fr. associated exclusively with roots of evergreen, ericaceous, woody ornamentals in Rhode Island, U.S.A. This consistent association possibly indicates a mycorrhizal relationship. The morphology of the fungal fruiting body resembles but is not identical with that reported by Seviour, Willing and Chilvers (1973), who postulated a mycorrhizal association between a *Clavaria* sp. and ericaceous plants in Australia. No synthesized mycorrhizal association with a *Clavaria* sp. has been reported. The availability of *Clavaria* fruiting bodies for experimentation is limited to 3 to 4 weeks in the autumn when they develop beneath the canopy of ericaceous plants.

Reciprocal translocation patterns of carbon and phosphorus have been demonstrated in ericaceous mycorrhizas by Pearson and Read (1973b) and Stribley and Read (1974), who synthesized mycorrhizal associations between *Pezizella ericae* and *Vaccinium* and *Calluna* (Pearson and Read, 1973a). We have investigated a possible relationship between host roots and the *Clavaria* fruiting bodies by studying the transport of ^{32}P from fungal fruiting bodies into roots and the transport of [^{14}C]photoassimilate from the plant into the fruiting bodies.

MATERIALS AND METHODS

^{14}C studies

Six-year-old field-grown plants of *Rhododendron* cv. 'English Roseum' and *Pieris japonica* D. Don. were used *in situ* for ^{14}C transfer studies. Numerous *Clavaria* fruiting bodies developed beneath these plants each autumn. Plants of each genus were exposed to $^{14}CO_2$ using techniques described by Hull and Leonard (1964) and similar plants served as unlabelled controls. The terminal portion of a shoot on each plant

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selected for ^{14}C treatment was enclosed in a transparent polythene bag. Two pieces of Tygon tubing were inserted into the polythene bag along the stem and wrapped with a strip of polyurethane foam so that, when the mouth of the bag was tied, a gas-tight seal was made. Both sections of tubing protruding from the bag were attached to a CO_2 generator and a rubber aspirator pump containing a one-way valve was inserted in the line, so that air could be circulated from the bag through the generator and returned to the bag. The CO_2 generator consisted of a stoppered vial with three inlets: two for the inlet and outlet tubes from the bag and a third with a removable plug for inserting acid. The generator initially contained 50 μCi $Ba^{14}CO_3 \cdot {}^{14}CO_2$, liberated by the addition of several drops of 85% lactic acid, was circulated through the polythene bag by repeatedly manipulating the aspirator pump. Shoots were exposed to $^{14}CO_2$ for about 30 min, after which the remaining $^{14}CO_2$ was removed by circulating the gas from the bag through a gas scrubber containing a $Ba(OH)_2$–$BaCl_2$ solution. The length of the path from treated shoots down the stems to the soil line was 91 ± 5 cm. After various periods following treatment, fruiting bodies of *Clavaria* and foliage of other plants (both monocotyledons and dicotyledons) growing beneath treated and untreated ericaceous plants were harvested. Sampled tissue was dried at 70 °C and pulverized with a mortar and pestle. The resulting powder was placed in tared planchets and compressed with a piston to produce a flat surface for radioassay. Radioassays, performed in a Tracerlab TGC-14 thin-window, proportional gas-flow counting system, were corrected for self-absorption.

^{32}P studies

The prerequisite that ^{32}P studies be performed in the greenhouse necessitated that plants, with *Clavaria*, were sufficiently small to allow transplanting with minimal disturbance to the roots and fungal structures. As *Clavaria* fruits infrequently beneath young plants, only six such associations, with 2-year-old *Rhododendron* cv. 'English Roseum' growing in a nursery bed, were found for this study. Large clumps of soil, each encompassing a cluster of fruiting bodies and one or two plants, were carefully lifted and placed in individual containers in the greenhouse. Each of the six fungus-plant associations served as a replicate (sites I–VI in Table 3).

The ^{32}P was applied as small droplets (3 to 4 μl) to the upper surface of the mature, club-like fruiting bodies of *Clavaria*. These droplets, which remained at the point of application, had disappeared through absorption and evaporation within 3–5 min. Each cluster of fruiting bodies was treated with a total of 20 or 40 μCi ^{32}P as $NaH_2{}^{32}PO_4$ in 10 to 20 μl distilled water. A protective canopy of aluminium foil was placed loosely over treated fruiting bodies and no water was applied to soil or plants for the duration of the study to minimize non-biological transport of ^{32}P into the soil and adjacent plants.

Prior to sampling, ^{32}P-treated fruiting bodies and the basal weft of hyphae with associated soil to a depth of 3 mm were removed and discarded. Root samples from plants near ^{32}P-treated fruiting bodies and untreated control plants were obtained 10 to 11 days after application of the isotope by removing a core of soil 40 mm in diameter and 40 mm deep. Cores from directly beneath treated fruiting bodies were divided into two equal layers, one representing the 20 mm nearest the soil surface and the other the lower 20 mm. Cores containing roots taken at the midpoint between treated fruiting bodies and the stem of adjacent plants were processed as a single sample, designated 'midpoint roots'.

Roots were separated from soil by shaking the core in a flask of water, pouring the contents on to a no. 20 sieve (841 μm pores) and flushing with water to remove the soil. Roots retained on the sieve were transferred to a dish and adhering soil was carefully removed with the camel-hair brush under a dissecting microscope. Cleaned roots were cut into short segments and samples were placed in tared planchets and dried under a sunlamp. Their dry wts were determined prior to radioassay.

In a simultaneous study, 40 μCi ^{32}P were applied in 20 μl solution directly to the surface of the soil near plants, without associated fruiting bodies, from the same source described above. Before sampling these sites, 3 mm of surface soil was removed and discarded. Then, samples were taken at 3 mm increments to a total depth of 40 mm. Samples of soil and roots were placed in tared planchets, dried, weighed, and radioassayed. 'Midpoint roots', centrally located along a line between sites of application to soil and the stem of adjacent plants, were cleaned and prepared for assay as described above.

Foliar samples were prepared by removing duplicate three-leaf samples from each plant from which root samples had been taken, 14 days after ^{32}P application, drying at 70 °C, grinding in a Wiley Mill and compressing samples in tared planchets.

Radioassays were performed as described above. No corrections for decay were made since all root and foliage samples in the ^{32}P study were assayed within a 2-day period.

Results

Translocation of ^{14}C to Clavaria *fruiting bodies*

Fruiting bodies of *Clavaria* were assayed 3 days after application of $^{14}CO_2$ to *Rhododendron* plants. Those which had expanded beneath one plant during the 3-day post-treatment interval had levels of radioactivity substantially higher than the background level in weeds beneath this plant and fruiting bodies beneath untreated plants. Under the labelled plant, fruiting bodies that had expanded prior to treatment had radioactivity similar to the background level (Table 1). Beneath two other plants, no fruiting bodies expanded within 3 days of treatment since dry, cold weather had arrested the development of additional fruiting bodies.

Table 1. *Specific activity of* Clavaria *fruiting bodies and weed foliage beneath treated and untreated* Rhododendron cv. *'English Roseum' 3 days after $^{14}CO_2$ application*

Plant	Sample description	Distance from stem (cm)	Specific activity (ct min^{-1} dry wt^{-1})
Rhododendron (^{14}C-treated)	Fruiting bodies, expanded after treatment	0–10	0·94
		0–10	1·36
		10–20	2·14
		30–40	1·73
	Fruiting bodies, expanded prior to treatment	30–40	0·24
	Monocotyledonous weeds	10–40	0·07
	Dicotyledonous weeds	10–40	0·13
Rhododendron (untreated)	Fruiting bodies	10–40	0·04
	Monocotyledonous weeds	10–40	0.09
	Dicotyledonous weeds	10–40	0.17

In tests the following year a longer interval of time (8 and 27 days) between treatment and sampling was allowed to ensure development of new fruiting bodies after treatment. These new fruiting bodies beneath labelled *Rhododendron* and *Pieris* plants exhibited levels of radioactivity consistently higher than fruiting bodies beneath untreated plants (Table 2). Foliage of actively growing weeds from beneath labelled plants had levels of radio-activity similar to the background level measured in weed foliage and *Clavaria* fruiting bodies beneath untreated plants. [^{14}C]Photosynthate was translocated and distributed within the root system of treated plants and the radioisotope was detected in fruiting bodies at various locations around these plants, with quantitative differences undoubtedly influenced by such factors as the vascular anatomy of the roots and stems, and the age, depth and direction of growth of roots in the soil. This experiment, performed initially in 1976, was repeated in 1978 with similar results.

Table 2. *Specific activity of* Clavaria *fruiting bodies and weed foliage beneath* $^{14}CO_2$*-treated and untreated* Rhododendron *cv.* '*English Roseum*' *and* Pieris japonica *plants*

Sample and location	Days after treatment	Host plant: *Rhododendron* (ct min^{-1} dry wt)	Host plant: *Pieris* (ct min^{-1} dry wt)
Clavaria directly beneath treated shoot, 10–20 cm from stem	8	*8·70 ± 4·15	12·83 ± 2·50
	27	5·20 ± 1·75	14·41 ± 5·91
Clavaria directly beneath treated shoot 30–40 cm from stem	8	8·47 ± 3·00	10·35 ± 3·65
	27	9·73 ± 3·60	5·07 ± 0·82
Clavaria on side of plant opposite treated shoot, 20–40 cm from stem	8	5·73 ± 1·19	24·16 ± 4·33
	27	8·51 ± 0·67	28·44 ± 13·25
Weeds beneath treated shoot	8	0·36 ± 0·22	0·23 ± 0·10
	27	0·22 ± 0·15	0·09 ± 0·001
Clavaria beneath untreated plant	8	0·31 ± 0·16	0·13 ± 0·02
	27	0·10 ± 0·09	0·28 ± 0·22
Weeds beneath untreated plant	8	0·42 ± 0·19	0·23 ± 0·02
	27	0·22 ± 0·17	0·15 ± 0·08

* Each specific activity value is the mean of 4 to 6 samples from each of 3 plants, followed by the standard error of the mean.

Translocation of ^{32}P *from* Clavaria *fruiting bodies to* Rhododendron *roots*

Roots sampled at various depths beneath ^{32}P-labelled fruiting bodies and 'midpoint roots' exhibited elevated levels of radioactivity (Table 3, sites I to VI) compared with unlabelled controls (sites X to XII). Roots 23 to 43 mm below treated fruiting bodies also showed elevated levels, whereas at distances approximately 12 mm or greater beneath sites where ^{32}P had been applied directly to soil only background radiation levels were detected (Table 3, sites VII to IX). Similarly, most 'midpoint roots' between treated fruiting bodies and adjacent plants were substantially more radioactive than controls (i.e. 'mid-point roots' between sites of application to soil and adjacent plants. There was no correlation between radioactivity levels of roots and the initial dose of ^{32}P applied to fruiting bodies; 20 μCi ^{32}P was applied to fungus at sites I and VI, and 40 μCi was applied at sites II–V. There was no radioactivity detected above background levels in the foliage from plants whose roots had been assayed in the ^{32}P studies.

Table 3. *Specific activity of soil and roots at various depths beneath the site of application of* ^{32}P *to* Clavaria *fruiting bodies or soil, and 'midpoint roots', equidistant between treated sites and the* Rhododendron (*cv. 'English Roseum'*) *stem. Samples from unlabelled sites are included for comparison. (Values as ct* min^{-1} *mg dry wt.)*

	Sample description				
	Root sample depths (mm)				
^{32}P application	3–23	23–43			'Midpoint roots'
Fruiting bodies					
I	4·60	1·83			0·84 (6)*
II	24·97	2·44			—†
III	4·24	3·76			0·92 (4)
IV	38·90	17·80			1·31 (10)
V	380·93	66·11			‡12·60 (7) 0·97 (10)
VI	128·44	3·66			‡0·35 (7) 0·18 (7)
	Soil/root sample depths (mm)				
	3–6	6–9	9–12	12–15§	'Midpoint roots'
Soil surface					
VII	5·32	0·55	0·34	0·09	0·08 (4)
VIII	0·79	0·22	0·19	0·13	0·12 (5)
IX	3·93	0·94	0·12	0·10	0·03 (5)
	Root samples				Soil/root samples
Unlabelled controls					
X	0·17				0·21
XI	0·10				0·17
XII	0·14				0·06

* Figures in parentheses represent distance (cm) of 'midpoint roots' from the treated fruiting bodies.

† Application site too close to stem for 'midpoint roots' to be sampled.

‡ Two specific activity values refer to 'midpoint root' samples from separate plants adjacent to treated site.

§ Specific activity of all samples below this depth were similar to values at 12 to 15 mm depth.

Discussion

Pearson and Read (1973b) demonstrated, with *Pezizella ericae* in aseptic, synthesized, ericaceous mycorrhizas, a pathway for the translocation of nutrients from the mycorrhizal fungus into plant roots and the transfer of host photosynthate to the fungus. These translocation phenomena offered experimental methods for studying the relationship between *Clavaria* and ericaceous plant roots. Owing to our lack of success in culturing the fungus, which necessitated working with naturally occurring associations, fruiting bodies had to be the treated donor or the sampled recipient of test materials.

The transfer of [^{14}C]photosynthate to fungal fruiting bodies depends on (a) movement from roots to hyphae and (b) the continued movement through the hyphae to fruiting bodies. The path by which labelled carbon may reach the hyphae is speculative. Carbon compounds leached or CO_2 respired from roots rapidly could be incorporated by microorganisms in the root zone. Rhizoplane soil sampled from roots of labelled plants, even if radioactive, would not serve to identify the recipient of ^{14}C,

which may be either *Clavaria* hyphae or other microorganisms. No rhizoplane fungal associations were found during extensive microscopic examinations. Rather, there were numerous thin wefts of hyphae external to the root, connected to apparently healthy root cells which contain hyphal 'knots' similar to those described as typical of ericaceous mycorrhizas (Pearson and Read, 1973a; Seviour *et al.*, 1973). Positive identification of mycelium of our *Clavaria* within roots of ericaceous plants has not been successful, although some of the hyphae found in epidermal and cortical cells have basidiomycetous characteristics. The recovery of ^{14}C in *Clavaria* fruiting bodies 3 days following exposure of *Rhododendron* leaves to $^{14}CO_2$ indicates that metabolite transfer between plant and fungus is indeed efficient and probably involves more than fungal assimilation of root leachate.

That ^{14}C-labelled photosynthate from the host plant moved into *P. ericae* hyphae and was translocated some distance toward developing hyphal tips (Pearson and Read, 1973b) suggested that this material might also move into developing *Clavaria* fruiting bodies once assimilated by the hyphae. Pearson and Read (1973b) postulated that the extent of movement of ^{14}C-labelled material was limited by the fact that hyphal tips may have provided an insufficient metabolic sink. However, expanding *Clavaria* fruiting bodies may be a more effective sink and thus account for the accumulation in these structures of the significant amounts of ^{14}C reported here. It should be noted that only fruiting bodies which continued growing after the host foliage was labelled had elevated levels of radioactivity, whereas fruiting bodies which were fully expanded at the time the plants were exposed to $^{14}CO_2$ did not accumulate radioactivity (Table 1).

Although there is evidence for the bi-directional movement of ^{32}P in the hyphae of the endomycorrhizal fungus, *P. ericae* (Pearson and Read, 1973b), we had no prior indication that this radioisotope would be mobilized from *Clavaria* fruiting bodies into hyphae and roots. Fully expanded fruiting bodies were chosen for ^{32}P application sites, since their height (1 to 2 cm) allowed reasonable spatial separation from the soil and thereby reduced the probability of soil contamination with the radioisotope. The use of mature fruiting bodies may have permitted reabsorption of labelled phosphate by the connecting soil hyphae more than might have occurred from labelled, immature fruiting bodies. ^{32}P may have been transferred from fungus to plant by leakage into, and diffusion through, the soil and subsequent absorption by roots or by direct transfer from hyphae in or on root cells. However, despite wide variation in radioactivity levels between similar samples at sites I to VI (which may be attributed to the variability in efficiency of phosphate transfer from fungus to host plant roots), specific activity values of root samples taken 23–43 mm below treated fruiting bodies and of 'midpoint roots' were much greater than would be expected from non-biological transport of phosphorus through the soil. This is in accord with our control data (application of ^{32}P to soil – Table 3, sites X to XII) and documentation in the literature of the characteristic immobility in soil of the phosphate anion (Vasey and Barber, 1963; Rhodes and Gerdemann, 1975).

In ^{32}P experiments the lack of radioactivity in foliar samples from plants with some radioactivity in roots may be due to dilution beyond detectable levels as the radioisotope was distributed throughout the tissue of the plant, or possibly, insufficient time was allowed for the labelled phosphate to move into the foliage. Additional leaf samples were not taken after a longer interval, owing to the destructive nature of root harvesting at the initial sampling time.

Evidence for bidirectional nutrient transfer between ericaceous plant roots and *Clavaria argillacea* has been presented. The fungus is in some manner deriving a measure of carbon nutrition from the plant. This may have been obtained through a saprotrophic, necrotrophic or mycorrhizal relationship. Support for the latter phenomenon comes from data showing that this fungus can contribute phosphorus to the plant. Presently, we are restricted by our dependence upon naturally occurring associations, with formation of fruiting bodies requiring environmental conditions that are met for only a short period each year. Further work on the physiology of nutrient exchange will be facilitated by pure culture synthesis of the putative mycorrhiza. Microscopic studies are needed to clarify the relationship of the fungus and plant roots and to assess the correlation of this species with that of Seviour *et al.* (1973), who presented serological evidence for the presence of *Clavaria* peletons in ericaceous roots.

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