DIFFUSION AND TRANSLOCATION IN SOME Fungal culture systems

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(With 1 Text-figure)

Experiments were designed to study the effects of different types of diffusion barrier on the movement of solutes in an endomycorrhizal fungus (Pezizella erlcae Read) and an ectomycorrhizal fungus (Suillus luteus Fries). Up to 87% reduction of transport occurred when a lanolin layer was placed on a glass or plastic 'barrier'. Application of propylene oxide and inhibitors indicated that reduction was due to elimination of diffusion and capillary movement which was particularly pronounced in semi-aqueous pure culture systems in which fungal hyphae acted as 'wicks'. The findings suggest that care must be taken in the extrapolation of results from aseptic culture systems to field situations.

Studies of nutrient movement through fungal mycelium are of particular interest to those investigating mutualistic relationships between fungi and higher plants. In mycorrhizal studies, for example, nutrient exchanges have been shown to occur between host and fungal symbiont growing together in pure culture (Melin & Nilsson, 1950, 1952, 1958; Smith, 1967). The ecological significance of nutrient movement in such systems is often stressed but it is important to realise that the conditions are highly artificial. Sterile culture conditions eliminate competition and yield dense aggregates of fungal hyphae which usually grow from a point source and more or less in parallel. Such growth forms might be expected to lead to abnormally large amounts of passive external transport since capillary channels are present between the walls of hyphae which can therefore act as a wick. This makes it extremely difficult to distinguish between passive movement of nutrients and true translocation within the hyphae.

Several workers have been aware of this difficulty and have introduced trenches (Lucas, 1960; Lyon & Lucas, 1969), double dishes (Schutte, 1956; Thrower & Thrower, 1968; Milne & Cooke, 1969) or combinations of the two (Littlefield, Wilcoxson & Sudia, 1965) as diffusion barriers between nutrient sources and sinks. With fungi like Rhizoctonia solani Kuhn these barriers appear to be effective since movement ceases if the mycelium is killed. This implies that all nutrient transfer through such fungi is by translocation. In other fungi however, such simple barriers are quite ineffective. Studies of nutrient transport in cultures of ericaceous mycorrhiza (Pearson & Read, 1973b) revealed that much of the movement over glass barriers was by diffusion. For this reason experimental systems were designed to give better discrimination between transport and translocation.

This paper compares the efficiency of such systems when used with the
endomycorrhizal fungus *Pezizella ericae* Read and the sheathing mycorrhizal fungus *Suillus luteus* (Fr) S. F. Gray (*=* Boletus luteus Fries). It was hoped by obtaining such discrimination to gain a more realistic understanding of the ecological importance of nutrient movement in these types of mycorrhiza.

**MATERIALS AND METHODS**

The mycorrhizal endophyte of ericaceous plants was isolated by procedures described by Pearson & Read (1973a), and the *S. luteus* isolate was obtained from a young fruit body. Uniform disks of inoculum were transferred to culture systems of various types, each one being designed to facilitate study of nutrient movement over a particular kind of diffusion...
barrier. The basic systems are described in Fig. 1. They represent the glass cup method, similar to that of Melin & Nilsson (1950) (Systems A and D) the divided dish method (System B) (Pearson & Read, 1973b) and the agar trench method (System C) of Lucas (1960).

Agar cultures consisted either of Robbins dilute mineral nutrient medium (Robbins & White, 1936) supplemented with 1% glucose, or of pure 1% glucose medium. The concentration of agar employed was either 0.5 or 1%. These media were selected because they were found to yield optimal development of mycorrhiza in seedling cultures and were therefore to be employed for later nutrient transfer studies. In all cases the initial nutrient status of the medium was identified on both sides of the ‘barrier’.

The fungi were allowed to grow from the inoculum or source position across the barrier and to colonize the medium on the other side which was termed the sink.

A layer of anhydrous lanolin was applied to the barrier wall in some dishes of each system just prior to the application of tracer nutrient to the source side. Systems with lanolin are indicated by the subscript L in Tables 1-4. The lanolin was first heated at 100° for 24 h and then cooled to 40° before being dispensed from a sterile syringe onto the fungus growing over the barrier.

Other treatments to the mycelium included exposure to the lethal vapour of propylene oxide (subscript PO in Tables) and to the inhibitors 2,4-dinitrophenol (DNP) (10^-4 M) and sodium azide (10^-3 M). In some dishes the mycelium was severed (subscript S) either inside the source dish as in System A, or by passing a sterile razor blade through the hyphae along the top of the barrier in System B.

Movement of solutes in the various systems

Fluorescent dye

Fluorescein was first used to provide a rapid visual estimate of the rate of movement of material. A few drops of a 0.1% solution of fluorescein were added to the source vessel and the culture was observed under UV light. The distance of the fluorescent front from the barrier was measured after 30 min, 1 h and 24 h and in two concentrations of non-nutrient agar. The systems and fungi employed are shown in Table 1.

32P-orthophosphate

In these experiments Robbins medium was supplemented with 1% glucose. The agar concentration was either 0.5 or 1%. Labelled orthophosphate was diluted in Robbins solution to give a final activity of 10 μCi/ml and 0.2 ml of labelled solution was added to each dish. The systems employed are shown in Table 2. Radioactivity was estimated by removing replicate sample disks with a 6 mm cork borer at two distances from the diffusion barrier after 24 h. These were dried under an infra-red lamp and counted on planchettes using a Nuclear Chicago gas flow counter. In the case of the S. luteus sand culture systems (System D),
replicate 100 mg samples were taken from the margin of the fungal colony (4 cm) and from the sand around the colony (8 cm) and dried before planchette counting as before.

\(^{14}\text{C}-\text{glutamine}\)

Dishes of System B were used for these experiments but they were slightly modified by flattening of the central barrier (see B\(_2\) in Fig. 1). The medium used was a 1\% glucose solution in 0.5\% agar. \(^{14}\text{C}\)-labelled glutamine was added to cultures of \(P.\) \(ericae\) and the movement of the label was followed. The isotope was first dissolved in 0.08 M McIlvaine’s (phosphate-citrate) buffer at pH 5.5 and its concentration was adjusted to \(10^{-3}\) M by the addition of the appropriate quantity of unlabelled compound. Each dish received 0.2 \(\mu\)Ci of isotope in 0.2 ml of solution. The isotope was added to a trough (3.0 \(\times\) 0.3 \(\times\) 0.3 cm) cut in the agar parallel to the barrier and 1 cm away from it. A small glass well which contained 0.2 ml of 5.0 N-KOH was placed on the agar of each dish to absorb any \(^{14}\text{CO}_2\) evolved by the fungus.

Disks of mycelium for \(^{14}\text{C}\) assay were cut from the periphery of the colony with a 6 mm cork borer. The disks were dried as before but their radioactivity was determined by the combustion technique of Gupta (1968) which liberates \(^{14}\text{CO}_2\) for liquid scintillation counting.

\text{RESULTS}

\text{Movement of fluorescein}

The results of the dye experiment are presented in Table 1. The most striking result is the ease and rapidity with which the dye moves over a simple glass or plastic barrier with either fungus. A number of facts suggest that this movement is largely by passive diffusion. The movement was virtually eliminated by the lanolin barrier and it was most rapid in the more aqueous systems (0.5\% agar) in which it even extended into the agar beyond the fungal colony after 2 h. If the fungal hyphae were severed on the inside of the source dish no transport occurred whereas severing on the barrier surface allowed some movement to continue, presumably because some physical contacts were maintained. These facts together confirm that the fungus acted largely as a wick facilitating the movement of the dye along a diffusion gradient.

The pattern of movement was similar in both fungi employed but the rate of diffusion through \(S.\) \(luteus\) mycelium was slower probably because of its more lax growth form.

\text{Movement of} \(^{32}\text{P-orthophosphate}\)

The results for \(P.\) \(ericae\) are presented in Table 2 and those for \(S.\) \(luteus\) in Table 3. As in the fluorescein experiments, the lanolin barrier considerably reduced movement. In \(P.\) \(ericae\) on 0.5\% agar the maximum movement through the lanolin barrier was only 19\% of that without the barrier. The figure for \(S.\) \(luteus\) cultures was 37\%. These figures probably
Table 1. Distance of fluorescein front (mm) from the diffusion barrier at different time intervals following dye application in two different agar concentrations

<table>
<thead>
<tr>
<th>System</th>
<th>0.5%</th>
<th>1%</th>
<th>0.5%</th>
<th>1%</th>
<th>0.5%</th>
<th>1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pezizella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>18</td>
<td>12</td>
<td>28</td>
<td>20</td>
<td>60†</td>
<td>38</td>
</tr>
<tr>
<td>A&lt;sub&gt;L&lt;/sub&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>16</td>
<td>10</td>
<td>19</td>
<td>14</td>
<td>29†</td>
<td>16</td>
</tr>
<tr>
<td>B&lt;sub&gt;L&lt;/sub&gt;</td>
<td>6</td>
<td>4</td>
<td>10</td>
<td>11</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>B&lt;sub&gt;L&lt;/sub&gt;</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td></td>
<td>Trace</td>
<td></td>
</tr>
<tr>
<td>Suillus luteus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>7</td>
<td>7</td>
<td>14</td>
<td>10</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>A&lt;sub&gt;L&lt;/sub&gt;</td>
<td>0</td>
<td>0</td>
<td>Trace</td>
<td></td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

* For details see Fig. 1 and text.
† Represents movement into agar beyond the margin of the colony.

Table 2. Movement of 32P across different types of diffusion barrier in Pezizella ericae

(Measurements were made at two distances from the barrier on the sink side and in two concentrations of agar. Figures are in counts per minute per sample disk. Each figure represents a mean of 12 samples (six dishes, 2 samples per dish).)

<table>
<thead>
<tr>
<th>System</th>
<th>0.5%</th>
<th>1%</th>
<th>0.5%</th>
<th>1%</th>
<th>0.5%</th>
<th>1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>196±241</td>
<td>—</td>
<td>1751±164</td>
<td>—</td>
<td>1402±96</td>
<td>—</td>
</tr>
<tr>
<td>A&lt;sub&gt;L&lt;/sub&gt;</td>
<td>336±42</td>
<td>17</td>
<td>230±41</td>
<td>13</td>
<td>448±101</td>
<td>31</td>
</tr>
<tr>
<td>A&lt;sub&gt;L&lt;/sub&gt;</td>
<td>21±7</td>
<td>—</td>
<td>19±10</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A&lt;sub&gt;L&lt;/sub&gt;</td>
<td>1654±190</td>
<td>—</td>
<td>126±348</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A&lt;sub&gt;L&lt;/sub&gt;</td>
<td>3011±186</td>
<td>—</td>
<td>286±99</td>
<td>—</td>
<td>1710±219</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>499±111</td>
<td>16</td>
<td>284±41</td>
<td>19</td>
<td>510±88</td>
<td>29</td>
</tr>
<tr>
<td>B&lt;sub&gt;L&lt;/sub&gt;</td>
<td>9±4</td>
<td>—</td>
<td>12±3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B&lt;sub&gt;L&lt;/sub&gt;</td>
<td>4907±160</td>
<td>—</td>
<td>4233±210</td>
<td>—</td>
<td>2614±215</td>
<td>—</td>
</tr>
<tr>
<td>C</td>
<td>411±98</td>
<td>—</td>
<td>348±56</td>
<td>—</td>
<td>303±61</td>
<td>—</td>
</tr>
<tr>
<td>C&lt;sub&gt;L&lt;/sub&gt;</td>
<td>1045±186</td>
<td>—</td>
<td>207±17</td>
<td>—</td>
<td>207±17</td>
<td>—</td>
</tr>
</tbody>
</table>

* For details see Fig. 1 and text.

Table 3. Movement of 32P across different types of diffusion barrier in Suillus luteus

<table>
<thead>
<tr>
<th>System</th>
<th>4 cm</th>
<th>6 cm</th>
<th>8 cm saturated sand only</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>453±79</td>
<td>26</td>
<td>652±70</td>
</tr>
<tr>
<td>A&lt;sub&gt;L&lt;/sub&gt;</td>
<td>121±16</td>
<td>—</td>
<td>246±36</td>
</tr>
<tr>
<td>A&lt;sub&gt;L&lt;/sub&gt;</td>
<td>75±46</td>
<td>—</td>
<td>12±6</td>
</tr>
<tr>
<td>4 cm mycelium + sat. sand</td>
<td>849±198</td>
<td>—</td>
<td>640±75</td>
</tr>
<tr>
<td>D</td>
<td>284±17</td>
<td>33</td>
<td>28±16</td>
</tr>
<tr>
<td>D&lt;sub&gt;L&lt;/sub&gt;</td>
<td>284±17</td>
<td>—</td>
<td>28±16</td>
</tr>
</tbody>
</table>

In System A, figures are in counts per minute per sample disk. Each figure represents the mean of 12 samples.
In Systems D each figure represents counts per 100 mg sample with replication as in system A.
Table 4. Effects of various treatments on movement of \(^{14}\text{C}\) by the endophyte in System B

(Figures represent activity in sample disk (cpm), Figures in brackets are the percentages of counts attributable to translocation.)

<table>
<thead>
<tr>
<th>Culture treatment</th>
<th>Without lanolin barrier</th>
<th>With lanolin barrier</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48 h, 192 h</td>
<td>48 h, 192 h</td>
</tr>
<tr>
<td>Untreated mycelium</td>
<td>282 ± 42, 717 ± 72</td>
<td>92 ± 12 [32]</td>
</tr>
<tr>
<td>DNP</td>
<td>158 ± 29, 416 ± 70</td>
<td>* 52 ± 12</td>
</tr>
<tr>
<td>Azide</td>
<td>197 ± 19, 575 ± 72</td>
<td>* 43 ± 11</td>
</tr>
<tr>
<td>Propylene oxide</td>
<td>80 ± 12, 209 ± 50</td>
<td>10 ± 4</td>
</tr>
<tr>
<td>Mycelium severed</td>
<td>20 ± 7, 70 ± 14</td>
<td>* 29 ± 9</td>
</tr>
</tbody>
</table>

Each figure is a mean of 17 samples and is quoted with standard errors.

* Indicate counts not significantly above background.

The rates of translocation in *S. luteus* are similar to those found in the endophyte but the proportion of the total movement which this represents is higher because of the smaller amounts of diffusive and capillary movement in this fungus. A significant amount of such movement occurs when *S. luteus* is cultured in an aqueous sand medium (System D). In the absence of a lanolin barrier this activity becomes freely distributed in the nutrient medium away from the region of mycelial colonization. Very low levels of activity are detected in this position when a lanolin barrier is employed. It is not possible to determine from these experiments whether this activity arises as a result of leakage through the barrier or whether it is leaked out of the hyphae themselves, after translocation through the lanolin.

Comparisons of movement through the endophyte at 0.5 and 1.0 % agar concentrations show that passive movement is considerably restricted at the higher agar concentration. The relative proportion of translocated material is approximately doubled in the higher concentration.

**Movement of \(^{14}\text{C}\) labelled material**

The results are presented in Table 4. In the absence of a lanolin barrier movement of \(^{14}\text{C}\) could be detected even when the isotope was added in the presence of 2,4-DNP, or sodium azide. Both these substances are known to be powerful inhibitors of amino acid uptake in fungi. Moreover labelled material still moved through mycelium which had been killed by an 8 h exposure to propylene oxide (100 ppm). Such treatments, however, prevented movement of \(^{14}\text{C}\) for up to 192 h if a lanolin barrier was used. After this period a slight activity could be detected in sample disks. The general
Diffusion and translocation D. J. Read and D. P. Stribley 387

pattern of the results is comparable with that obtained with $^{32}$P but in the 0.5 % agar a higher proportion of the label (32–34 %) moves by translocation when glutamine is applied than when the tracer is $^{32}$P (16–19 %).

DISCUSSION

The results demonstrate that great care must be taken in the interpretation of data obtained in fungal culture systems employing diffusion barriers. It is plain that, with some fungi at least, simple glass or plastic walls present no obstacle to nutrient diffusion along the hyphae. This is particularly true in more aqueous systems. Observations using a fungus and systems similar to those of Melin & Nilsson (1950) indicate that a proportion at least of the nutrient movement obtained may have been due to diffusive and capillary movement. Such transport might help to account for the quite large amounts of radio-activity recorded in the bathing medium by these workers.

Of course, diffusive and capillary movement might be of importance in nature and so should not be disregarded, but it must be stressed that the amount of such transport in the semi-liquid conditions of agar culture and dense fungal growth might be quite unrepresentative of that to be expected in the natural environment.

The important distinction between the two types of movement is that whereas the capacity to translocate is likely to be a characteristic of the fungus in both laboratory and field situations, a significant proportion of the passive movement may be an artefact of the culture system. It is therefore necessary to discriminate between the two modes of nutrient movement before possible ecological inferences are drawn.

In the case of ericaceous mycorrhizas, the hyphal systems developed on agar are very different from those found in the field. On agar the fungus characteristically produces fascicles of hyphae which though undifferentiated and consisting of only 10–20 parallel hyphae are reminiscent of rhizomorphs. These structures which might be expected to provide particularly effective channels for capillary movement are not seen in the field where the extra-matrical mycelium of the endophyte is normally diffuse.

It thus seems likely that while a limited amount of passive movement might occur when soil around the root is saturated, at other times the only significant means of nutrient transfer must be translocation. Studies of vesicular arbuscular mycorrhizas in the field suggest that a similar situation must apply. In these endo-mycorrhizas, therefore, the systems likely to be most representative of the field condition is one which eliminates the passive movement. Such systems have been employed in recent studies of ericaceous mycorrhizas (Pearson & Read, 1973 b; Stribley & Read, in press), and are now being used in studies on vesicular arbuscular mycorrhizae (Pearson, personal communication).

In sheathing mycorrhizas the situation is probably more complex since elaborate fascicled and rhizomorphic structures often link the soil with the hyphal mantle around the roots. Capillary movement through such structures may occur and the relative importance of such transport of
nutrients compared to translocation will probably depend on conditions
around the root at any time.

Both the endo- and ectomycorrhizal fungi examined in these experiments
are clearly capable of translocating nutrients in the direction of a nutrient
sink. While some of the characteristics of the culture systems employed
here, for example, the dense growth of fungal mycelium, might exaggerate
the apparent efficiency of translocation, others, in particular the absence
of a sink in the form of a host plant, might decrease it.

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