DINITROGEN FIXATION ASSOCIATED WITH SPOROPHORES OF FOMITOPSIS PINICOLA, FOMES FOMENTARIUS, AND ECHINODONTIUM TINCTORIUM

M. J. LARSEN

U. S. Department of Agriculture, Forest Service, Center for Forest Mycology Research, Forest Products Laboratory, Madison, Wisconsin 53705

M. F. JURGENSEN

Department of Forestry, Michigan Technological University, Houghton, Michigan 49931

A. E. HARVEY

U. S. Department of Agriculture, Forest Service, Forestry Sciences Laboratory, Missoula, Montana 59801

AND

J. C. WARD

U. S. Department of Agriculture, Forest Service, Improvements in Drying Technology, Forest Products Laboratory, Madison, Wisconsin 53705

SUMMARY

Fixation of atmospheric dinitrogen by bacteria associated with contextual tissues of sporophores of Fomitopsis pinicola, Fomes fomentarius, and Echinodontium tinctorium is reported. Since nitrification rates are low, the data indirectly support the view that an autolysis-recycling mechanism of nitrogen from large volumes of woody tissue is the principal means by which wood-destroying fungi obtain nitrogen for sporophore production and sporulation.

In a series of papers on nitrogen requirements of fungi associated with wood decay summarized by Cowling (1970), the nitrogen-deficient

1 Maintained in cooperation with the University of Wisconsin, Madison, Wisconsin.

2 Maintained in cooperation with the University of Montana, Missoula, Montana.
nature of woody materials for fungal growth was emphasized. Merrill and Cowling (1966) and Cowling (1970) indicated that wood-destroying fungi can apparently recycle nitrogen from older fungal tissues and wood (also see Levi et al., 1968) and use it elsewhere. Evidence has been presented (Seidler et al., 1972; Cornaby and Waide, 1973; Sharp and Millbank, 1973; Aho et al., 1974a, b) for the presence or activity of dinitrogen-fixing bacteria associated with decaying wood, indicating that wood-decay fungi may rely, in part, on an extramural source of nitrogen. Herein, we present data that suggest nitrogen requirements of fungal sporophores are apparently met, in small part, by the activity of a nitrogenase function in sporophores of *Fomitopsis pinicola* (Swartz ex—Fr.) Karst., *Echinodontium tinctorium* (Ell. & Ev.) Ell. & Ev., and *Fomes fomentarius* (L. ex Fr.) Kickx.

**METHODS**

Live sporophores of *Fomitopsis pinicola*, *Echinodontium tinctorium*, and *Fomes fomentarius* were obtained from several forest stands on the Coram Experimental Forest in northwest Montana. Each *F. pinicola* sporophore was collected from individual dead and fallen boles of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and of subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.). *Fomes fomentarius* sporophores were collected from three standing boles of dead paper birch (*Betula papyrifera* Marsh.). Three standing live western hemlock trees were selected on the basis of occurrence of *E. tinctorium* sporophores, which were collected at each of three heights (0–3 m, 6–9 m, and 12–15 m) from each of the three stems.

Three conks of each fungal species on each host were sectioned into small pieces (ca. 0.5 cm³) and placed into 20-ml glass Vacutainer (Becton-Dickinson Div., Becton-Dickinson) tubes. Five replicate-tube samples of each conk were prepared. The outer surfaces of the conks were removed to eliminate surface contamination of N₂-fixing organisms.

Nitrogen-fixation rates in sporophores were estimated using an adaptation of the acetylene-reduction technique described and discussed by Hardy et al. (1968). Within 2 h of sampling, the tubes were evacuated five times with the aid of syringe needles, flushed with argon five times, then filled with a gas mixture of 10% acetylene and 90%
argon. Final oxygen concentrations in the tubes approximated 0.05%. Acetylene was omitted from one tubed sample of a sporophore of each species for monitoring endogenous ethylene production; these values were subtracted from those obtained from samples incubated with acetylene.

The sample tubes containing sporophore tissues were incubated in the dark for 24 h at ambient field temperature (12–15°C). Gas subsamples were removed by 2-ml Vacutainer tubes and analyzed for acetylene and ethylene levels using a Varian Series 2800 gas chromatograph fitted with an 80–100 mesh Poropak R column. The column was run at 50°C using nitrogen as the carrier gas. After gas sampling, the sporophore samples were dried at 105°C to determine dry weight and moisture contents.

Tissue isolates were made from all sporophores before the fungus was cut into pieces for the acetylene-reduction test. Pieces of context tissue were placed on malt agar (2% w/v) and incubated at 18°C. Fungal growth typical of *F. pinicola*, *F. fomentarius*, and *E. tinctorium* indicated the presence of viable and active hyphae within the sporophores tested.

**RESULTS AND DISCUSSION**

Our data (Tables I and II) demonstrate a nitrogenase function in fungal sporophores by the acetylene-reduction technique. No significant differences were detected between *Echinodontium tinctorium* sporophores (Table II) horizontally or vertically. Differences (P = 0.001) were significant between sporophores of *F. pinicola* on hemlock and all other fungus-substrate associations. However, these differences were not reflected by moisture contents of the various species because *F. fomentarius* and *E. tinctorium* contained significantly less moisture (P = 0.001 and P = 0.01, respectively) than *F. pinicola* on Douglas-fir, subalpine fir, and hemlock. Moisture contents of sporophores were not highly correlated with increased dinitrogen fixation.

The fixation rates reported here are similar to those reported for "soil" from Georgia by Cornaby and Waide (1973) as 1.18 × 10⁻⁹ mol/g/24 h and by Larsen et al. (1978) as 7 × 10⁻⁹ mol/g/24 h from a variety of woody substrates in various stages of decay in western Montana.

Whether or not the amounts of dinitrogen fixed actively contribute to the nitrogen regimes of actively sporulating sporophores of *F. pinicola*...
Table I

Mean number moles (× 10⁻⁹) per g oven-dry weight of fungal tissue of net ethylene produced in 24 h in sporophores of *Fomitopsis pinicola* on Douglas-fir, western hemlock, and subalpine fir, and *Fomes fomentarius* on paper birch

<table>
<thead>
<tr>
<th>Fungus and associated tree species</th>
<th>Percent moisture content (g/g)</th>
<th>Moles produced in 24 h (× 10⁻⁹ mol/g odw)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fomitopsis pinicola</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Douglas-fir</td>
<td>166.6 ± 25.4</td>
<td>0.98 ± 0.40</td>
</tr>
<tr>
<td>Subalpine fir</td>
<td>162.15 ± 12.13</td>
<td>0.75 ± 0.20</td>
</tr>
<tr>
<td>Western hemlock</td>
<td>176.3 ± 19.9</td>
<td>2.20 ± 0.30</td>
</tr>
<tr>
<td><em>Fomes fomentarius</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White birch</td>
<td>111.4 ± 95.8</td>
<td>0.98 ± 0.12</td>
</tr>
</tbody>
</table>

cola, *F. fomentarius*, and *E. tinctorium* is a question that awaits resolution.

If we assume from our work an average rate of net ethylene production for sporophores as 1.12 × 10⁻⁹ mol/g odw (oven-dry weight) fungal tissue/24 h, then for 200 da at that level of fixation in a 300-g sporophore (assumed average weight) 0.6 × 10⁻³ g of N would be fixed annually. Upon reviewing the figures of Merrill and Cowling (1966) on the regimes and the amounts of nitrogen they reported for fungi, the amount of nitrogen fixed in our experiments appears to represent only a small fraction of the nitrogen requirements for sporophore formation and sporulation. Merrill and Cowling (1966) reported that 15.6 g of N would be required for 100 da of sporulation by *Ganoderma applanatum* (Pers. ex Wallr.) Pat., and if sporulation occurred for as long as White (1920) observed (6 mo), 28 g of N would be required annually. The data of Meyer (1936), as interpreted by Merrill and Cowling (1966), are even more impressive because she reported the astounding figure of more than

Table II

Mean number moles (× 10⁻⁹) per g oven-dry weight of fungal tissue of net ethylene produced in 24 h in sporophores of *Echinodontium tinctorium* on western hemlock

<table>
<thead>
<tr>
<th>Boles position of sporophore (m above ground level)</th>
<th>Percent moisture content (g/g)</th>
<th>Moles produced (× 10⁻⁹ mol/g odw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–3</td>
<td>97.9 ± 34.4</td>
<td>1.07 ± 0.14</td>
</tr>
<tr>
<td>6–9</td>
<td>131.6 ± 20.7</td>
<td>1.0 ± 0.09</td>
</tr>
<tr>
<td>12–15</td>
<td>132.7 ± 35.9</td>
<td>0.98 ± 0.13</td>
</tr>
<tr>
<td>Total, all sporophores</td>
<td>120.75 ± 34.33</td>
<td>1.01 ± 0.12</td>
</tr>
</tbody>
</table>
1 kg of spores produced in a 20-da period by *F. fomentarius*, requiring 35 g of N (assuming N content of spores to be 3%).

We may conclude that the autolysis-recycling mechanism(s) of nitrogen from large volumes of woody materials proposed by Merrill and Cowling (1966) is the primary means by which wood-decay fungi are provided with the necessary amounts of nitrogen for sporophore formation and subsequent sporulation.

Although we have not demonstrated the physical presence of N₂-fixing bacteria in sporophore tissue, we may reasonably assume the fixation phenomenon is not caused by the fungus (Millbank, 1968), but instead by an intimately associated bacterial population. Preliminary results from isolations of sporophores and examination by scanning-electron microscopy of contextual tissues indicate that a bacterial population does exist. Our inability to date to cultivate the organism(s) indicates that it is extremely fastidious in its requirements for axenic culture.

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LITERATURE CITED


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