INTERNAL REPORT 15

BASIDIOMYCETES IN THE PRIMARY DECOMPOSITION OF FOREST FLOOR LITTER

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INTRODUCTION

Decomposition is known to be a vital step in the cycling of energy and nutrients within a coniferous forest ecosystem. Little is known, however, about the specific organisms that play major roles in the primary processes of decomposition.

Fungi of the basidiomycete class have been closely associated with decaying forests. Members of this group of fungi especially have been found to degrade cellulose and lignin, the primary components of trees. Little information, outside of observations on occurrence, has specifically been reported on basidiomycetes functioning as primary decomposers within a forest ecosystem.

Waksman (1916, 1917, 1944) conducted studies on the fungi present in soil. Warcup (1950, 1951) described several basidiomycetous fungi isolated from a grassland soil. Warcup and Talbot (1962, 1963) continued work on soil-inhabiting basidiomycetes.

Other workers have not been as successful as Warcup in demonstrating basidiomycetes in soil. Hodges (1962) reported isolating no basidiomycetes from southern forest tree nursery soils. Brandsberg (1966) recently published a thesis on the fungi associated with the decomposition of coniferous litter. He reported no basidiomycetes among the fungi identified. He did isolate and identify an Oedocephalum sp., which has been reported by Batshi (1952) as being a conidial stage of Fomes annosus. Members of the genus Oedocephalum, however, are also commonly found as the conidial stages of other basidiomycetes and discomycetes.

Several studies have been conducted on methods of inducing basidiomycetes to fruit in culture. Tamblyn and DeCosta (1958) developed a technique for producing fruiting bodies of wood-decaying basidiomycetes. Warcup and Talbot (1962) also described methods, one of which is a variation of Tamblyn's and DeCosta's technique. Another somewhat more complicated technique is described by Parkinson and Williams (1961). The technique is to remove the spores of the heavily sporulating fungal species from the soil by washing and then plating out the remaining soil. By this method, they have obtained basidiomycetes in culture.

These reports serve to illustrate the paucity of good methods to isolate and identify basidiomycetes as significant forest decomposers. Our study was undertaken to determine the place of basidiomycetous fungi as primary decomposers within a coniferous forest ecosystem.

MATERIALS AND METHODS

A significant portion of the initial biomass on the coniferous forest floor is needles. We determined, therefore, to study the primary decomposition of Douglas-fir needles.

The needles were collected in three ways. The first method was to catch the needles in traps placed above the forest floor. The traps consist of wooden frames 18 inches square and 2 inches deep. Screen wire served as the bottom of the frame. The traps were attached to a tower at random intervals, starting just below the crown of the trees and ending at ground level. Needles were collected aseptically from the traps, placed in sterile containers and transported to the laboratory on the day of collection.

A second technique consisted or removing needles from the traps and placing thein small, sterile nylon mesh bags. The bags were placed on the forest floor and collected for isolation trials at 30-day intervals for a period of one year.

The third technique consisted of simply collecting needles from the forest floor, transporting them immediately to the laboratory, and culturing them.

Basidiomycete fructifications were collected whenever observed and identified.

In an effort to obtain growth of basidiomycetes, we tested several methods of culture. The simplest method was to place the needles on 2-percent malt agar. Another technique was to place the needles on moist, sterile filter paper in sterile petri dishes that were incubated in a moisture chamber. A third procedure was to place one gram of needles in sterile, distilled water, allow it to start for 10 minutes, shake, and make a serial dilution (1:10, 1:100, 1:1,000). Each dilution was plated on 2- percent Difco malt extract agar. Three replications for each dilution were made.

In an effort to induce fruiting in cultures that remained sterile on 2-percent malt extract agar, we sterilized the needles with propylene oxide in petri dishes, inoculated with a sterile culture, and placed them in a moisture chamber.

Two special media were tried for culturing basidiomycetes. The first contained 1 ml of a 1:30,000 dilution of Rose Bengal in 1,000 ml of 3-percent diastate malt agar. The second was identical to the first but contained, in addition, 0.2 ml of a 250 mg/cc dilution of streptomycin.

To determine whether temperature affected fungi isolated, needles were placed on 2-percent malt agar in petri dishes and then placed in incubators. Two temperature ranges were tested--10 to 15°C and 20 to 30°C. Cultures were also made at room temperature.

Cultures obtained with the above methods were examined microscopically to determine the class of fungi--particularly to determine whether clamp connections were present or absent.

To determine what constituents of the needles were utilized in the decomposition processes, we used the following procedures. The needles were ovendried for 24 hours at 105°C, and a weighed amount of needles was placed in a Soxhlet extractor and extracted with n-hexane for 5 hours. Needles were then removed, dried for 24 hours at 105°C, and weighed to determine weight loss. This

procedure was carried out on needles that were exposed to the following treatments: (1) exposed on the forest floor for 6 months, (2) exposed for 12 months, and (3) stored under laboratory conditions after air-drying (control).

A flow chart of the handling of the needles is shown in Figure 1.

RESULTS

Basidiomycete fructifications collected at the research site were Cortina sp., Amanita sp., Armillaria sp., Polyporus abietinus, Fomes annosus, Poria spp., Fomes pinicola, Coprinus spp., and Cantharellus cibarius.

Fungal organisms isolated from partially decomposed needles were phycomycetes and Fungi Imperfecti, and organisms isolated from undecomposed needles were primarily yeasts.

Organisms that remained sterile in culture and had clamp connections will be put through special cultural techniques to induce fruiting.

DISCUSSION

The results indicate that basidiomycetous fungi either do not play an active role in primary decomposition of needle litter or, if they do, they will not grow in the artificial cultures tested.

The chemical analyses (Figure 2) of undecomposed and decomposed needles indicate that the associated organisms either utilize the oils or simply change their structure to more soluble forms, which makes them available to other microorganisms in the decomposer chain.

Figure 1 shows that the greatest loss of weight from needles was after 6 months of decomposition. Possibly the decay organisms only attack the cuticle and, after 6 months, the cuticle was reduced to the point where extraction of the oils was much easier.

Work is continuing on associating the organisms isolated with specific functions of the decomposition processes. The methods developed and modified thus far will be employed in studies underway on the decomposition of the wood component of the Douglas-fir ecosystem.

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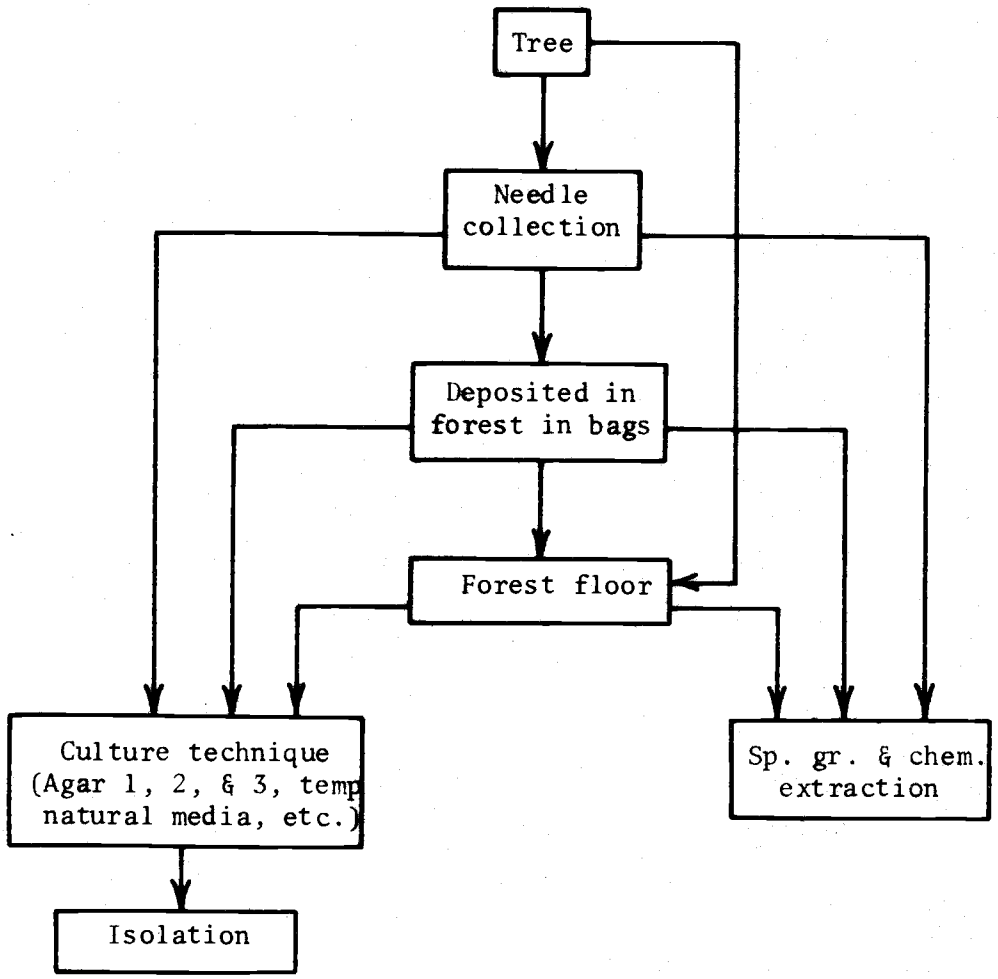

Figure 1. Flow chart of the handling of Douglas-fir needles.

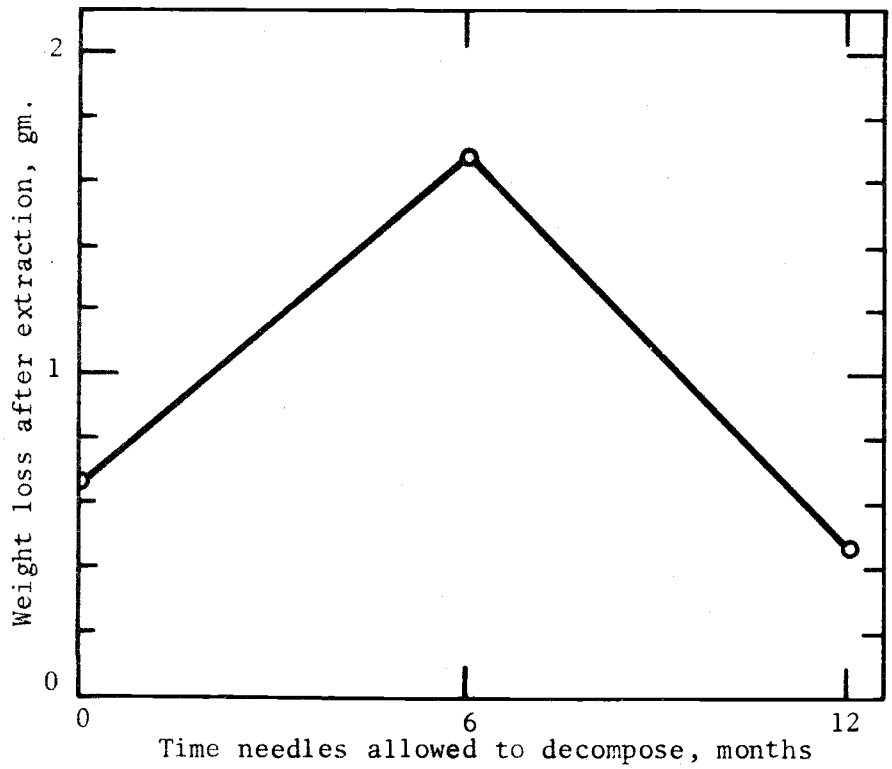

Figure 2. Results of extraction of oils from needles. Weight of all samples before extraction was 8.000 gm.