Basidiospores of *Armillaria mellea* Survive an Alaskan Winter on Tree Bark

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ABSTRACT

Shaw, C. G., III. 1981. Basidiospores of *Armillaria mellea* survive an Alaskan winter on tree bark. Plant Disease 65:972-974.

Sterile distilled water was applied to the outer bark of 28 western hemlock (*Tsuga heterophylla*) and 26 Sitka spruce (*Picea sitchensis*) trees growing at three different locations in southeast Alaska. Runoff was incubated for up to 5 wk on Kuhlman's medium. Colonies of *Fomes annosus* developed from runoff collected from 2 hemlock and 1 spruce, and colonies of *Armillaria mellea* from 12 hemlock and 8 spruce. Most *A. mellea* cultures had morphological characteristics commonly associated with single spore isolates. Because collections were made in March and April, 3–4 mo after sporophore production by *A. mellea*, basidiospores of *A. mellea* apparently survived the winter on the outer bark of sampled trees.

Armillaria mellea (Vahl. ex Fr.) Quél. is a serious root pathogen of forest and orchard trees that spreads mainly by vegetative means. Rishbeth (11) demonstrated infection of coniferous stumps by basidiospores of *A. mellea*, and Hintikka (3) suspected basidiospores to be the inoculum source in some coniferous infections. Kile (4) and Rishbeth (12) considered basidiospores to be the inoculum source for certain infections in hardwoods. However, these authors and other observers generally conclude that although infection occurs from basidiospores, it is rather uncommon.

Determining frequencies of infection in the forest by *A. mellea* basidiospores is complicated by the prevalence of vegetative mycelia and their rapid invasion of stumps. As Rishbeth (12) noted, trapping of airborne basidiospores of *A. mellea* has been insufficient to determine their adequacy as inoculum. Evidence from limited spore trappings (7,10,14) and reports of sometimes prolific sporophore production, however, suggest that spores are sometimes readily available for colonizing stumps (11).

In this study, I collected washings from the outer bark of live trees and used trappings to detect possible airborne basidiospores of *A. mellea* several months after fruiting of *A. mellea* in southeast Alaska.

MATERIALS AND METHODS

This study began as an extension of work to detect inoculum of *Fomes annosus* (Fr.) Cke. in southeast Alaska (13). For that reason, a medium developed specifically for germination of *F. annosus* spores was used (6). All bark washings and airborne samples were incubated in 90-mm plastic petri dishes containing 30 ml of this freshly prepared medium.

Bark washings were obtained by placing flanges around living trees at about 25 cm above ground level, or the height of stumps left after precommercial thinning. The flange consisted of a radiator hose clamp pop-riveted to the outside of a 5-cm-wide band of galvanized sheet metal (Fig. 1). The upper half of the band was cut into fingers, with the end farthest from the tightening screw loose from the clamp. The interior face of the sheet metal band was covered with duct tape. This configuration allowed the unit to fit uniformly around the tree stem when tightened. Bands of several different lengths were constructed. Stem diameter at the point of attachment, which varied from 7 to 15 cm, determined the size used on each tree.

A 2.5-cm-wide band of inner tube rubber was fitted firmly to the tree and the taped clamp tightened around it. This created a dam that held applied water long enough to allow collection.

With the flange in place, about 10 ml of sterile distilled water was squirted onto the bark around the stem to a height of 10 cm above the flange. As water collected in the dam, it was removed with a medicine dropper and placed in sterile, screw-cap culture tubes, one tube per tree. Volume of water collected varied per tree, but it was usually at least 3 ml.

After each tree was sampled, the strips of inner tube rubber and taped clamp were sprayed with ethanol, wiped, and rinsed with sterile distilled water. The medicine droppers were sterilized before

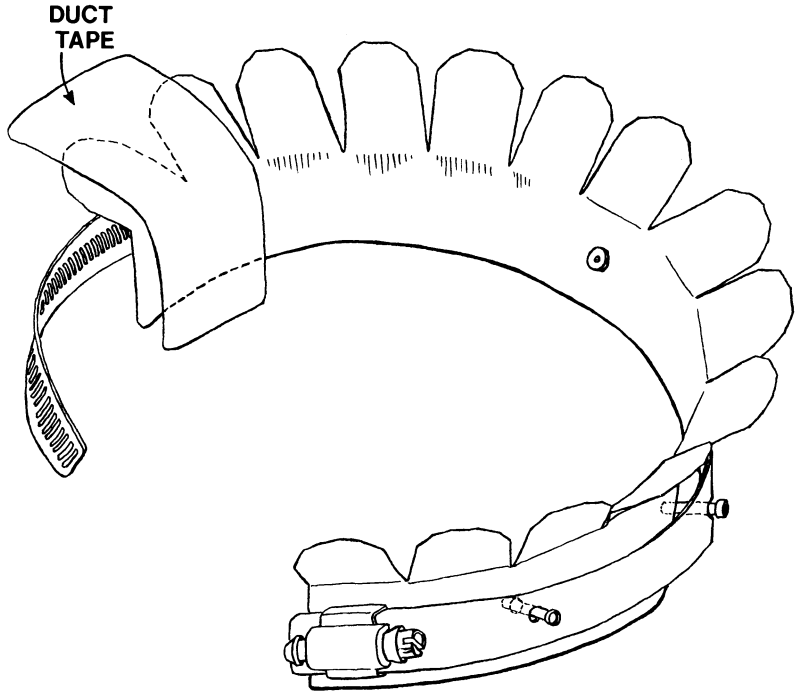

Fig. 1. Flange used to gather washings from the outer bark of living trees. Strips of duct tape were placed as shown over the entire interior face of the flange. When tightened firmly on a tree stem, the flange held applied water long enough to allow collection with a medicine dropper.

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use and thoroughly rinsed between uses with sterile distilled water.

The young-growth stands described previously (13) at Petersburg, Juneau, and Hollis were used as study sites. In March 1980, washings were collected at Petersburg from the outer bark of 10 young-growth Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and 10 young-growth western hemlock (*Tsuga heterophylla* (Raf.) Sarg.). In April 1980, washings were collected from an additional 5 trees of each species at Petersburg, from 10 trees of each species at Hollis, and from 5 Sitka spruce and 6 western hemlock at Juneau. During each collection of washings, 10 petri plates of Kuhlman's medium were exposed to the air for 8 min, which was about the time required to collect washings from one tree. These plates served as traps to detect possible airborne spores of *A. mellea, F. annosus,* or both.

Washings from each tree were poured without dilution onto petri plates of Kuhlman's medium, using three or four plates per tree (six to eight plates were used for the second sampling at Petersburg). Washings were spread across the agar surface with a sterilized inoculation loop.

All petri plates, including those directly exposed to the air, were incubated at room temperature (20–25 C) for 3–5 wk and periodically examined for colonies of *A. mellea* or *F. annosus*. When found, colonies were subcultured onto malt agar medium. A tree was considered to have had spores of *A. mellea* or *F. annosus* on its surface if one or more fungal colonies developed. Petri plates containing washings from four spruce and three hemlock trees from the first sampling at Petersburg were inadvertently destroyed during incubation; thus, full data were available from only six spruce and seven hemlock trees.

In October 1980, spore prints were obtained from several *A. mellea* sporophores freshly collected around Juneau. Spore suspensions were made by washing each spore print with sterile distilled water. From each suspension, a dilution series was plated onto Kuhlman's medium. After germination, single spore germlings were transferred to Kuhlman's medium and incubated at room temperature for 3 wk.

RESULTS

Colonies of *A. mellea* developed from washings collected from several trees of both species at Petersburg, from two hemlock at Juneau, and from one spruce at Hollis (Table 1). When *A. mellea* was present on a petri plate, the number of individual colonies varied from one to five. Initial colony development consisted of fluffy white, aerial mycelia frequently accompanied by light-colored rhizomorphs (Fig. 2). Twenty-nine separate isolates were maintained for several weeks on malt agar medium. Twenty of these maintained fluffy white, aerial mycelia, while nine developed brown, crustose mycelia. No colonies of *A. mellea* occurred on petri plates directly exposed to air.

Colonies of *F. annosus* developed from washings collected from two hemlock and one spruce at Petersburg. The spruce and one hemlock had also yielded colonies of *A. mellea*. *F. annosus* occurred on three petri plates exposed directly to the air at Hollis but not at other locations. Colonies of *F. annosus* formed within the first 10 days of incubation, while most colonies of *A. mellea* took considerably longer to reach an identifiable stage.

Spores of *A. mellea* obtained directly from sporophores germinated readily on Kuhlman's medium. Transfers of single spore germlings developed light-colored rhizomorphs and predominately fluffy white, aerial mycelia in 2 to 3 wk, as had isolates obtained from bark washings.

DISCUSSION

In Alaska, sporophores of *A. mellea* are generally produced from July to October (1). In southeast Alaska, mycophagists have collected fresh sporophores of *A. mellea* in mid-November. During December through March, the forest floor in this region is generally frozen or snow covered. When washings were collected at Juneau and initially at Petersburg, patches of snow were still present. The snow had just melted at Hollis.

These events, coupled with the absence of *A. mellea* colonies on culture medium directly exposed to air, make it highly unlikely that sporophores had been present on any of the sample sites since early in the preceding December, at leasts 3 mo before sampling. Consequently, the basidiospores most likely survived the winter on the outer bark of sample trees.

Another explanation for the survival of basidiospores over the winter might be that the cultures originated from vegetative mycelia, but the known types and growing locations of vegetative

Table 1. *Armillaria mellea* in washings collected from the outer bark of young-growth Sitka spruce and western hemlock trees in southeast Alaska

Location[a]	Trees sampled/ trees with *A. mellea*[b] (no.)	
	Sitka spruce	Western hemlock
Petersburg-1	6/4	7/6
Petersburg-2	5/3	5/4
Juneau	5/0	6/2
Hollis	10/1	10/0
Total	26/8	28/12

[a] Petersburg-1 washings collected in March 1980, the rest in April 1980.

[b] One or more colonies of *A. mellea* developed from washings collected from tree.

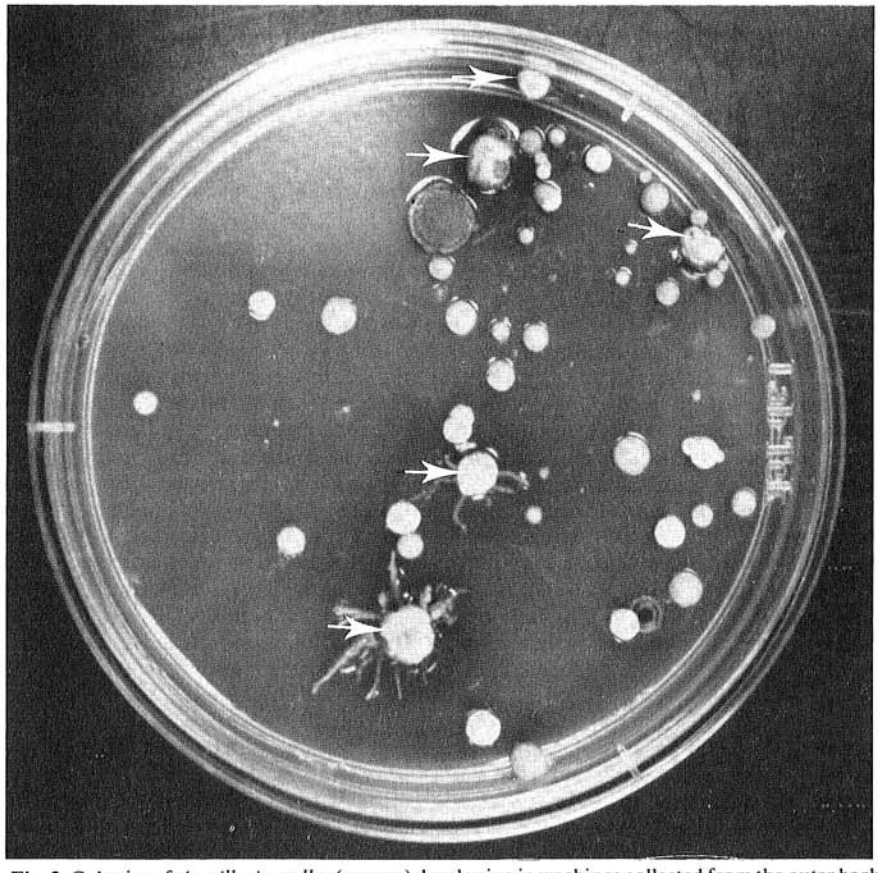

Fig. 2. Colonies of *Armillaria mellea* (arrows) developing in washings collected from the outer bark of a living tree after 3 wk of incubation on Kuhlman's medium. Other, unidentified fungal colonies are also present.

mycelia of *A. mellea* make this unlikely. Rhizomorphs are common in forest soil, and rhizomorphs and mycelial felts commonly develop within decaying wood and within the cambial region of freshly killed trees; however, neither is known to occur 25 cm above the ground on the outer bark of living trees.

The fluffy white, aerial mycelia that developed in most of these cultures are typical of single spore isolates of *A. mellea* (2,15). This characteristic, however, may not always be a reliable indicator of single spore origin. Raabe (9) found considerable variation in cultural appearance of single spore isolates of *A. mellea*. This variation included the development of brown, crustose, surface mycelium in some of his single spore isolates, a characteristic that some of these cultures developed with age and that has generally been associated with diploid isolates (2,15). Perhaps my cultures with brown, crustose mycelium developed from two or more spores germinating in close proximity and uniting.

The ability of *A. mellea* basidiospores to survive the rather harsh winter of southeast Alaska enables them to establish infections at times other than during active sporulation. Viable spore inoculum is present at stump height on trees that might be cut in spring or early summer, which is a common time for precommercial thinning in young stands of this region. This spore survival and direct access to freshly cut stump surfaces could increase the probability of infection from spores and the subsequent development of new fungal clones (5,15), regardless of how rare the individual event.

Rishbeth (12), discussing the occurrence of *A. mellea* on first rotation hardwoods in Great Britain, stated, "Since the thinning did not necessarily coincide with the period of [spore] discharge, the presence of [disease] foci in four of the six plantations was unexpected." Rishbeth (12) concluded that it was highly unlikely that these foci arose from vegetative mycelia. The means of infection could have been basidiospores that survived on the bark at stump height, as indicated in this study.

Infection of freshly cut stump surfaces by spores of *F. annosus* previously deposited on tree bark and drawn across the stump face during tree cutting has been suggested as the mechanism for stump infection during periods of tree cutting when levels of airborne inoculum are low (8,13). My data confirmed the presence of *F. annosus* spores on the outer bark of young trees and showed that spores of both *A. mellea* and *F. annosus* may occur on the same tree.

This study also showed that Kuhlman's medium (6), developed specifically for germination of *F. annosus* spores, was suitable for germination of *A. mellea* spores obtained directly from sporophores or washed from tree bark. Incubating samples should not be discarded too early, because colonies of *A. mellea* may take 3 or more weeks to develop.

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