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Surface Morphology of Basidiospores from Decay Fungi That Are Common in Pacific Northwest Forests

Abstract

Hymenomycetes are a worldwide group of fungi, several species of which cause serious decay in the roots and stems of conifers. New infections by these fungi are often established by airborne spores; however, species identification based solely on these spores has proven difficult, especially when only light microscopy is used. We thus employed scanning electron microscopy (SEM) to characterize spore types. Basidiospores and conidia of *Heterobasidion annosum* were compared with basidiospores from nine other species of wood decay fungi: *Fomitopsis cajanderi*, *F. pinicola*, *Phellinus robustus*, *Ganoderma applanatum*, *G. oregonense*, *Phaeolus schweinitzii*, *Trichaptum abietinum*, *Bjerkandera adusta*, and *Trametes versicolor*. Light microscopy demonstrated differences in spore size but did not consistently resolve variations in shape and ectosporal structure. SEM provided a more reliable means for distinguishing among basidiospores and identifying species. Structure beyond the resolution of light microscopy was resolved with the SEM, which should improve the accuracy of taxonomic descriptions. Comparisons among all spore types demonstrated distinct differences that aid in identification of spores collected in airborne samples—including types of inocula for *H. annosum*, the cause of a major root disease in Pacific Northwest forests. The latter information should help to clarify the role of asexual conidia in development of annosus root disease.

Introduction

Hymenomycetes are common decay organisms in forests worldwide. One of these fungi, *Heterobasidion annosum* (Fr.) Bref. [*Fomes annosus* (Fr.) Karst.] causes serious root and butt rot in many species of conifers (Bega 1963, Gilbertson and Ryvarden 1986), including several in the Pacific Northwest (Hadfield *et al.* 1986).

Previous studies on establishment of new infections by *H. annosum* have dealt with airborne inocula in a collective sense because this fungus produces conidia and basidiospores that appear similar when viewed with light microscopy (LM). Using techniques of scanning electron microscopy (SEM), however, Shaw and Florance (1979) were able to distinguish between these conidia and basidiospores. This technique allows for the identification of specific spore types in samples of airborne inocula, an area of research for which the critical need for study has been noted for some time (Bega 1963, Kallio 1970, Lowe 1957, Morris and Knox 1962, Roll-Hansen 1940, Stambaugh *et al.* 1962), but not adequately addressed (Florance *et al.* 1981, Leslie 1983, Shaw and Florance 1979).

Before spores in samples of airborne inocula can be accurately identified, however, the

characteristics of other spores that might appear in the samples require elucidation. The objective of this study was to characterize the features of ectosporial wall morphology visible with LM and SEM for decay fungi common to forests in the Pacific Northwest where *H. annosum* causes disease (Russell and others 1973). The spore features were then compared to those of *H. annosum* (Roll-Hansen 1980, Shaw and Florance 1979).

Materials and Methods

Fomitopsis pinicola (Swartz: Fr.) Karst. [*Fomes pinicola* (Swartz ex Fries) Cooke], *Phellinus robustus* (Karst.) Bourd. & Galz. [*Fomes robustus* Karst.], *Fomitopsis cajanderi* Karst. [*Fomes cajanderi* Karst.], *Ganoderma oregonense* (Murr.) Kauffman, *G. applanatum* (Pers. ex Wallr.) Pat., *Phaeolus schweinitzii* Fries, *Trichaptum abietinum* (Dicks.: Fr.) Ryv. [*Polyporus abietinus* Dicks ex Fries], *Bjerkandera adusta* (Willd.: Fr.) Karst. [*Polyporus adustus* Willd. ex Fries], and *Trametes versicolor* (L.: Fr.) Pilat. [*Polyporus versicolor* L. ex Fries] are common decay fungi in forests of the Pacific Northwest (Gilbertson and Ryvarden 1986, 1987, Partridge and Miller 1974). Fresh basidiocarps of these fungi were collected from forest sites in the Coast and Cascade

Ranges of Oregon in autumn. Samples for LM were macerated on a glass slide and mounted in a Lactophenol medium for viewing with a Nikon Microflex Model EFM Microscope. Spores were examined with an oil immersion objective (NA = 0.65) and photographed with Kodak Technical Pan 2415 (ASA = 125) film.

Spores for SEM were obtained from the same basidiocarps by removing pieces of the hymenophore about 0.5 cm² and fixing them with 4 percent glutaraldehyde, 6 percent paraformaldehyde, and 1 percent acrolein in a 0.2 molar cacodylate buffer. Subsequently, samples were rinsed twice in fresh buffer, transferred to Flor-thru tissue carrier vials (American Optical Co.) and dehydrated in an ascending series of four ethanol-distilled water solutions (25, 50, 75, and 100 percent), followed by an ascending series of four ethanol-trichlorotrifluoro-ethane (TF) solutions (25, 50, 75, and 100 percent TF). Spores and tissue remained in each solution, including fixative, for 10 minutes. After treatment in 100 percent TF, samples were dried by the critical point method (Cohen *et al.* 1968), mounted on SEM studs, coated with a platinum-palladium alloy, and viewed with an AMR 1200 Scanning Electron Microscope. Data were recorded on Polaroid Type PN55 (4 by 5 in, 10.1 by 12.7 cm) land film.

Results

Basidiospore Structure

With LM, basidiospores of *F. cajanderi* appeared hyaline, smooth, elongate-cylindrical, sometimes allantoid, and measured 2-2.5 by 5-7 μm (Fig. 1A). The SEM clearly showed an apiculus (= hilar appendix) not resolvable with LM (Fig. 2A) that protrudes 200 nanometers (nm) from the spore surface and is 500 nm in diameter. In scanning electron micrographs, spores appear smooth and primarily allantoid (Fig. 2A); however, when they are rotated away from the curve, they appear straight (Fig. 2A).

With LM, basidiospores of *F. pinicola* appeared ovoid to subglobose, hyaline, smooth, and measured 5-7 by 3-4 μm (Fig. 1B). Spores appeared similar to those seen with SEM, including the smooth surface (Fig. 2B); however, the eccentrically located and abruptly protruding apiculus is more distinct with SEM. The apiculus protrudes 315 nm from the spore surface and is 420 nm in diameter.

With LM, basidiospores of *Phellinus robustus* appeared globose, smooth, hyaline, apiculated, and measured 5-6 μm in diameter (Fig. 1C). With SEM, smooth biconcave or triconcave spores were revealed (Fig. 2C). The apiculus protrudes 400-500 nm from the spore surface and is about 200 nm in diameter. Concavities viewed with the SEM cannot be distinguished with the LM, although there was some indication of their presence.

With LM, spores of *G. applanatum* appeared ovoid, smooth to slightly echinulated, brown, and measured 6-9 by 5-6 μm with LM (Fig. 1D). Some spores also appeared truncated. With SEM, a foveate ectosporium was visible on each spore (Fig. 2D). A concavity was also apparent at the distal end on spores separated from the sterigmata (Fig. 2D).

With LM, basidiospores of *G. oregonense* appeared ovoid, truncated, possibly echinulated, brown, and had a thick outer wall (Fig. 1E). The spores are relatively large compared with the others and measured 10-14 by 7.5-9 μm . With SEM, the ectosporium appeared somewhat foveate—similar to that of *G. applanatum*; however, the depressions were not as deep. A feature resolved by SEM was small, irregularly sized pits or pores in the ectosporium (Fig. 2E). The largest pit measured 416 by 332 nm, just slightly above the resolving power of LM; however, most pits are smaller and would not be resolved with LM.

With LM, Basidiospores of *Phaeolus schweinitzii* appeared ellipsoid to ovoid, smooth, hyaline, apiculate, and measured 5.5-8 by 4-5 μm with LM (Fig. 3A). With SEM, these spores appear similar; however, more accurate measurements of the apiculus can be made with SEM (Fig. 4A). The apiculus protrudes about 480 nm from the spore surface and is about 720 nm in diameter.

Spores of *Trichaptum abietinum* appeared cylindrical to allantoid, hyaline, smooth, and measured 4-6 by 1.5-2.5 μm with LM (Figure 3B). With SEM, a very wrinkled ectosporium was visible (Fig. 4B) that was not resolvable with LM (Fig. 3B).

With LM, basidiospores of *B. adusta* appeared oblong to oblong-ellipsoid, smooth, hyaline, and measured 4-5 by 2-3 μm (Fig. 3C). These spores appear almost identical to those of

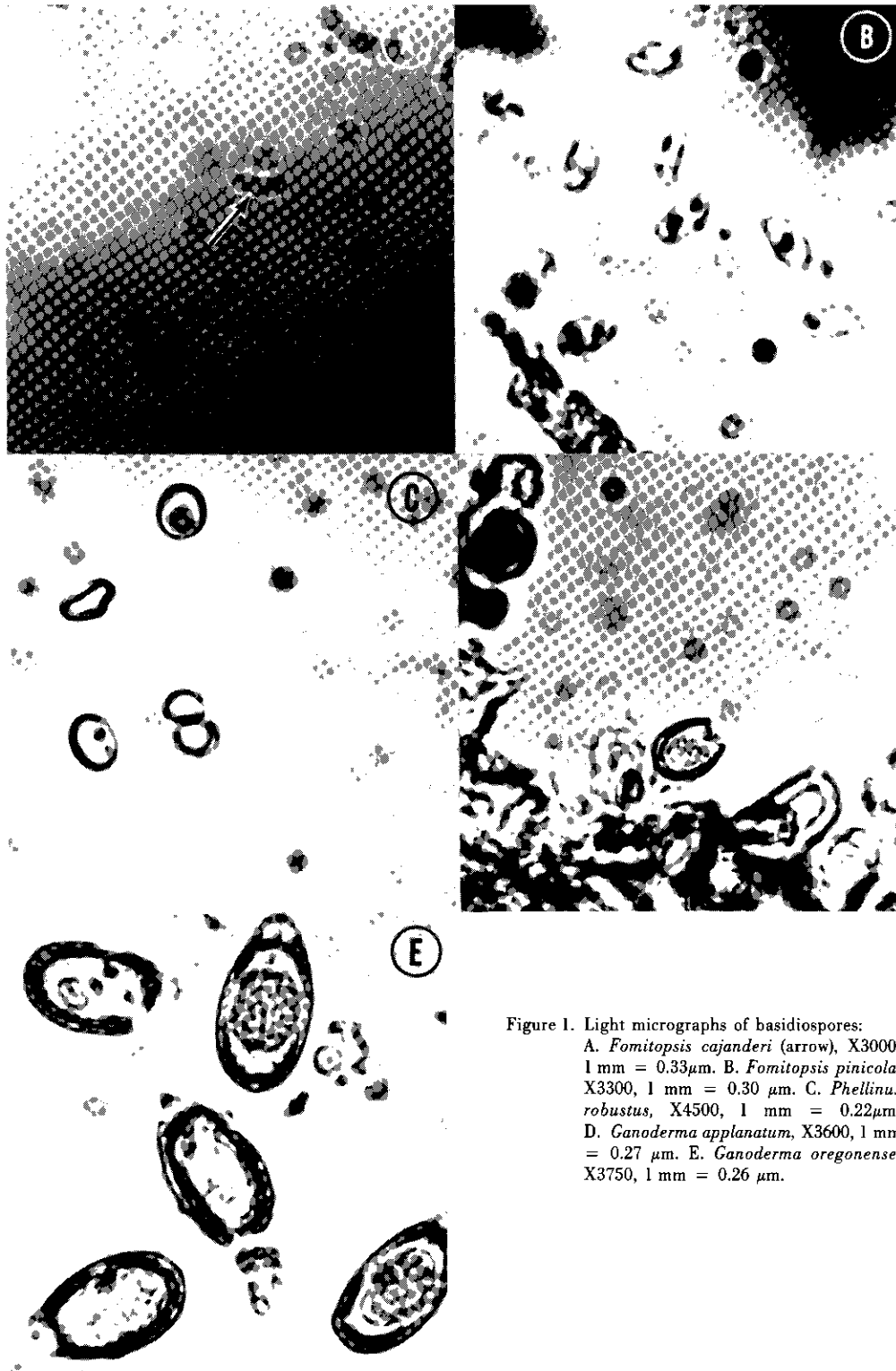


Figure 1. Light micrographs of basidiospores:
 A. *Fomitopsis cajanderi* (arrow), X3000, 1 mm = 0.33 μ m. B. *Fomitopsis pinicola*, X3300, 1 mm = 0.30 μ m. C. *Phellinus robustus*, X4500, 1 mm = 0.22 μ m. D. *Ganoderma applanatum*, X3600, 1 mm = 0.27 μ m. E. *Ganoderma oregonense*, X3750, 1 mm = 0.26 μ m.

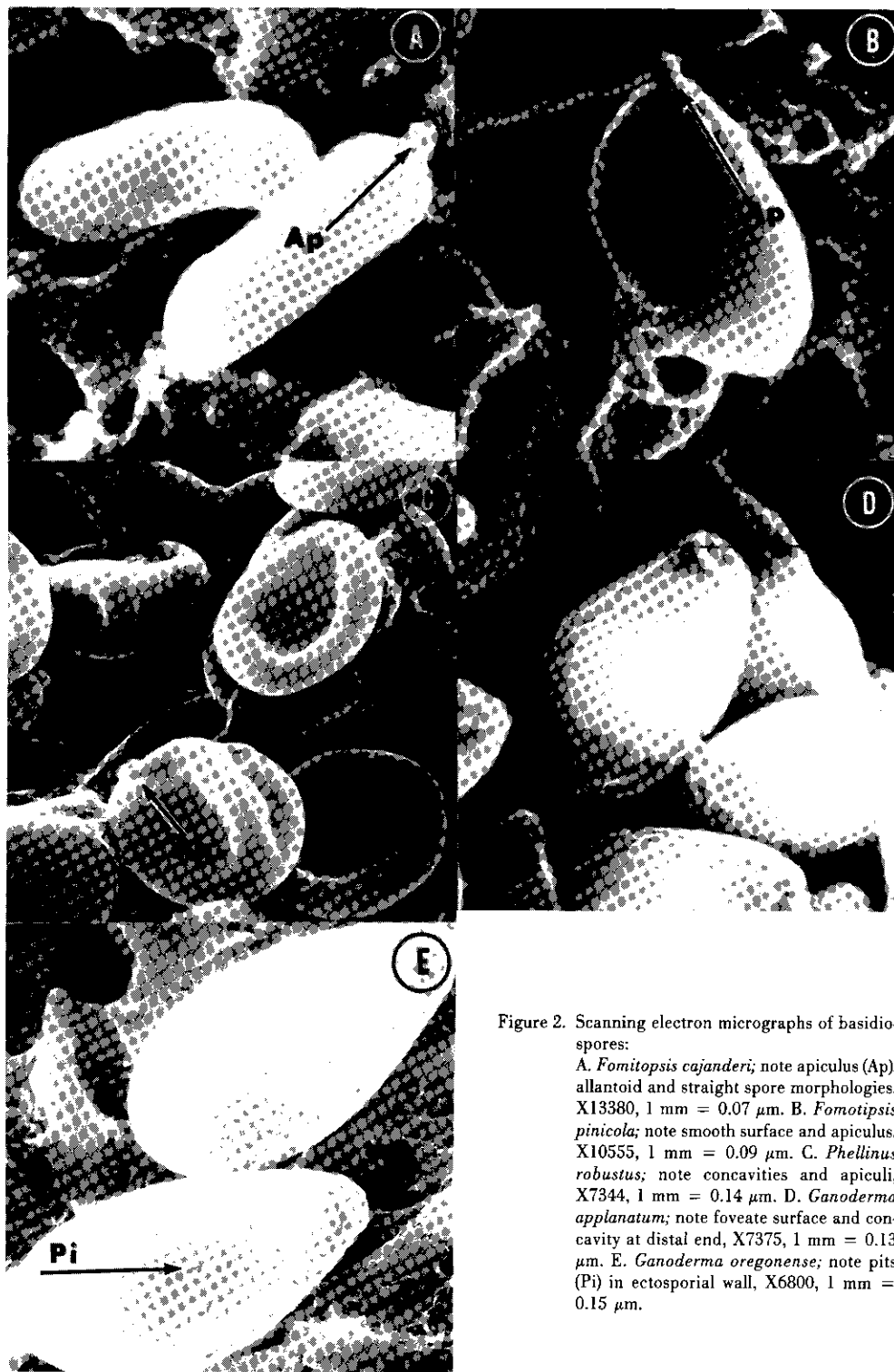


Figure 2. Scanning electron micrographs of basidiospores:

A. *Fomitopsis cajanderi*; note apiculus (Ap), allantoid and straight spore morphologies, X13380, 1 mm = 0.07 μ m. B. *Fomitopsis pinicola*; note smooth surface and apiculus, X10555, 1 mm = 0.09 μ m. C. *Phellinus robustus*; note concavities and apiculi, X7344, 1 mm = 0.14 μ m. D. *Ganoderma applanatum*; note foveate surface and concavity at distal end, X7375, 1 mm = 0.13 μ m. E. *Ganoderma oregonense*; note pits (Pi) in ectosporial wall, X6800, 1 mm = 0.15 μ m.

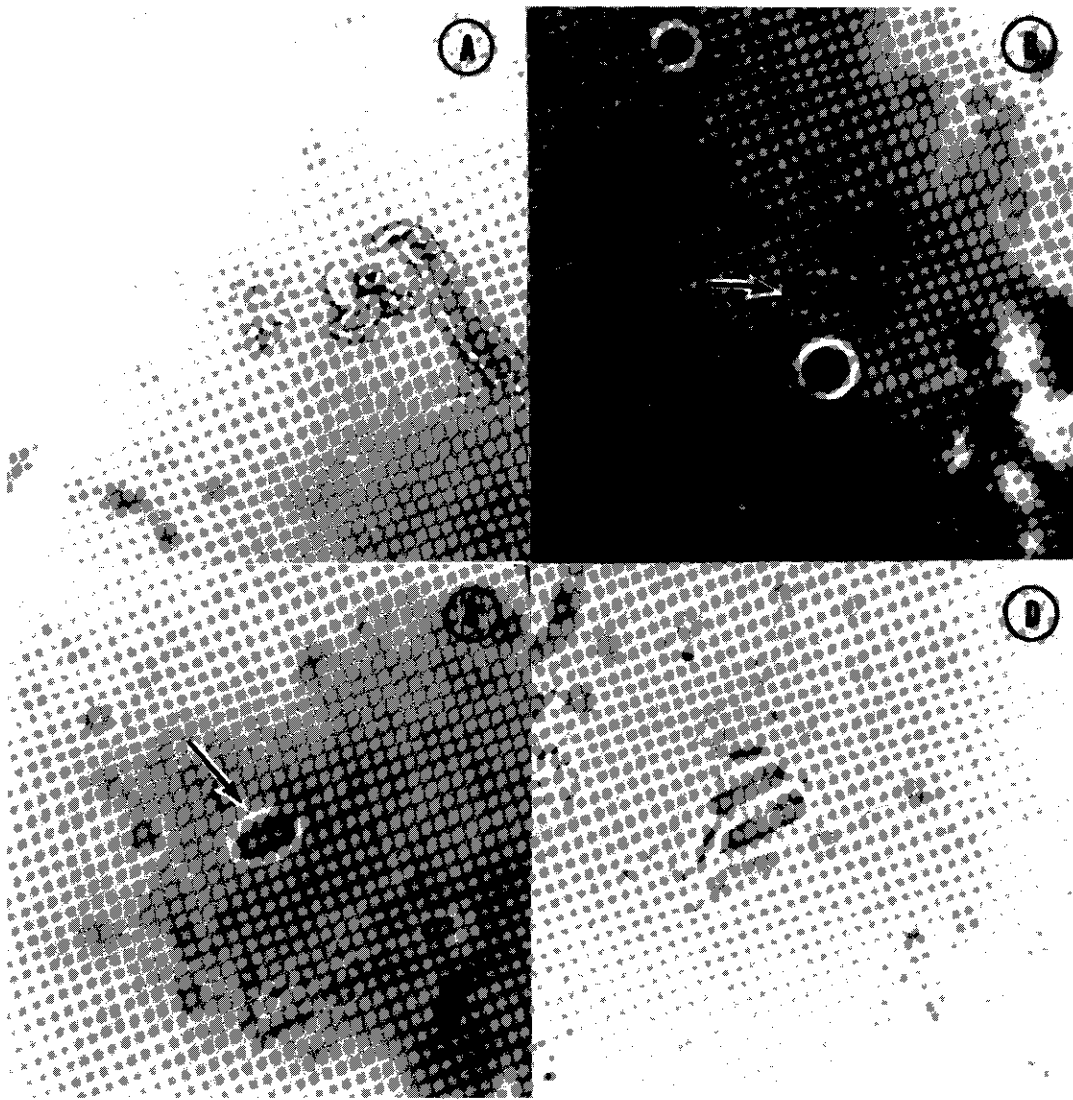


Figure 3. Light micrographs of basidiospores:

A. *Phaeolus schweinitzii*, X4000, 1 mm = 0.25 μ m. B. *Trichaptum abietinum* (arrow), X5000, 1 mm = 0.20 μ m.
 C. *Bjerkandera adusta* (arrow), X5000, 1 mm = 0.20 μ m. D. *Trametes versicolor*, X3000, 1 mm = 0.33 μ m.

T. abietinum. In contrast to LM, SEM revealed a very wrinkled ectosporium, again similar to that of *T. abietinum* (Fig. 4C).

Basidiospores of *Trametes versicolor* appeared cylindric, oblong or slightly allantoid, smooth, hyaline, and measured 4-6 by 1.5-2 μ m with LM (Fig. 3D). Except for a wrinkled ectosporial surface visible with SEM (Fig. 4D), all other spore features correlated with those observed by LM.

Comparisons with Spores of *Heterobasidion annosum*

Basidiospores of *H. annosum* show distinct differences from spores of each of these nine species (compare Fig. 5B with Figs. 2 and 4). Echinulations on basidiospores of *H. annosum* are a surface feature that makes this species unique among the 10 species (Fig. 5B). Contrarily, conidia of *H. annosum* are not as distinctive as



Figure 4. Scanning electron micrographs of basidiospores: A. *Phaeolus schweinitzii*; note apiculus, X3928, 1 mm = 0.25 μ m. B. *Trichaptum abietinum*, X19412, 1 mm = 0.05 μ m. C. *Bjerkandera adusta*, X10607, 1 mm = 0.09 μ m. D. *Trametes versicolor*, X9945, 1 mm = 0.10 μ m. Note wrinkled surface on spores in B, C, and D.

basidiospores (compare Fig. 5A with Figs. 2 and 4). Their smooth ectosporium is similar to basidiospores of *F. pinicola*, *Phellinus robustus*, and *Phaeolus schweinitzii*. Apicular structure and position are, however, different enough to allow for accurate separation of the species.

Certainly, when compared with basidiospores of *F. cajanderi*, *G. oregonense*, *G. applanatum*, and *Trichaptum abietinum*, *B. adusta*, and

Trametes versicolor, the conidia of *H. annosum* are distinct.

Discussion

Ectosporial ultrastructure and morphology were characterized and compared for nine species of decay fungi. Distinct differences were observed among most species, and in most, features were

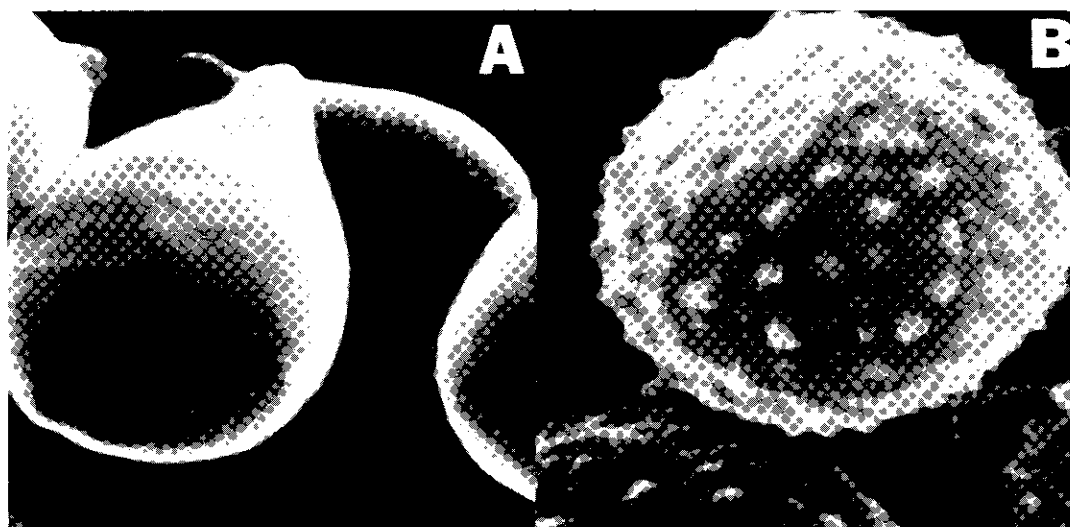


Figure 5. Scanning electron micrographs of:
 A. *Heterobasidium annosum* conidia, X15683, 1 mm = 0.06 μ m. B. Basidiospores, X20255, 1 mm = 0.05 μ m. Compare all scanning electron micrographs of basidiospores in Figures 2 and 4 with those in Figure 5.

resolved with SEM that could not be resolved with LM.

Spores of *Phellinus robustus* are reported to be globose or subglobose (Lowe 1957, Overholts 1977). SEM reveals that these spores have deep depressions and are biconcave or triconcave (Fig. 2C). Variation in the number of depressions likely contributed to reported differences in spore shape reported elsewhere (Lowe 1957, Overholts 1977). Gilbertson and Ryvar den (1987) indicate that the *P. robustus* complex is probably the most difficult taxonomic problem in the genus. By their new descriptions, our specimens may be *P. hartigii* (Allesch. & Schnabl) Bond. because this is the most common species in coniferous forests of the Pacific Northwest, and *P. robustus sensu stricto* causes primarily a heartwood decay of living hardwoods.

Overholts (1977) reported spore surfaces of *G. oregonense* to be "apparently echinulate." SEM shows that the echinulated appearance is caused by numerous pits in the ectosporial wall (Fig. 2E) and is similar to that of many of the British species of *Ganoderma* (Pegler and Young 1973). The pits cause constructive and destructive interference of light and thus contribute to the surface patterns visible with LM (Fig. 1E). Gilbertson and Ryvar den (1986) suggest that these pits result from points of outer wall connection with interwall pillars.

Spores of *G. applanatum* are reported to be smooth when observed by LM (Overholts 1977). Our Figures, and those in Nilsson (1983) and Pegler and Young (1973), indicate a foveate surface (Fig. 2D). In addition, spores released from sterigmata are concave at the distal end, a feature that likely contributes to the descriptions of truncate spores (Gilbertson and Ryvar den 1986, Overholts 1977).

A feature common to *Trichaptum abietinum*, *B. adusta*, and *Trametes versicolor* is the wrinkled ectosporium (Fig. 4B, C, D). Initially, we thought the wrinkling was caused by preparatory techniques; however, several techniques were used (air drying, fixing for different time periods, using different fixatives, omitting critical point drying) and results were identical. Also, samples of other species were not similarly affected by these preparatory techniques, nor was the basidium of *T. versicolor* wrinkled or distorted (Fig. 4D). Therefore, we conclude that spores of these species will always appear wrinkled when observed with SEM. The wrinkles are either not resolvable with LM (Fig. 3B, C, D), which would explain the lack of earlier observation (Gilbertson 1974, Gilbertson and Ryvar den 1986, 1987), or they are an artifact of our collection procedures or preparatory techniques for SEM. Possibly these fungi have an extremely delicate endosporial, episporial, and perisporial

structure that is altered regardless of preparatory procedures. A study of the genus using thin sectioning and transmission electron microscopy could answer this question; however, such a study is beyond the scope of this project.

Confusion about the distinction between spores of *F. cajanderi* and *Phellinus robustus* exists in the literature (Lowe 1957, Overholts 1977). Lowe (1957) states that *P. robustus* "is very similar to *F. cajanderi* except for the straight thicker spores." In contrast, Overholts (1977) states, "the significant difference between the two species lies in the size and shape of the spores." Emphasis is placed on the allantoid shape. Our results show that even though the spores of *F. cajanderi* are allantoid, this shape is not a reliable feature for separation. Figure 2A shows one spore where the curved morphology is obvious, whereas the other spore appears straight. The condition varies, depending on spore position, because spores viewed with LM are in liquid medium and thus could be variously oriented. This situation likely contributed to differences in earlier descriptions. Gilbertson and Ryvarden (1986, 1987) do not mention problems with distinguishing between spores of these two fungi; however, they do note that *F. cajanderi* has curved spores, whereas the closely related *F. rosea* (Alb. et Schw.: Fr.) Karst. has straight spores.

All nine spore types were compared to an earlier characterization of *H. annosum* spores (Roll-Hansen 1940, 1980, Shaw and Florence 1979) to evaluate the possibility of detecting the presence of this species by identifying its spores in a mixed sample of airborne inocula. Results demonstrate differences in ectosporial structure or morphology among the nine species examined and basidiospores of *H. annosum* (compare Figs. 2 and 4 with Fig. 5). The smooth ectosporium on conidia of *H. annosum* is similar to that on basidiospores of *F. pinicola*, *Phellinus robustus*, *F. cajanderi*, and *Phaeolus schweinitzii*. We believe, however, that there are enough differences in overall morphology, ectosporial structure, and apicular attachment to accurately identify these conidia in samples of airborne inocula. Since conidia may be an important source of inoculum for *H. annosum* in West Coast forests

(Hunt *et al.* 1976), knowledge of their abundance in airborne inocula could be important to forest management.

Attempts have been made to collect and analyze spore types present in airborne inocula of *H. annosum*. We made collections at two forest sites in northwest Oregon from September through November with a vacuum device that pulls air through a nucleopore filter. The filters were removed and prepared for viewing with the SEM. Basidiospores of *H. annosum* were observed but conidia were not. In contrast, Leslie (1983) determined, by using similar collection procedures and our SEM techniques (Florance *et al.* 1981, Shaw and Florance 1979), that 18 percent of the *H. annosum* spores deposited in October at a site in coastal Washington state were conidia. If Leslie's (1983) contention that conidia of *H. annosum* are more resistant than basidiospores to heat and desiccation is correct, then the moderately high level of conidia in airborne inocula could be significant to disease development in Pacific Northwest forests.

Both of these data sets are very preliminary and many more sampling attempts need to be made before definitive results can be obtained; however, such information should be useful to researchers in the expanding field of aerobiology. Scanning electron micrographs of specific basidiospores also should help to clarify uncertainties in some current taxonomic descriptions.

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