

Ascospore dispersal of *Ceratocystiopsis ranaculosus*, a mycangial fungus of the southern pine beetle

John C. Moser

Thelma J. Perry

Southern Forest Experiment Station, U.S. Department of Agriculture, Forest Service, 2500 Shreveport Hwy., Pineville, Louisiana 71360

J. Robert Bridges

Forest Insect and Disease Research, U.S. Department of Agriculture, Forest Service, 14th and Independence, S.W., P.O. Box 96090, Washington, D.C. 20090-6090

Hui-Fen Yin

Institute of Zoology, Academia Sinica, Beijing, China

Abstract: Ascospores of the heterothallic fungus *Ceratocystiopsis ranaculosus* were found in sporothecae of three mite species of the genus *Tarsonemus*. These mites were phoretic on the coniferous bark beetles *Dendroctonus frontalis*, *D. brevicomis*, and *Ips acuminatus*. *Ceratocystiopsis ranaculosus* inhabits the mycangium of both *Dendroctonus* species as conidia in a budding yeast-like form. Ascospores are not known to occur in mycangia of bark beetles, and the means of ascospore dispersal has not previously been reported. It is postulated that ascospores transported by phoretic mites may be responsible for establishing sexually compatible colonies of the fungus in beetle galleries either by themselves or in combination with the mycangial fungus type.

Key Words: *Ceratocystiopsis*, southern pine beetle

One of the fungi found in mycangia of females of both the southern pine beetle, *Dendroctonus frontalis* Zimmermann (SPB), and the western pine beetle, *Dendroctonus brevicomis* LeConte (WPB), is a budding, yeast-like organism; the other is an unidentified basidiomycete. Barras and Taylor (1973) demonstrated that the yeast-like isolates from the mycangium of SPB express a *Sporothrix* Hektoen & Perkins morphology in galleries of the beetle and in agar culture. On the basis of serology and virulence studies, they determined that this fungus was close to *Ceratocystis minor* (Hedgcock) Hunt (= *Ophiostoma minus* (Hedgcock) H. & P. Sydow), the cause of bluestain disease in pine, and they pro-

posed *C. minor* var. *barrasii* J. Taylor for the nonstaining mycangial fungus. Bridges et al. (1985) noted that SPB infestations developed in the absence of *O. minus* and thus other fungi could be responsible for tree death. Bridges and Perry (1987) found a second ascomycete associated with beetle infestation that they described as *Ceratocystiopsis ranaculosus* Perry & Bridges¹. Agar cultures of *C. ranaculosus* produced a *Sporothrix* anamorph, but the authors were not aware of a role for their fungus in the biology of the bark beetle.

Harrington and Zambino (1990) rejected *C. minor* var. *barrasii* because it was not properly typified. They found that the *Sporothrix* anamorph described by Barras and Taylor (1973) was more similar to that of *C. ranaculosus* than to the anamorph of *Ophiostoma minus*. Using isozyme and mating analyses, Harrington and Zambino (1990) concluded that the isolate referred to by Barras and Taylor in 1973 as *Ceratocystis minor* var. *barrasii* was actually the anamorph of the heterothallic *Ceratocystiopsis ranaculosus*. The mycangial *Sporothrix* of the WPB is reputed to be *Ophiostoma nigrocarpum* (Davidson) deHoog (= *Ceratocystis nigrocarpum* Davidson) (Barras and Perry, 1972), but mating studies conducted by Harrington (1993) have shown that this anamorph is actually *Ceratocystiopsis ranaculosus*. This is not surprising to us given that we have found ascospores of this species in sporothecae of *Tarsonemus* mites phoretic on WPB in California. Thus, key elements in the southern pine beetle/fungus symbiosis were joined. Females of both *Dendroctonus* species distribute conidia of the *Ceratocystiopsis* from their mycangia, initiating new infection with the formation of galleries. The fungus grows in the beetle galleries beginning its development in loblolly pine (*Pinus taeda* L.) trees. One element missing from this story, however, has been how ascospores are dispersed and how two mating types for the fungus are inoculated into the galleries. In the current paper, we report finding ascospores of *C. ranaculosus* associated with mites phoretic on southern pine beetle, and speculate on the role of the fungus/mite association in the sexual cycle.

Mature ascospores of *Ceratocystiopsis ranaculosus* have

¹ Hausner et al. (1993) have reduced *Ceratocystis* Upadhyay & Kendrick to synonymy with *Ophiostoma* H. & P. Sydow. We are not entirely in agreement with their conclusions and maintain the genus for now.

the distinctive shape of a tadpole or sperm, with an enlarged head and an elongated tail (see Bridges and Perry, 1987). We have observed these ascospores, as well as immature ascospores, in sporothecae of the tarsonemid mites (Acari, Tarsonemidae) *Tarsonemus krantzi* Smiley & Moser and *T. ips* Lindquist, both of which occur on SPB, and two individuals of *T. endophloeus* Lindquist (Cedar Valley, California) on WPB. The mites had been removed from male and female SPB. From 1 to 40 ascospores were seen in sporothecae of 42 individuals of both species of mite. Several mite individuals carried ascospores both of *C. ranaculosus* and *Ceratocystis minor* (Bridges and Moser, 1983). Fifteen mite species are commonly phoretic on SPB that attack *Pinus* species in the southern United States (Kinn, 1976), but so far we have only found ascospores associated with two tarsonemid species in about 5000 specimens examined.

Fungal spores within the sporotheca may be protected from exposure to oleoresins exuding from beetle-induced wounds in the tree. This would be analogous to the protection afforded conidia within the mycangium of female SPB. We do not know when the mites leave their beetle hosts, or when ascospores are discharged from sporothecae, but because most blue staining caused by *O. minus* occurs close to the point of beetle entry (Nelson, 1934), it is possible that ascospores as well as conidia from the mycangium are available for germination soon after the beetle enters the tree. The phoretic mite *Dendrolaelaps quadrisetus* Berlese detaches from its beetle host once the beetle begins to excavate a gallery (Kinn, 1967).

According to Lindquist (1969), Smiley and Moser (1974), and Moser et al. (1974), the geographic distribution of the mite *T. ips* is Europe and North America, south to Honduras. It is associated with a large number of bark beetle species; however, *T. krantzi* is associated only with *Dendroctonus* and *Ips* bark beetles attacking pines in the southern United States and Central America. Of the several hundred microscope slides of *T. ips* and *T. krantzi* examined by us, we found ascospores of *C. ranaculosus* only from the municipalities of Clarks, Olla, and Williana in Louisiana, and the Sam Houston and Sabine National Forests located in East Texas. More extensive investigations should reveal this fungus throughout the range of SPB. Like the ascospores of *Ophiostoma minus*, ascospores of *C. ranaculosus* appear to be located only in the mite sporotheca (Moser, 1985), an area under tergite 1.

We also found one specimen of *Tarsonemus* sp. near *subcorticalis* Lindquist from *Ips acuminatus* (Gyllenhal) from China. We note here that all three bark beetle species possess a mycangium (Francke-Grossman, 1967). Hence, the phenomenon of *Tarsonemus* mites transporting ascospores of *C. ranaculosus* as well as

those of *O. minus* (Moser, 1985) in sporothecae may extend geographically at least to China with a broad range of conifer, bark beetle, and mite hosts.

It is important to point out that the beetles and the mites acquire the fungal spores in very different ways. The callow adult beetle mycangium is inoculated with one or more spores from fungal growth in pupal chambers in the outer bark that contain only conidia (Paine and Birch, 1983). On the other hand, the tarsonemid mites are in intimate contact with the ascomata, allowing the females access to high concentrations of ascospores of both *C. ranaculosus* (Bridges and Moser, 1983; Moser and Bridges, 1986) and *O. minus* (Moser, 1985).

We have not looked for ascospores on the surface of beetles, but when unsterile beetle mycangia were dissected (Baras and Perry, 1972) or the external surfaces of beetles were scraped (Baras, 1975) or isolates from exoskeletons were prepared (Zambino and Harrington, 1988), and then plated on agar media, cultures of *Sporothrix* developed. This mycangial anamorph exhibited pleomorphic and ambrosial characteristics but the production of ascospores on artificial media has never been observed.

Sporothrix has been observed in SPB galleries but this anamorph could belong to any of several *Ophiostoma* or *Ceratocystiopsis* species (Baras and Taylor, 1973; Harrington and Zambino, 1990).

Assuming that the mite-associated ascospores of *Ceratocystiopsis ranaculosus* and *O. minus* are viable (we have not attempted to germinate them), they may play a significant role in the life cycle of the fungi. Unlike the homothallic *O. minus*, *C. ranaculosus* is heterothallic, as demonstrated by the work of Harrington and Zambino (1990), and ascomata are found within beetle galleries (Bridges and Perry, 1987) indicating the presence of the two mating types in the same gallery. However, the gallery is initiated by a single female beetle, whose mycangial fungal population may have developed from only one propagule or conidium which divided, multiplied, and filled the mycangium with only one mating type, which would result in no ascomata in galleries. If two or more spores initiated the mycangial fungal population, it is possible that both mating types developed. But isolations from the mycangium onto artificial media have never been observed to produce mature ascomata except where opposite mating types were crossed (Harrington and Zambino, 1990). The assumption, then, is that the mycangium from single beetles is of only one mating type of the fungus, and that the other mating type must come from a source other than the single beetle that initiates the gallery.

Thus the fortuitous attachment of ascospores into the mite sporothecae might be more indiscriminate

than the beetle's selection of mycangial fungus. In any event, it is at least possible that the ascospores in the mites represent the two mating types necessary for ascromatal formation and, ultimately, genetic recombination with either themselves or with the mycangial fungus allele in the beetle gallery.

We thank Drs. Meridith Blackwell, Thomas C. Harrington and D. N. Kinn for reading and commenting on the manuscript, and J. W. Monahan for preparation of the slide material.

LITERATURE CITED

- Barras, S. J. 1975. Release of fungi from mycangia of southern pine beetles observed under a scanning electron microscope. *Z. Angew. Entomol.* 79: 173–176.
- _____, and T. Perry. 1972. Fungal symbionts in the prothoracic mycangium of *Dendroctonus frontalis* (Coleopt.: Scolytidae). *Z. Angew. Entomol.* 71: 95–104.
- _____, and J. J. Taylor. 1973. Varietal *Ceratocystis minor* identified from mycangium of *Dendroctonus frontalis*. *Mycopathol. Mycol. Appl.* 50: 293–305.
- Bridges, J. R., and J. Moser. 1983. Role of two phoretic mites in transmission of bluestain fungus, *Ceratocystis minor*. *Ecol. Entomol.* 8: 9–12.
- _____, W. A. Nettleton, and M. D. Connor. 1985. Southern pine beetle (Coleoptera: Scolytidae) infestations without the bluestain fungus, *Ceratocystis minor*. *J. Econ. Entomol.* 78: 325–327.
- _____, and T. J. Perry. 1987. *Ceratocystiopsis ranaculosus* sp. nov. associated with the southern pine beetle. *Mycologia* 79: 630–633.
- Francke-Grossman, H. 1967. Ectosymbiosis in wood-inhabiting insects. Pp. 142–205. In: *Symbiosis*. Ed., S. M. Henry. Academic Press, New York and London.
- Harrington, T. C. 1993. Diseases of conifers caused by species of *Ophiostoma* and *Leptographium*. Pp. 161–172. In: *Ceratocystis and Ophiostoma. Taxonomy, ecology and pathogenicity*. Eds., M. J. Wingfield, K. A. Seifert, and J. F. Webber. APS Press, St. Paul, Minnesota.
- _____, and P. J. Zambino. 1990. *Ceratocystiopsis ranaculosus*, not *Ceratocystis minor* var. *barrasii*, is the mycangial fungus of the southern pine beetle. *Mycotaxon* 38: 103–115.
- Hausner, G., J. Reid, and G. R. Klassen. 1993. *Ceratocystiopsis*: a reappraisal based on molecular criteria. *Micol. Res.* 97: 625–633.
- Kinn, D. N. 1967. Notes on the life cycle and habits of *Digamasellus quadrisetus* (Mesostigmata: Digamasellidae). *Ann. Entomol. Soc. Amer.* 60: 862–865.
- _____. 1976. Key to mites commonly associated with the southern pine beetle. *Res. Notes, S.O., U.S. Forest Serv.* 214: 1–11.
- Lindquist, E. E. 1969. New species of *Tarsonemus* (Acarina: Tarsonemidae) associated with bark beetles. *Canad. Entomol.* 101: 1291–1314.
- Moser, J. C. 1985. Use of sporothecae by phoretic *Tarsonemus* mites to transport ascospores of coniferous bluestain fungi. *Trans. Brit. Mycol. Soc.* 84: 750–753.
- _____, and J. R. Bridges. 1986. *Tarsonemus* (Acarina: Tarsonemidae) mites phoretic on the southern pine beetle (Coleoptera: Scolytidae): attachment sites and numbers of bluestain (Ascomycetes: Ophiostomataceae) ascospores carried. *Proc. Entomol. Soc. Wash.* 88: 297–299.
- _____, R. C. Wilkinson, and E. W. Clark. 1974. Mites associated with *Dendroctonus frontalis* Zimmerman (Scolytidae: Coleoptera) in Central America and Mexico. *Turrialba* 24: 379–381.
- Nelson, R. M. 1934. Effect of bluestain on southern pines attacked by bark beetles. *Phytopathol. Z.* 7: 327–353.
- Paine, T. D., and M. C. Birch. 1983. Acquisition and maintenance of mycangial fungi by *Dendroctonus brevicomis* LeConte (Coleoptera: Scolytidae). *Environm. Entomol.* 12: 1384–1386.
- Smiley, R. L., and J. C. Moser. 1974. New tarsonemids associated with bark beetles. *Ann. Entomol. Soc. Amer.* 67: 639–665.
- Zambino, P. J., and T. C. Harrington. 1988. A reexamination of the mycangial fungi of the southern pine beetle. *Micol. Soc. Amer. Newslett.* 39: 56.