Parasitization of Pine Stem Rust Fungi by *Monocillium nordinii*

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ABSTRACT

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Monocillium nordinii that occurred in sori of *Cronartium coleosporioides* and *Endocronartium harknessii* in Alberta, Canada, was a destructive mycoparasite of these pine stem rust fungi. Detailed study of the parasitic process in the *E. harknessii-M. nordinii* interaction showed that hyphae of *M. nordinii* grew between the surface wart layers of rust spores and that swollen appressoriumlike bodies often were formed where contact was made. The host cytoplasm occasionally developed a papilla beneath the area of contact during the early stages of parasitism; however, the host cell eventually lost most of its cytoplasm, except oil bodies, in most cases. Penetration of the rust spores by the *Monocillium* hyphae usually appeared to occur after the host cells were killed. *Monocillium* generally formed many conidiophores and conidia on the host spores. Eventually the surface morphology of the infected rust spores was greatly degraded. It is suggested that antifungal metabolites of *M. nordinii* are involved in this parasitic process.

Additional key word: mycoparasitism.

Western gall rust (caused by *Endocronartium harknessii* [J. P. Moore] Y. Hiratsuka) and stalactiform blister rust (caused by *Cronartium coleosporioides* Arth.) are major pine stem rusts in Western Canada that cause severe damage to hard pines (7,16). Outbreaks of these rusts have been severe in some years, but usually there is an interval of several years between major outbreaks. Field surveys in Alberta in 1977–1979 revealed that not only abiotic factors, but also some mycoparasites play important roles in suppressing the rust epidemics. In 1979, for example, *Scytalidium uredinicola* Kuhlman et al destroyed more than 80% of *E. harknessii* galls in some localities in Alberta (8,13). *Cladosporium gallicola* Sutton also occurred frequently on the rust and reduced its inoculum potential (12). Although *Monocillium nordinii* (Bourchier) W. Gams (≡*Cephalosporium nordinii* Bourchier) (6) occurred less frequently than these mycoparasites, it also was found in sori of both *E. harknessii* and *C. coleosporioides*, where it appeared to produce strong antifungal metabolites. This fungus commonly has been isolated from the heartwood of lodgepole pine, *Pinus contorta* Dougl. var. *latifolia* Engelm. (4); however, this is the first report on its mycoparasitic activity.

MATERIALS AND METHODS

Rust sori of *C. coleosporioides* and *E. harknessii* on lodgepole pine were collected at several locations in Alberta. All the collected samples of *E. harknessii* sori with natural infections of *M. nordinii* also were colonized by other mycoparasites, usually *C. gallicola*, but most samples of *C. coleosporioides* parasitized by *M. nordinii* were free from other mycoparasites. Sori of *C. coleosporioides* that were naturally infected by *M. nordinii* and those of *E. harknessii* that were artificially inoculated with *M. nordinii* were used for scanning electron microscopy (SEM). Artificial inoculation was performed by spraying young sporulating rust galls with about 1 ml of *Monocillium* spore suspension (about 10^7 spores per milliliter of distilled water) per gall. These galls were incubated at 22–25 C in sterile beakers lined with moist filter paper. Small pieces (about 5 × 5 × 5 mm) taken from samples of both rusts were fixed and dehydrated by either air drying or freeze-drying, as described previously (12,15). The dried materials were shadowed with gold, and at least six pieces of each rust were examined with a Cambridge Stereoscan S_4 scanning electron microscope.

For light microscopy, 0.5 ml of the spore suspension of *M. nordinii* was spread evenly on a cellophane membrane disk (8-cm diameter) that was placed on 2% water agar in a petri dish. Mature spores of *E. harknessii* were dusted onto the membrane, and the petri dish was kept at 20 C in the dark. After incubation for 48 or 96 hr, the cellophane membrane was removed, cut into strips, mounted in water or in cotton blue-lactophenol on a microscope slide, and examined with a Leitz Orthoplan microscope with Nomarski interference-contrast optics.

RESULTS AND DISCUSSION

Sori of *C. coleosporioides* that were naturally infected by *M. nordinii* had a whitish, fluffy appearance because of the numerous conidiophores and conidia produced by the mycoparasite. Many annulated warts ornamented the surface of noninfected aeciospores (Fig. 1), but the warts of infected spores appeared degraded. In extreme cases, warts were not discernible, and hyphae of *M. nordinii* were covered with an amorphous material that appeared to be the degraded wart material (Fig. 2). Wart degradation may be either an autolytic process induced in the host by the parasitic attack, the result of activity of the cell wall hydrolyzing enzymes of the mycoparasite, or both. Similar wart degradation occurs commonly in *E. harknessii* spores parasitized by other mycoparasites (12,13).

Hyphae of *M. nordinii* grew profusely in sori of *E. harknessii* that were sprayed with *M. nordinii* spore suspensions. The mycoparasite hyphae grew between the surface wart layers of the rust spores and were appressed to the host cell wall (Fig. 3). Swollen appressoriumlike bodies often were formed at these contact points (Fig. 4). Host cytoplasm occasionally formed a papilla beneath the appressoriumlike body (Fig. 4), but only where contact was made during the very early stages of infection. Papillae are not induced in the cytoplasm of *E. harknessii* by *C. gallicola* or *S. uredinicola* (12,13). We speculate, therefore, that *E. harknessii* develops the structure in response to a specific metabolite released by *M. nordinii*. Similar structures have been reported for fungal (9–11, 14, 15) and vascular plant cells (1,5) infected by fungal parasites. These structures are thought either to have a protective role in delaying or preventing penetration by the parasites (5,15) or to represent a sign of necrosis without any positive role in host resistance (11). Induction of a papilla in *E. harknessii* by *M. nordinii* occurs only while the host cells are fully viable, suggesting that the structure is not simply a product of necrosis.

Formation of conidiophores and conidia by *M. nordinii* occurred frequently on the host spores (Fig. 5). The conidiophores

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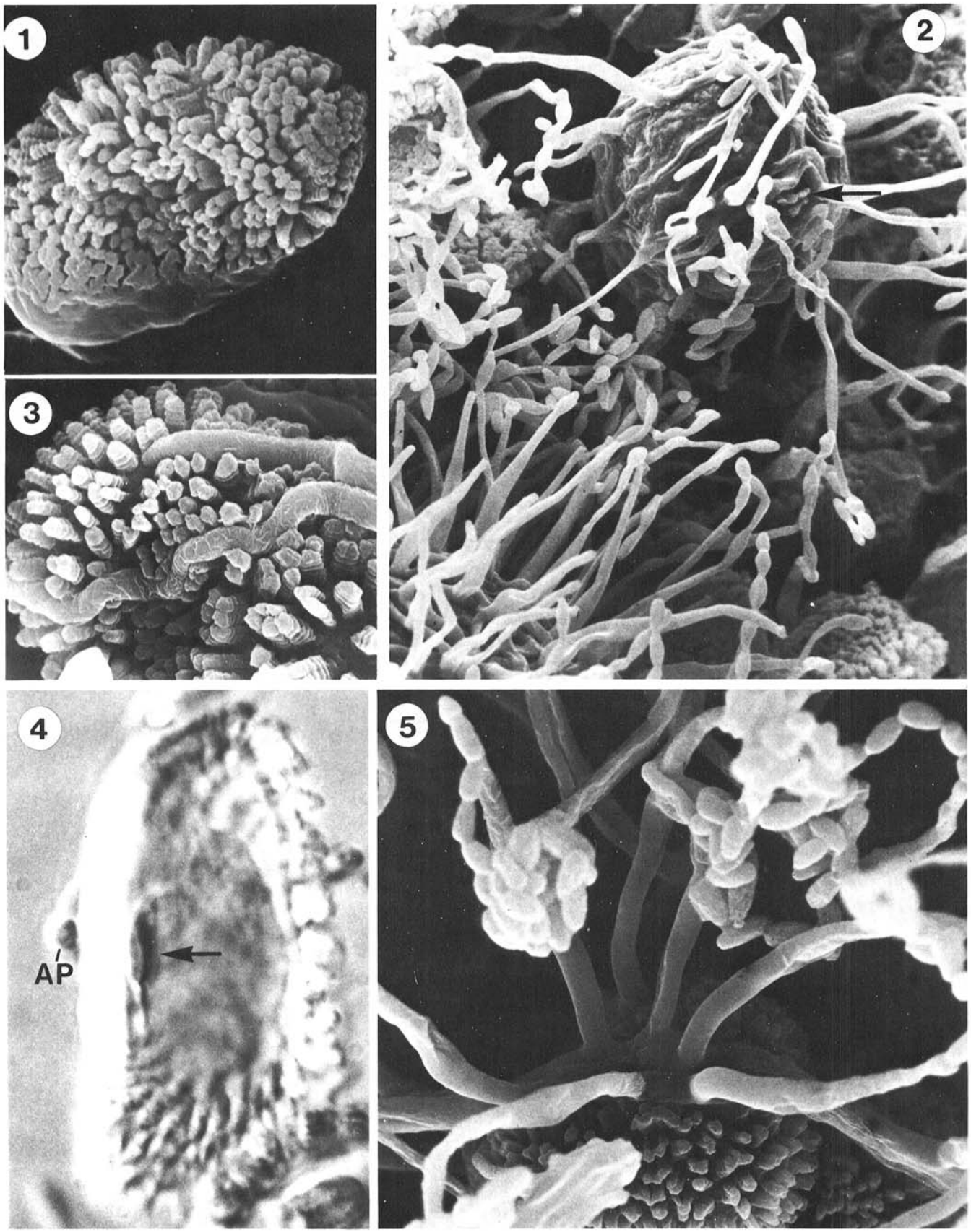
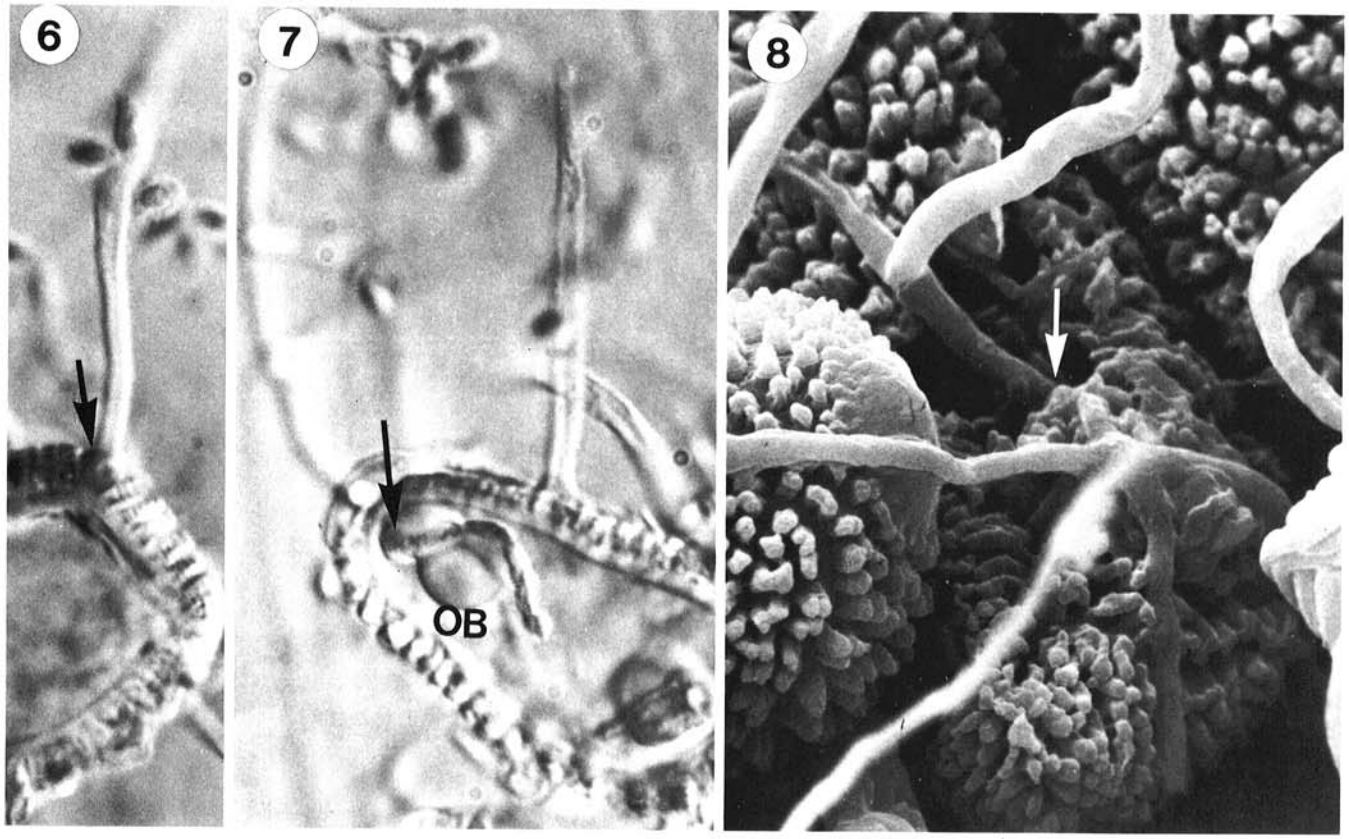


Figs. 1–5. Host-parasite interactions of pine stem rust fungi and *Monocillium nordinii*. **1,** Noninfected aeciospore of *Cronartium coleosporioides* with normal annulated warts on the surface (× 3,000). **2,** Portion of a *C. coleosporioides* sorus showing an advanced stage of natural infection by *M. nordinii*. Note a rust spore showing a severely degenerated surface with a few warts (arrow) of nearly original shape (× 1,800). **3,** *M. nordinii* hyphae growing between the surface wart layers of a peridermioid teliospore of *Endocronartium harknessii* (× 5,000). **4,** Papilla (arrow) in cytoplasm of *E. harknessii* formed in response to contact by an appressoriumlike body (AP) of *M. nordinii* (Nomarski light optics ×3,500). **5,** Formation of conidiophores and conidia by *M. nordinii* on a spore of *E. harknessii* during a late stage of infection (× 3,600).



Figs. 6–8. Spores of *Endocronartium harknessii* parasitized by *Monocillium nordinii*. **6,** Conidiophore of the parasite swollen at the base (arrow) in contact with the host (Nomarski light optics ×2,800). **7,** *E. harknessii* spore penetrated by an *M. nordinii* hypha (arrow). Oil bodies (OB) remain within the host hypha (Nomarski light optics ×2,800). **8,** Severely degenerated *E. harknessii* spore penetrated by an *M. nordinii* hypha (arrow) (×4,000).

usually became swollen at the base and anchored themselves between the warts of the host spore (Fig. 6). Although penetration was common (Figs. 7 and 8), it rarely occurred from appressoriumlike bodies and was not observed at the points where the papillae were present. Penetration appeared to occur usually after the host cells were killed. The host cytoplasm eventually lost most of its contents, except oil bodies (Fig. 7).

Similar destructive mycoparasitism (3) also has been reported in the interactions between *E. harknessii* and its two other mycoparasites, *C. gallicola* (12) and *S. uredinicola* (13). The detailed mode of parasitism, especially the importance of penetration, however, varies in these interactions: *Cladosporium gallicola* often penetrates and kills living host spores, *M. nordinii* appears to penetrate only after the host is killed, and no penetration is involved in the parasitism by *S. uredinicola*. *Cladosporium gallicola* forms appressoria and often develops penetration pegs from them, while penetration seldom occurs from the appressoriumlike bodies of *M. nordinii*.

Antifungal metabolites of *M. nordinii* apparently are involved in its parasitic process on pine stem rusts. Our preliminary experiments indicated that the ether extract of an *M. nordinii* culture medium had a strong inhibitory effect on the rust fungi as well as other fungi such as *Ceratocystis ulmi* (Buism.) C. Moreau, the pathogen of Dutch elm disease. Subsequently six compounds, mainly consisting of monorden ($C_{18}H_{17}O_6Cl$) and a new antibiotic, monocillin I ($C_{18}H_{18}0_6$), were isolated from liquid culture of *M. nordinii* (2).

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