THE FUNGUS *BEAUVERIA BASSIANA* (DEUTEROMYCOTINA: HYPHOMYCETACEAE) IN THE WESTERN BALSAM BARK BEETLE, *DRYOCOETES CONFUSUS* (COLEOPTERA: SCOLYTIDAE)[1]

H. S. WHITNEY
Pacific Forest Research Centre, Canadian Forestry Service, Victoria, British Columbia V8Z 1M5

D. C. RITCHIE, J. H. BORDEN
Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia V5A 1S6

A. J. STOCK
British Columbia Ministry of Forests, Prince Rupert Region, Smithers, British Columbia V0J 2N0

Abstract

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The natural occurrence of beauveriosis in *Dryocoetes confusus* (Swaine) in *Abies lasiocarpa* (Hook.) Nutt. in British Columbia is reported, and the pathogenicity of *Beauveria bassiana* (Bals.) Vuill. isolated from *D. confusus* was confirmed experimentally. Also, direct observations of newly established pairs of *D. confusus* in glass – phloem sandwich cultures showed that cadavers of *B. bassiana*-killed females were walled-off with frass by healthy males.

Résumé

L'incidence naturelle de la beauveriose chez *Dryocoetes confusus* (Swaine) qui parasite *Abies lasiocarpa* (Hook.) Nutt., en Colombie-Britannique, est signalée. La pathogénicité de son agent, *Beauveria bassiana* (Bals.) Vuill., obtenu de *D. confusus* a été confirmée expérimentalement. En outre, des observations directes de paires de *D. confusus* récemment établies dans des sandwiches de verre et de phloème ont montré que les mâles sains ont emmuré les cadavres des femelles tuées par *B. bassiana* derrière leurs débris et leurs excréments.

Beauveria bassiana (Bals.) Vuill. has been recognized as an entomopathogen for more than 100 years but there is relatively little information on its life cycle and ecology on native insects in the field (Rockwood 1916; Nuorteva and Salonen 1968; Taylor and Franklin 1973; Evans 1974; Anon. 1980). Its mode of action in epizootics is virtually unknown. *Beauveria bassiana* occurs in several species of Scolytidae (Coleoptera) (Whitney 1982; Doberski 1978). It is a moderate to severe pathogen and its potential as a pest-management tool for bark beetles is being evaluated. To date it has not been reported from the western balsam bark beetle, *Dryocoetes confusus* (Swaine), the principal insect pest of subalpine fir, *Abies lasiocarpa* (Hook.) Nutt. (*sensu* Hosie 1979), in British Columbia (Cottrell *et al.* 1979; Doidge 1981).

Our objectives were: (*i*) to determine if *B. bassiana* occurs in field populations of *D. confusus*; (*ii*) to test its pathogenicity to *D. confusus*; and (*iii*) to observe the effect of infected beetles on the behavior of survivors within the gallery system.

Materials and Methods

Field observations. Collections of *A. lasiocarpa* bark or logs containing brood of *D. confusus* were made in 1979–81 from 3 well-separated localities in British Columbia: Thynne Mountain, near Brookmere; Headwater Lakes, near Peachland; and McKendrick Pass, near Smithers. Mycotic beetles excised from the bark were examined microscopically for *B. bassiana*, which, if present, was identified according to DeHoog (1972). Detailed microscopic observations were also made of larvae, pupae, and adults in their bark habitat collected from live trees, fresh windfalls, and cut logs from Thynne Mountain. Pure cultures of *B. bassiana* were established on 2% yeast extract / malt extract agar (YM)

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from hyphal tips transferred from a mass of conidia germinating on YM diluted 1:10 with 2% water agar.

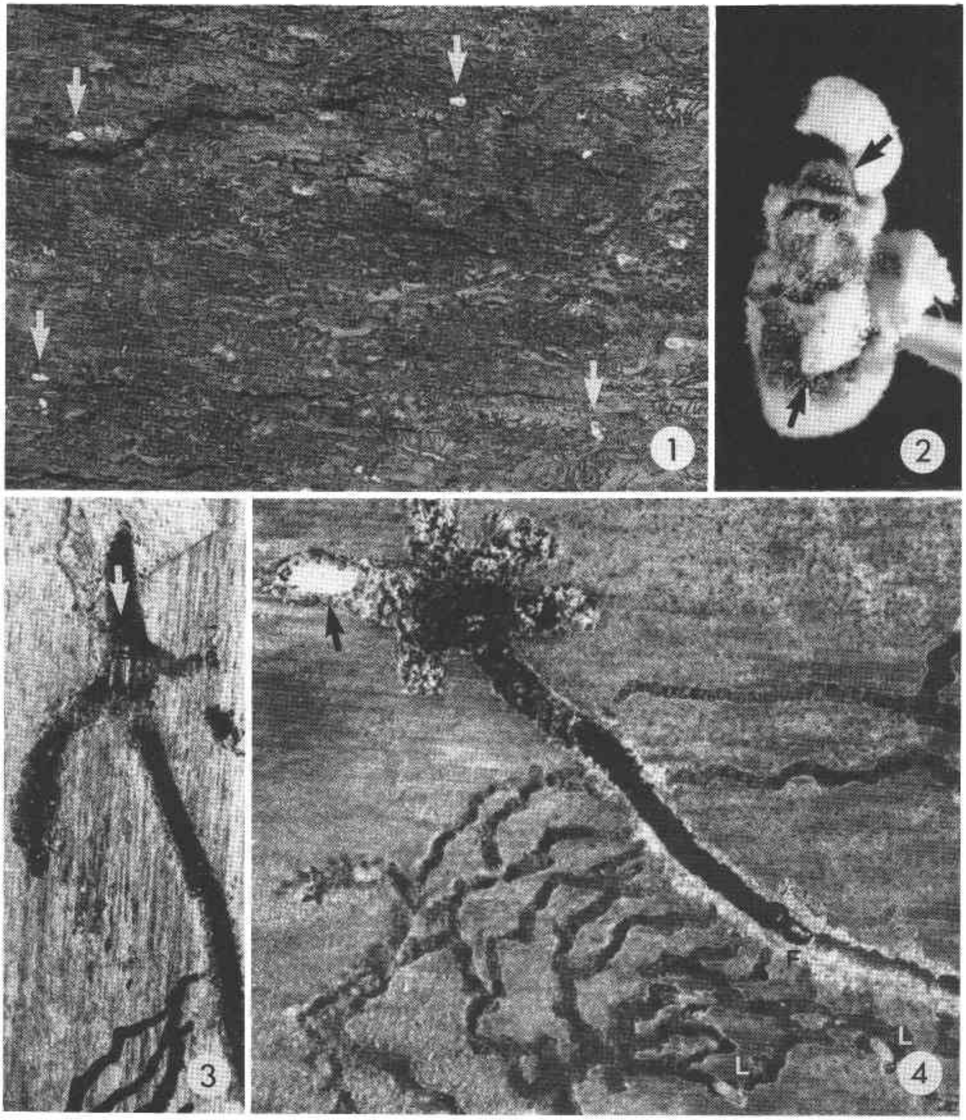
Pathogenicity studies. Pathogenicity tests of *B. bassiana*, isolated from dead *D. confusus* from Thynne Mountain, were conducted on two separate collections of *D. confusus* also from Thynne Mountain. Collection 1 was of pre-emergent adults excavated from their habitat and collection 2 was of freshly emerged adults. Beetles were reared from larvae in field-infested bolts in an insectary at Simon Fraser University. Experimentally inoculated and control beetles were enclosed in glass shell vials (19×55 mm) with size-18 stainless-steel Morton® culture closures. Each vial for collection 1 contained approximately 1 cm^3 of *D. confusus*-mined phloem and frass from an *A. lasiocarpa* brood bolt. The mined phloem and frass were taken from locations in the brood habitat where there were no dead beetles. In the absence of fresh, unmined *A. lasiocarpa* phloem known to be free of *B. bassiana*, each vial for collection 2 contained coarse-ground (2–5 mm^3) fresh-frozen lodgepole-pine phloem. Vials were kept in a crystallizing dish in a loosely closed polyethylene bag at 24 ± 1°C.

Beetles were experimentally inoculated by rolling them in conidia produced at 21 ± 1°C on a 15–21 day old 2% YM petri-plate culture of *B. bassiana*. Thoroughly dusted beetles were placed individually in the vials. Control beetles were similarly rolled in a clean, empty petri plate and transferred to separate vials. All beetles were examined 6 and 14–15 days after inoculation and recorded as living or dead. Some dead beetles were dissected and examined ($10\times$ magnification) and if mycotic, the fungus was identified.

Walling off. Observations of live beetles walling off dead beetles, made during study of reproductive behavior, suggested an experiment in which death of the beetles was artificially induced by beauveriosis. Glass–phloem sandwiches (Schmitz 1972) that allowed direct observation of beetle activity in phloem tissue were used to observe the walling-off behavior. Twenty-five female *D. confusus* were experimentally inoculated with *B. bassiana* and held for 2 days in ground pine phloem at 24 ± 1°C to permit infection. Each female was then allowed to enter a separate nuptial chamber established by a control male introduced into a phloem sandwich 4 days earlier. Twenty-five control females were similarly paired with 25 control males. The sandwiches were maintained in a vertical position at 20–24°C. The sandwiches were kept in the dark except during observations which were made weekly for 3 weeks.

Results and Discussion

Infection of *D. confusus* by *B. bassiana* was confirmed from all three locations. Twelve infected beetles (502 dm^2 of bark area observed) were obtained from McKendrick Pass and 31 infected beetles (317 dm^2 of bark area observed) from Headwater Lakes. At Thynne Mountain detailed observations of carefully dissected portions of several attacked trees and logs disclosed that this entomopathogen was common in the beetle population. However, a specific survey to ascertain the incidence of beauveriosis was not conducted. The fungus killed adults after they established egg galleries (Figs. 1, 2). The cadavers were found in egg galleries from 1 to several cm long but the brood habitat was too disrupted by larval mining to ascertain how soon adult beetles died after they penetrated the tree. Numerous other egg galleries contained either apparently healthy adults or no adults, and brood developing in the vicinity of the pathogenic fungus. *Beauveria bassiana* did not grow extensively into the habitat from mycotic cadavers. Apparently it competes poorly with other fungi such as *Ceratocystis dryocoetidis* Kendrick and Molnar or *Pezizella chapmanii* Whitney and Funk in the egg and larval galleries. Nevertheless, beauveria-killed larvae and teneral adults were found occasionally. They were often near or in contact with mummified adult cadavers bearing sporulating *B. bassiana*. This observation suggests that beauveriosis spreads to offspring that venture near dead parents.



FIGS. 1–4. 1, advanced brood galleries with several mycotic cadavers (arrows) of *Dryocoetes confusus* adults (×0.5). 2, detail of mycotic, mummified cadaver of *D. confusus*. Mass of white mycelium and spores has been partially removed, revealing dense clusters of conidiogenous cells (arrows) scattered on the integument (×12). 3–4, live, and walled-off dead adult *Dryocoetes confusus* in a glass-phloem sandwich: 3, adult male (arrow) in typical nuptial chamber, with three feeding tunnels, constructed in a glass-phloem sandwich. Note relative absence of frass compared with Fig. 4 which shows a male, killed by *Beauveria bassiana*, isolated at the end of a short feeding tunnel by a frass plug (arrow) constructed by a healthy female (F). The more densely packed frass nearest the cadaver contrasts to the less densely packed frass nearer the nuptial chamber and in the aborted feeding tunnels. Also, note the larvae (L). There is no sign of *B. bassiana* growing in the rest of the gallery system (×2).

A collembolan (Order Arthropleona) was observed apparently feeding on *B. bassiana* spores produced on an adult beetle cadaver. These spores are well within the size range of food particles eaten by Collembola of many families (Butcher *et al.* 1971). Regardless of the effect of *B. bassiana* on the collembolan, and whether or not the animal was actually feeding on the fungus, this behavior would aid in dispersal of these spores. Other tiny animals such as mites, nematodes, and insects other than *D. confusus* were in the brood habitat and could also have been involved in the spread of *Beauveria* spores. Some, such as phoretic mites, might have been active vectors (Schabel 1982).

A *B. bassiana*-killed male *D. confusus* was found in a rudimentary nuptial chamber on a live tree in the Thynne Mountain study area. The minor amount of mining done by this beetle indicated that it probably became inoculated and infected in its former habitat. If this was true, it could be important in host-tree colonization due to competition for establishment sites between short-lived unhealthy beetles and longer-lived healthy ones.

Pathogenicity tests revealed that *B. bassiana* killed 85–96% of inoculated beetles (Table I). In the test of collection 2, most beetles were dead 6 days after artificial inoculation with *B. bassiana*. Observations at 14 days confirmed that the mortality at 6 days was mostly due to *Beauveria*. The high (75%) incidence of beauveriosis in control-excavated compared with control-emerged beetles (Table I, collections 1 and 2) probably resulted from natural *B. bassiana* inoculation and infection in the brood-rearing bolts. Additionally, the mined phloem and frass used in the test of excavated beetles may have contained *B. bassiana* spores in which case the excavating procedure would likely aid in dispersal of this natural inoculum. Furthermore, excavated beetles may have been stressed and thereby predisposed to infection and disease development. By comparison, emerged beetles (Table I collection 2) kept in clean (*B. bassiana*-free) coarse-ground lodgepole-pine phloem had much lower beauveriosis (10%). In this test, the few emerged beetles that became diseased without having been experimentally inoculated were also probably naturally inoculated and infected prior to emergence. Natural pre-emergence infection could account for the field observation of beauveriosis of a male beetle that died in the process of establishing its nuptial chamber.

Preliminary observations of phloem sandwiches intended for observations of reproductive behavior disclosed two galleries in which the males had died apparently from beauveriosis. The behavior of the surviving females was remarkable. In each case the female constructed a frass plug in the tunnel in which the male had died, thus walling it off from the rest of the gallery (Figs. 3, 4).

Of the 25 experimentally inoculated females that were introduced into nuptial chambers in phloem sandwiches, 60% died from *B. bassiana* (Table II). In one case, both beetles in one pair succumbed to the fungus, the male either was naturally inoculated earlier, or became inoculated from the artificially inoculated female. Of the 14 cases in which only the female died, 10 were walled-off with frass plugs by the males. Three additional dead females for which the cause of death was unknown were also walled-off as was one dead control female (Table II).

This burial behavior adds another dimension to the extremely complex activity of scolytids in their galleries (Schmitz 1972). Similar frass-plug construction has also been observed in *Alniphagus aspericollis* (Le Conte) (Borden 1969), suggesting that it may be widespread in the Scolytidae. There was no obvious walling-off of dead adults in brood habitats examined from the field. Such structures, however, would likely be obscured or destroyed as the brood develops. This burial habit would be most effective in reducing the spread of *Beauveria* during early stages of brood development, because the plug would tend to prevent access to inoculum by bark beetle larvae and other phloem-habitat animals.

The geographic distribution and apparent persistence of this fungus in natural *D. confusus* infestations of relatively undisturbed *A. lasiocarpa* in British Columbia suggest that *B. bassiana* is enzoic in this insect. This circumstance may offer an opportunity to gain important information about the biology of this fungus, including its potential as a bioinsecticide for bark beetles.

Table I. Pathogenicity of *Beauveria bassiana* on adult *Dryocoetes confusus* in vitro

Collection	Examination (days after inoculation)	Inoculated			Controls		
			Dead			Dead	
		Living (%)	Beauveriosis[a] (%)	Cause uncertain (%)	Living (%)	Beauveriosis (%)	Cause uncertain (%)
1 (20 excavated beetles)	15	0	85	15	5	75	20
2 (50 emerged beetles)	6	6	46	48	90	6	4
	14	0	96	4	78	10	12

[a]Diagnosis based on production of characteristic conidia externally on mycotic cadavers. The first appearance of this sporulation occurred from 3 to 7 days after inoculation.

Table II. Incidence of walling-off of female *Dryocoetes confusus* killed by *Beauveria bassiana* in glass–phloem sandwich galleries

	Control beetles		Treated beetles	
	Males	Females	Males	Feamales[a]
Total beetles	25	25	25	25
Dead, beauveriosis	0	0	1	15
Dead, cause unknown	0	2	0	3
Dead with frass plugs[b]	0	1	0	13

[a]Artificially inoculated with *B. bassiana*.
[b]One additional frass plug constructed by a control beetle to wall-off a mass of conidia at the end of a gallery.

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