NOTE

Fungal associates of *Buprestis aurulenta* in western Oregon[1]

Carlos M. Garcia and J.J. Morrell

Abstract: Associations between the golden metallic beetle, *Buprestis aurulenta* L., and wood-destroying fungi were explored with adult beetles collected from log decks. A variety of fungi were isolated from the beetle bodies by four methods. A total of 863 isolations were made from 59 females and 21 males. There was no significant difference between the microflora associated with male and female adult beetles. Hyphomycetes constituted 91.4% of all isolates. Zygomycetes and yeasts represented 5.8% of the total number of isolates. Basidiomycetes were infrequently isolated (2.8%), suggesting that this buprestid does not consistently vector decay fungi into woody debris.

Résumé : Les relations entre le bupreste doré (*Buprestis aurulenta* L.) et les champignons qui décomposent le bois ont été étudiées avec des insectes adultes récoltés dans des empilements de billes. Différents champignons ont été isolés du corps des insectes en utilisant quatre méthodes. Au total, 863 isolations ont été effectuées sur 59 femelles et 21 mâles. Il n'y avait pas de différence significative entre les microflores associées aux mâles et aux femelles. Les hyphomycètes représentaient 91,4% de tous les isolats. Les zygomycètes et les levures représentaient 5,8% du nombre total d'isolats. Les basidiomycètes étaient peu fréquents (2,8%), suggérant que ce bupreste n'agit pas systématiquement comme vecteur pour les champignons de carie dans les débris ligneux.

[Traduit par la Rédaction]

Introduction

Bark and wood-boring beetles are important in the breakdown of coarse woody debris in natural forests (Carpenter et al. 1988; Edmonds and Eglitis 1989; Schowalter et al. 1992) and may provide a method of introducing wood-colonizing fungi. Their larvae penetrate bark, providing avenues of entry for fungi, and their galleries in the wood structure allow moisture ingress, creating conditions suitable for microbial growth. Many wood-degrading fungi cannot directly penetrate the bark of a freshly killed trees without assistance from insect vectors and are thus unable to exploit that largely unoccupied resource. Conversely, the wood substrate, which contains copious energy, has relatively low levels of some nutrients, notably nitrogen (Merrill and Cowling 1966); decay fungi colonizing the wood in advance of beetle larvae may solubilize and concentrate nutrients for larval consumption. Thus, beetles associated with decay fungi may have a higher probability of successfully exploiting the substrate than those without such associates. Perhaps the closest associations between beetles and fungi identified in wood invasion are those of ambrosia and bark beetles with selected ascomycetes, in which the beetle carries spores and hyphal fragments in specialized mycangia for dissemination to new galleries (Baker 1963; Batra 1963; Batra and Batra 1967; Graham 1967; Norris and Baker 1967; Barras and Perry 1971; Barras and Taylor 1973; Brand et al. 1976; Crowson 1984; Beaver 1986; Furniss et al. 1987). Interactions between fungi and other wood-boring beetles are less well understood.

This study explores the relationship between wood-inhabiting fungi and the golden metallic wood borer (*Buprestis aurulenta* L.), a widely distributed beetle in the Pacific Northwest of the United States (Every and Rudinsky 1975). The female of the species is active between June and October and oviposits its eggs on fire-damaged or wounded living trees as well as on freshly fallen logs that retain their bark (Burke 1918; Helfer 1941). Larval development normally takes 1–4 years but can extend to several decades (Linsley 1943; Chamberlin 1947; Smith 1962). The beetle can survive in large sections of wood that are subsequently processed into poles and timbers, and it is during such processing that it is most frequently encountered as internal galleries are exposed (Spencer 1930; Franz 1936; Smith 1962). Larval damage is not the only concern; wood damaged by *B. aurulenta* also often contains evidence of fungal deterioration. The source of the damage is uncertain, but one possibility is that it is a fungus introduced by the female beetle during oviposition. An association between *B. aurulenta* and various wood-degrading fungi, notably basidiomycetes,

Received June 18, 1998. Accepted January 16, 1999.

C.M. Garcia and J.J. Morrell.[2] Department of Forest Products, Oregon State University, Corvallis, OR 97331, U.S.A. e-mail: garciacm@frl.orst.edu and morrellj@frl.orst.edu.

[1]Paper No. 3211 of the Forest Research Laboratory, Oregon State University, Corvallis, Oreg.
[2]Author to whom correspondence should be addressed.

Table 1. The percentage frequency of isolates of the genera obtained from 80 live, adult *Buprestis aurulenta* beetles by four methods of isolation.

Fungi	Percentage of total isolations	Crawling dissemination	Streaking of washings	Other body parts					
				Gut	Legs	Head	Thorax	Elytra	Total
Basidiomycetes									
Heterobasidion annosum	0.6	0	20	60	20	0	0	0	20
Trametes versicolor	0.2	50	0	0	50	0	0	0	50
Basidiomycete No. 1	0.3	0	67	0	33	0	0	0	33
Basidiomycete No. 2	1.0	33	0	0	11	0	11	44	67
Basidiomycete No. 3	0.6	20	20	0	0	40	20	0	60
Total percentage by group		0.6	0.5	0.3	0.5	0.2	0.2	0.5	1.4
Hyphomycetes									
Trichoderma	31.6	18	6	13	24	11	11	17	63
Penicillium	22.1	13	18	33	13	7	8	8	36
Alternaria	11.4	5	0	20	18	10	18	28	74
Cladosporium	5.2	7	24	13	29	9	4	13	56
Aspergillus	4.2	11	3	14	50	6	11	6	72
Neurospora	2.9	32	0	0	52	12	0	4	68
Scytalidium	2.7	13	30	17	9	4	0	26	39
Drechslera	2.1	11	0	11	11	22	22	22	78
Curvularia	1.2	0	0	0	50	10	10	30	100
Aureobasidium	0.9	12	50	12	12	0	0	12	25
Torula	0.8	14	14	0	0	0	43	29	71
Nigrospora	0.8	0	0	14	0	29	29	29	86
Gliomastix	0.7	17	33	0	17	17	17	0	50
Rhinocladiella	0.7	0	0	50	17	17	0	17	50
Ulocladium	0.6	0	0	0	20	0	40	40	100
Torulomyces	0.5	0	25	25	0	25	0	25	50
Epicoccum	0.3	0	0	0	0	33	67	0	100
Graphium	0.2	0	50	0	0	50	0	0	50
Botrytis	0.1	100	0	0	0	0	0	0	0
Stachybotrys	0.1	100	0	0	0	0	0	0	0
Hyphomycete No. 1	0.2	0	0	50	0	50	0	0	50
Hyphomycete No. 2	0.8	0	14	0	29	0	43	14	86
Hyphomycete No. 3	1.3	9	36	27	0	0	18	9	27
Total percentage by group		12.2	9.8	16.9	19.4	8.7	10.3	14.1	52.5
Zygomycetes									
Rhizopus	1.3	9	9	27	36	0	18	0	54
Total percentage by group		0.1	0.1	0.3	0.5	0.0	0.2	0.0	0.7
Yeasts	4.5	15	36	44	0	0	3	3	5
Total percentage by group		0.7	1.6	2.0	0.0	0.0	0.1	0.1	0.2
Total percentage frequency	100.0	13.6	12.1	19.6	20.3	8.9	10.9	14.7	54.8

Note: Values may not add to 100 because of rounding.

might benefit both fungus and beetle and is of interest both economically and ecologically.

The objective of this study was to determine whether specific basidiomycetes were consistently associated with various body parts of adult *B. aurulenta*.

Materials and methods

Eighty adult *B. aurulenta* beetles were collected from a Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) log deck in Mill City, Oreg., between July and August of 1995. Each beetle was placed in a sterile screw-cap vial (70 mm long by 20 mm in diameter), and its sex was determined by examining the posterior abdominal segments and the last abdominal sternum (Hatch 1971).

Four methods were used to isolate fungi from the beetles: (*i*) allowing beetles to crawl on media; (*ii*) washing body surfaces; (*iii*) extracting body parts; and (*iv*) dissecting the gut. The cultural medium was either 1% malt extract agar (MEA) alone or MEA plus 10 ppm benomyl (to retard growth of ascomycetes and hyphomycetes).

Twenty beetles were used for each method, with equal numbers of beetles or their respective body parts placed on each medium.

For the crawling method, beetles were allowed to crawl on the media in plastic Petri dishes for 30 min (one beetle per dish). For the washing method, each beetle was washed in 10 mL of sterile, distilled water in a test tube and shaken to dislodge any fungal propagules; water samples were then streaked on the surface of the media. For the body parts method, the legs, head, thorax, and elytra were cut with sterile razor and scissors, and each part was planted on the media. For the gut method, beetles were mounted on paraffin, and the abdominal integument was dissected either from

Table 2. Distribution of basidiomycetes, hyphomycetes, zygomycetes, and yeasts on female and male adult *Buprestis aurulenta*.

Fungal group	No. of isolates: Females (59 adults)	No. of isolates: Males (21 adults)	T^a
Basidiomycetes	17	7	0.3685
Hyphomycetes	575	214	0.4452
Zygomycetes	4	7	0.2945
Yeasts	25	14	0.3940
Total	621	242	

[a]Wilcoxon rank sum test results.

the foregut to the aedeagus (for males) or from the foregut through the ovipositor (for females). Each whole gut was directly placed on the media.

All plates were incubated at 23–25°C in an area with intermittent light and were examined for evidence of fungal growth every 2 days. Any growth was immediately subcultured onto fresh media and later identified by means of the available literature (Nobles 1948; Ellis 1971, 1976; Wang and Zabel 1990). The data were then summarized by source of plating matter. Isolation frequencies of the various fungi on male and female beetles were further examined by means of the Wilcoxon rank sum test (Cody and Smith 1991).

Results and discussion

Beetles observed emerging from older logs stayed only a short time before migrating towards newer logs in nearby decks. Swarming was also noted around the debarker, confirming previous observations that the beetles are attracted to resins present on freshly exposed Douglas-fir (Garnett 1918; Smith 1962). The adults were observed ovipositing in checks or cracks in the ends of logs and along the bark surface. The simultaneous deposition of spores into these zones could provide insect-vectored, wood-inhabiting fungi a major competitive advantage over fungi dependent on wind dispersal of spores into such invasion courts.

Isolated fungi

We obtained 863 isolates from the 80 beetles sampled, the greatest number (54.8%) from body parts other than the gut, followed in order by those from the gut, disseminations from crawling insects, and washings (Table 1). Of the body parts other than the gut, the legs were the most frequent source of isolates (20.3%), suggesting that the beetles picked up spores and hyphal fragments as they exited larval galleries and moved over the log surfaces. Isolations from the legs are probably less important than those from other body parts as they can suggest only a casual relationship of beetle and fungi unless mycangia are present on these surfaces. A scanning electron microscope examination of selected beetles from this study failed to show the presence of such structures (Garcia 1996). Propagules were also deposited on the elytra, head, and thorax, from which they could be dislodged on a wood surface during oviposition.

A substantial number of fungi were also isolated from the gut (19.6% of isolates). The role of the gut in fungal dissemination is unclear. Presumably, spores surviving the digestive process could be deposited on fresh logs as the beetles explore the surface for oviposition sites. These transfers might not translate into larval-associated fungal invasions, since excretion might not coincide with oviposition, but they would provide a means for spores to be moved and deposited on the wood along with a potential nutrient source (fecal matter).

In addition to the differences in isolation frequency of fungi from the various body parts, there appeared to be differences between male and female beetles (Table 2), but the Wilcoxon rank sum analysis showed that the differences were not significant.

Wood-staining associates

A variety of wood-inhabiting fungi were isolated (Table 1). Members of the genera *Trichoderma*, *Penicillium*, and *Alternaria* made up 65.1% of all isolates. All three of these genera commonly colonize sapwood. *Alternaria* spp. can cause blue stain in sapwood, while both *Trichoderma* and *Penicillium* spp. can grow through ray cells and rapidly use available stored nutrients. Some species in the latter two genera are antagonistic to decay fungi that might also colonize wood (Freitag et al. 1991).

Basidiomycete associates

While fungal frequency for body parts other than the gut suggests some association between fungi and *B. aurulenta*, basidiomycetes from the beetles represented only 2.8% of all isolates and comprised five taxa (Table 1). Of these, only two were positively identified: *Heterobasidion annosum* (Fr.:Fr.) Bref. and *Trametes versicolor* (L.:Fr.) Pilát. The former is a common agent of root rot of forest trees in the Pacific Northwest and was primarily isolated from the gut. These propagules may have been consumed as the larva fed on wood or inadvertently consumed as the beetle exited the wood after pupation. It produces copious spores that are capable of invading freshly exposed wood. The role of *B. aurulenta* in movement of *H. annosum* is questionable, although this fungus was among the most commonly isolated basidiomycetes in a recent log-decomposition study (Schowalter et al. 1992).

Trametes versicolor, a common decayer of both hardwood and softwood slash, was isolated from the legs and from insects crawling on agar (which also suggests origination on the legs). As noted earlier, the relevance of propagules on the legs in dissemination is questionable. The remaining three taxa, none isolated from the gut, could not be identified. The importance of *B. aurulenta* in disseminating basidiomycete propagules is difficult to assess. The number isolated in relation to all other fungi was small, but 24 of 80 beetles contained them. In separate tests, the basidiomycetes isolated were capable of 4–22% weight loss on pure blocks in a soil bottle test (Garcia 1996). These results indicated that the basidiomycetes isolated were capable of modifying the nutritional status of the wood.

The results show that a variety of fungi are present on and in adult *B. aurulenta*, but no species was consistently isolated in a manner that suggests an interactive association of fungus and insect. Adult beetles appear to collect propagules from many fungal species on their outer bodies. The roles of the various fungi in subsequent colonization of

wood substrates by the beetle larvae are unclear, but the absence of a consistent association of individual fungal species with the adults implies that the association is more casual than that found with other wood-boring beetles.

References

Baker, J.M. 1963. Ambrosia beetles and their fungi, with particular reference to *Platypus cylindrus* Fab. Symp. Soc. Gen. Microbiol. **13**: 232–265.

Barras, S.J., and Perry, T. 1971. Gland cells and fungi associated with prothoracic mycangium of *Dendroctonus adjunctus* (Col., Scol.). Ann. Entomol. Soc. Am. **64**: 123–126.

Barras, S.J., and Taylor, J.J. 1973. Varietal *Ceratocystis minor* identified from mycangium of *Dendroctonus frontalis*. Mycopathol. Mycol. Appl. **50**: 293–305.

Batra, L.R. 1963. Ecology of ambrosia fungi and their dissemination by beetles. Trans. Kan. Acad. Sci. **66**: 213–236.

Batra, S.W., and Batra, L.R. 1967. The fungus gardens of insects. Sci. Am. **217**(5): 112–120.

Beaver, R.A. 1986. The taxonomy, mycangia and biology of *Hypothenemus curtipennis* (Schedl), the first known cryphaline ambrosia beetle (Coleoptera: Scolytidae). Entomol. Scand. **17**: 131–135.

Brand, J.M., Bracke, J.W., Britton, L.N., Markovetz, A.J., and Barras, S.J. 1976. Bark beetle pheromones: production of verbenone by a mycangial fungus of *Dendroctonus frontalis*. J. Chem. Ecol. **2**: 195–199.

Burke, H.E. 1918. Biological notes on some flat-headed woodborers of the genus *Buprestis*. J. Econ. Entomol. **11**: 334–338.

Carpenter, S.E., Harmon, M.E., Ingham, E.R., Kelsey, R.G., Lattin, J.D., and Schowalter, T.D. 1988. Early pattern of heterotroph activity in conifer logs. *In* Fungi and ecological disturbance. *Edited by* R. Wating, A. Lyong, and L. Boddy. Proc. R. Soc. Edinburgh, **94B**: 33–43.

Chamberlin, W.J. 1947. The golden buprestidae: a household pest. Oreg. State Univ. Agric. Exp. Stn. Circ. No. 417.

Cody, R.P., and Smith, J.K. 1991. Applied statistics and the SAS programming language. 3rd ed. Prentice Hall, Englewood Cliffs, N.J.

Crowson, R.A. 1984. The associations of coleoptera with ascomycetes. *In* Fungus–insect relationships: perspectives in ecology and evolution. *Edited by* Q.D. Wheeler and M. Blackwell. Columbia University Press, New York. pp. 256–258.

Edmonds, R.L., and Eglitis, A. 1989. The role of the Douglas-fir beetle and wood borers in the decomposition and nutrient release from Douglas-fir logs. Can. J. For. Res. **19**: 853–859.

Ellis, M.B. 1971. Dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, England.

Ellis, M.B. 1976. More dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, England.

Every, R.W., and Rudinsky, J.A. 1975. The golden buprestid: a wood boring beetle. Oreg. State Univ. Ext. Serv. Circ. No. 713.

Franz, E. 1936. Ein nord-amerikanischer Käfer in einer Frankfurter Aurichte. Nat. Volk, **66**: 370.

Freitag, M., Morrell, J.J., and Bruce, A. 1991. Biological protection of wood: status and future prospects. Biodeterior. Abstr. **5**(1): 1–13.

Furniss, M.M., Woo, J.Y., Deyrup, M.A., and Atkinson, T.H. 1987. Prothoracic mycangium on pine-infesting *Pityoborus* spp. (Coleoptera: Scolytidae). Ann. Entomol. Soc. Am. **80**: 692–696.

Garcia, C.M. 1996. Fungal associates in the golden metallic beetle, *Buprestis aurulenta* L. M.S. thesis, Oregon State University, Corvallis.

Garnett, R.T. 1918. Notes on the genus *Buprestis* Linne. Ann. Entomol. Soc. Am. **11**: 90–92.

Graham, K. 1967. Fungal–insect mutualism in trees and timber. Annu. Rev. Entomol. **12**: 105–126.

Hatch, M.H. 1971. The beetles of the Pacific Northwest. *Rhipiceroides, Sternoxi, Phytophage, Rhynchophora,* and *Lamellicornia*. Vol. 16(5). University of Washington Press, Seattle.

Helfer, J.R. 1941. A revision of the genus *Buprestis* of North America north of Mexico. Entomol. Am. **21**(3): 123–199.

Linsley, E.G. 1943. Delayed emergence of *B. aurulenta* from structural timbers. J. Econ. Entomol. **36**(2): 348–349.

Merrill, W., and Cowling, E.B. 1966. Role of nitrogen in wood deterioration: amount and distribution of nitrogen in fungi. Phytopathology, **56**: 1083–1090.

Nobles, M.K. 1948. Studies in forest pathology. VI. Identification of cultures of wood-rotting fungi. Can. J. Res. **26C**: 281–431.

Norris, D.M., and Baker, J.K. 1967. Symbiosis: effects of a mutualistic fungus upon the growth and reproduction of *Xyleborus ferrugineus*. Science (Washington, D.C.), **156**: 1120–1122.

Schowalter, T.D., Caldwell, B.A., Carpenter, S.E., Griffith, R.D., Harmon, M.E., Ingham, E.R., Kelsey, R.G., Lattin, J.D., and Moldenke, A.R. 1992. Decomposition of fallen trees: effects of initial conditions and heteroph colonization rates. *In* Tropical ecosystems ecology and management. *Edited by* K.P. Singh and J.S. Singh. Wile Eastern Ltd., New Delhi. pp. 373–383.

Smith, D.N. 1962. A note on the longevity and behavior of adult golden buprestids, *B. aurulenta* L. (Coleoptera: Buprestidae) under artificial conditions. Can. Entomol. **94**(6): 590–591.

Spencer, G.C. 1930. Insects emerging from prepared lumber in building. Proc. Entomol. Soc. B.C. 1930: 6–10.

Wang, C.J.K., and Zabel, R.A. 1990. Identification manual for fungi from utility poles in the eastern United States. Allen Press Inc., Lawrence, Kan.