2008

Detection of Host Habitat by Parasitoids Using Cues Associated With Mycangial Fungi of the Mountain Pine Beetle, *Dendroctonus ponderosae*

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Detection of host habitat by parasitoids using cues associated with mycangial fungi of the mountain pine beetle, *Dendroctonus ponderosae*

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Abstract—Cues used by parasitoids to detect habitat of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae), were investigated by observing parasitoid attraction to logs infested with *D. ponderosae*, logs inoculated with one or both of the symbiotic fungi of *D. ponderosae* (*Grosmannia clavigera* (Rob.-Jeffr. & R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf. (Ophiostomataceae) and *Ophiostoma montium* (Rumbold) Arx (Ophiostomataceae)), logs containing no beetles or fungi, or empty screen cylinders. Captures of *Heydenia unica* Cook and Davis (Hymenoptera: Pteromalidae) and *Rhopalicus pulchripennis* (Crawford) (Hymenoptera: Pteromalidae) on logs with both *G. clavigera* and *O. montium* were greater than those from control treatments. These results suggest that characteristics of tree tissues simultaneously colonized by the two symbiotic fungi facilitate a detectable change in the volatile compounds released from *D. ponderosae*-attacked trees that may be used by parasitoids to locate hosts.

Résumé—Nous étudions les signaux utilizés par les parasitoïdes pour détecter l'habitat du dendroctone du pin ponderosa, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae), en observant l'attrait des parasitoïdes pour des troncs infestés par *D. ponderosae*, des troncs inoculés par l'un ou les deux champignons symbiotes de *D. ponderosae* (*Grosmannia clavigera* (Rob.-Jeff. & R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf. (Ophiostomataceae) et *Ophiostoma montium* (Rumbold) Arx (Ophiostomataceae)), des troncs ne contenant ni coléoptères ni champignons et des cylindres vides en grillage. Les captures d'*Heydenia unica* Cook et Davis (Hymenoptera: Pteromalidae) et de *Rhopalicus pulchripennis* (Crawford) (Hymenoptera: Pteromalidae) sur des troncs portant à la fois *G. clavigera* et *O. montium* sont plus importantes que sur les troncs témoins. Ces résultats indiquent que les caractéristiques associées aux tissus des arbres colonizés simultanément par les deux champignons symbiotes entraînent un changement perceptible dans la libération des substances volatiles par les arbres attaqués par *D. ponderosae* et que ces changements peuvent être utilizés par les parasitoïdes pour repérer leurs hôtes.

[Traduit par la Rédaction]

Natural enemies typically locate host habitat using chemical cues that are reliably associated with their host. Such olfactory cues may originate from host products such as frass (Sullivan *et al.* 2000) or pheromones (Payne *et al.* 1984), or from organisms associated with their host, including symbiotic microorganisms (Madden 1968) or plants fed upon by the host (Schnee *et al.* 2006).

Bark beetles (Coleoptera: Curculionidae: Scolytinae) are associated with fungi in the Ophiostomatales and include the genera *Ophiostoma*, *Grossmania*, and *Ceratocystiopsis* and their anamorphs (Upadhyay 1981). In culture, these fungi produce a number of volatile compounds, including oxygenated monoterpenes, cyclic sesquiterpenes, hydrocarbons, and short-chain alcohols and esters (Hanssen 1993). These compounds could be detected and exploited by natural enemies attacking bark beetle larvae. Bark colonized by *Ophiostoma* spp. associated with the bark beetles *Ips grandicollis* (Eichhoff) and *Dendroctonus frontalis* Zimmermann was attractive to the parasitoids *Roptrocerus xylophagorum*

Received 8 April 2007. Accepted 5 September 2007.

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(Ratzeburg) (Hymenoptera: Pteromalidae) and *Spathius pallidus* (Ashmead) (Hymenoptera: Braconidae) (Sullivan and Berisford 2004), respectively. Furthermore, larvae in phloem without fungi were less attractive to *R. xylophagorum* than larvae in phloem with their normal complement of microorganisms (Sullivan and Berisford 2004), suggesting that the origin of one attractant was the associated microorganisms.

Dendroctonus ponderosae Hopkins is consistently associated with two filamentous fungi, *Grosmannia clavigera* (Rob.-Jeff. & R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wingf. (Ophiostomataceae) (formerly *Ophiostoma clavigerum*) and *Ophiostoma montium* (Rumbold) Arx (Ophiostomataceae) (Six and Bentz 2007). With the exception of early instars, larvae are in physical contact with, and feed upon, the fungi throughout development (Adams and Six 2007). The consistent association of the growth of these fungi specifically with *D. ponderosae* brood may provide a reliable mechanism for fine-scale discrimination of host signals. The objective of this study was to determine if cues from the two primary fungal associates of *D. ponderosae* are utilized by parasitoids when locating host habitats.

An active infestation of *D. ponderosae* in lodgepole pine, *Pinus contorta* Dougl. *ex* Loud. var. *latifolia* Engelm. *ex* S. Wats. (Pinaceae), was located near Flat Rock Creek, Lolo National Forest, approximately 130 km west of Missoula, Montana (47°23′N, 115°14′W, 3200 ft elevation). This area had experienced outbreak population levels of *D. ponderosae* for 9 years prior to the inception of the study in 2003.

A complete randomized block design sticky-trap experiment was established with nine blocks within a 1 km × 2 km area, with each block located in zones of *P. contorta* that were currently infested with *D. ponderosae*. Six bait treatments were tested for parasitoid attraction: (1) empty screen cylinders, (2) logs experimentally infested with *D. ponderosae* and its natural complement of microorganisms, (3) logs without beetles or fungi, (4) logs inoculated with *G. clavigera*, (5) logs inoculated with *O. montium*, and (6) logs inoculated with both *G. clavigera* and *O. montium*. In each block, six traps were hung 1 m off the ground, 10 m apart, and no closer than 3 m to infested trees along a transect parallel to the slope. Blocks were no less than 30 m apart. Treatments were initially assigned at random to traps and re-randomized at sampling. Logs for treatment application were taken from four beetle-free *P. contorta* and cut into 40 cm lengths (diameters ranged from 20 to 30 cm and were balanced among treatments).

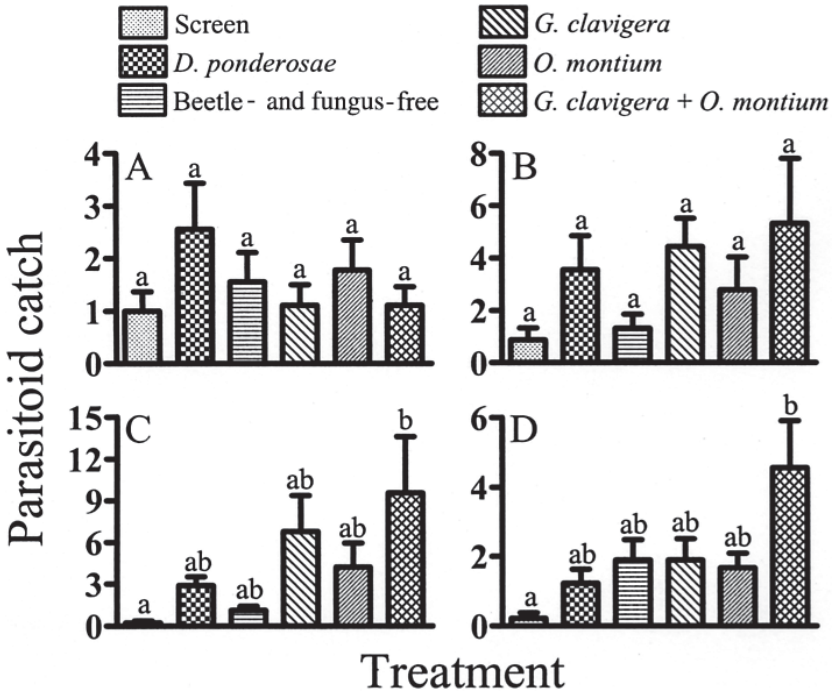
Strains of *G. clavigera* and *O. montium* used for inoculations were isolated from galleries of *D. ponderosae* in naturally attacked trees at one of the study sites. Isolates were purified and then identified using morphological characteristics.

Logs treated with fungi received inoculation at six points equally spaced around the circumference 5 cm from the top and 5 cm from the bottom of each log. A disk of bark (1.2 cm diameter) was removed and a disk of malt extract agar (4 mm diameter) colonized by either *G. clavigera* or *O. montium* was placed on the exposed sapwood. The bark plug was then replaced and sealed with paraffin. Inoculations for the treatment that included both fungi were prepared by alternating *G. clavigera* and *O. montium* among the inoculation points so that each fungus was evenly distributed around the log. Logs without fungi or beetles were treated in the same manner as logs inoculated with fungi; however, the disks of malt extract agar were sterile. All logs were enclosed with fine-mesh screen. Screen cylinders without logs were used as controls. Logs and screen cylinders were placed in the field and enclosed with hardware cloth coated with aerosol Tangle-Trap® (The Tanglefoot Company, Grand Rapids, Michigan). Captured insects were collected every 4 d from 7 July to 28 August.

Total numbers of parasitoids, by species, were summed over time within each block ($n = 9$) prior to one-way Kruskal–Wallis ANOVA on ranks (SigmaStat® version 2.03). Only captures of female parasitoids were analyzed. All *post-hoc* comparisons were made using Dunn's method.

Captures of *Heydenia unica* Cook and Davis (Hymenoptera: Pteromalidae) and *Rhopalicus pulchripennis* (Crawford) (Hymenoptera: Pteromalidae) on logs inoculated with both *G. clavigera* and *O. montium* were significantly greater than on controls (Dunn's method, $P < 0.01$ for both tests). Parasitoid captures on logs with *D. ponderosae* brood, logs inoculated with *G. clavigera*, and logs inoculated with *O. montium* were not significantly different than on the screen control or beetle- and fungus-free logs (Fig. 1). Captures of *Coeloides rufovariegatus* Provancher (Hymenoptera: Braconidae) and *Dendrosoter scaber* Muesebeck (Hymenoptera: Braconidae) did not differ significantly among treatments.

Fig. 1. Mean numbers (error bars represent the standard error) of *Coeloides rufovariegatus* (A), *Dendrosoter scaber* (B), *Heydenia unica* (C), and *Rhopalicus pulchripennis* (D) caught at each block (n = 9) over the course of the season on empty screen cylinders, logs containing *Dendroctonus ponderosae* and its mycangial fungi, beetle- and fungus-free logs, logs with *Grosmannia clavigera,* logs with *Ophiostoma montium*, and logs with both *G. clavigera* and *O. montium*. Bars with the same letter were not significantly different (Dunn's method, $P > 0.05$).



Release of oxygenated monoterpenes from beetle-infested trees is thought to be responsible for host-habitat detection by several parasitoid species (Pettersson 2001). Trees that are not colonized by beetles or fungi emit low levels of monoterpene hydrocarbons, whereas trees successfully attacked by bark beetles release oxygenated monoterpenes (Pettersson and Boland 2003). Release of oxygenated monoterpenes can result from microbial activity, including that of fungi (Leufvén *et al.* 1988), and thus account for attraction to logs containing both *G. clavigera* and *O. montium*. Alternatively, these compounds also form as a result of spontaneous resin oxidation (Birgersson and Bergström 1989). Resin oxidation would have occurred in all treatment logs used in this study, which may account for captures of parasitoids on logs without fungi (Camors and Payne 1972).

If fungi are the source of cues used to locate host beetles, then the most reliable signal should be produced by the most consistent associate of the beetles in both space and time. Since olfactory cues composed of many compounds have the potential to convey more information than cues consisting of one or a few compounds, the use of complex host-location cues may give parasitoids a greater capacity to distinguish profitable foraging sites.

We thank Andrew Christie, Cameron Patterson, David Chimo, Millie Bowman, Ted Benson, and Sandye and Oakleigh Adams for field and laboratory assistance. We also thank James Johnson for aid in identification of collected parasitoids. We also appreciate the help of Glenn Koepke of the Lolo National Forest, Superior Ranger District, Montana. The manuscript benefited greatly from the comments of three anonymous reviewers. This research was supported by the McIntire–Stennis Cooperative Forestry Program and was conducted as part of Regional Research Project W-187.

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