SCIENTIFIC NOTE

Ambulatory responses of *Laricobius nigrinus* (Coleoptera:Derodontidae), a hemlock woolly adelgid predator, to odors from prey, host foliage, and feeding conspecifics

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Behavioral interactions between insects and their environments are often mediated by volatile cues. Plant-produced chemical cues induced by herbivore activity are often more effective at attracting predators than are cues produced by the herbivore alone (Dicke and van Loon 2000). The presence of herbivore-induced plant volatiles makes foraging by predators more efficient than undirected hunting (Dannon *et al*. 2010), presumably because they indicate the presence of prey. Just as it is common for predators to find prey using olfactory cues, they can also use chemical cues to avoid intraguild competition or aggression (Janssen *et al*. 1995; Cakmak *et al*. 2006, Flowers *et al*. 2007), and evidence suggests that avoidance is a common response to the reception of a conspecific cue from a particular location (Janssen *et al*. 1995; Stout and Goulson 2001; Gnanvossou *et al*. 2003). Understanding these interactions may be important to the implementation of a biological control program in which a natural enemy complex is released and established, because they are integral to how predators find their hosts (Flowers *et al*. 2007). Information about predator interactions can inform the optimal release density of agents on the landscape and increase the efficiency and effectiveness of programs.

Classical biological control practices are being implemented in the eastern United States (U.S.A.) to control on invasive hemlock woolly adelgid (Hemiptera:Adelgidae, *Adelges tsugae*) populations on eastern hemlock (*Tsuga canadensis* (L.) Carriere) and Carolina hemlock (*Tsuga caroliniana* Engelmann) (DeBach 1974; McEvoy 1996; McDonald 2010). *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) is an adelgid predator associated with hemlock woolly adelgid on western hemlock (Tsuga heterophylla (Raf.) Sarg.) in the northwestern U.S.A. (Kohler *et al*. 2008) and British Columbia, Canada (Zilahi-Balogh *et al*. 2002). This predator is considered a promising candidate agent for biological control of hemlock woolly adelgid on eastern hemlock (Mausel *et al*. 2012). The objective of this study was to determine whether field-collected *L. nigrinus* responded to host and prey odors in an olfactometer, and to observe whether adding a conspecific individual feeding on hemlock woolly adelgid on host foliage would alter the orientation preferences of *L. nigrinus*.

In 2011 and 2012, *Laricobius* adults were field collected in the vicinity of Banner Elk, North Carolina, USA. (36.165643°N, 81.872118°W). Eastern hemlock foliage infested with hemlock woolly adelgid was obtained from trees near the *Laricobius* collection sites described previously, whereas uninfested eastern hemlock foliage was collected in South Burlington, Vermont, USA (44.4669° N, 73.1714° W). All clipped foliage was similarly handled and stored until used in the bioassays.

Two behavioral bioassays, conducted in the winters of 2011 and 2012, were used to test the ambulatory responses of adult *L. nigrinus* to various stimuli in a four-chambered olfactometer (Analytical Research Systems, #OLFM-4-C-2440PE, Gainesville, Florida, USA) identical to the one described in Wallin *et al*. (2011). One of the following four stimulus treatments was assigned to each glass chamber, which in turn was attached to a randomly chosen arm of the olfactometer: a

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Table 1

Ambulatory responses of *L. nigrinus* individuals to odors from host foliage, prey and conspecifics in a four-way olfactometer over two years of bioassays. Response values sharing the same letter are not significantly different (p>0.05).

Stimulus Field	Proportion of Choice by Year		
	2011 (N=55)	2012 (N=31)	Pooled years (N=86)
Feeding Beetle	0.13	0.16	0.14^{b}
E. Hemlock with HWA	0.35	0.29	0.33^{a}
E. Hemlock	0.24	0.26	0.24^{ab}
Blank Control	0.15	0.13	0.14^{b}
Center Field	0.15	0.16	0.15^{b}
	χ^2=9.273 p=0.055	χ^2=3.032 p=0.552	χ^2=11.791 p=0.019*

A Cochran Q test was completed for each year of data and the pooled data set. An asterisk indicates a significant difference in preference (p<0.05).

blank control (empty chamber), eastern hemlock foliage, eastern hemlock foliage infested with hemlock woolly adelgid, and eastern hemlock foliage with *Laricobius* feeding on hemlock woolly adelgid (hereafter called the feeding-beetle treatment). In the feeding-beetle treatment, a beetle was placed on a piece of adelgid-infested foliage and allowed to settle and begin feeding prior to placement of the foliage in the chamber. Foliage was infested with at least 10 adelgids per centimetre of twig length, and pieces of foliage with approximately equal densities of adelgids were used for each treatment and replicate. The foliage containing the feeding beetle was not placed into the glass chamber until the feeding beetle was visibly nestled among the adelgid wax and no longer moving. Between each replicate, foliage and the feeding beetle were replaced, and the chambers were cleaned with ethanol and allowed to dry. Chambers were randomly reassigned for each replicate.

Responses of individual *Laricobius* to treatments were measured using methodology similar to Wallin *et al*. (2011) and Arsenault (2013). A single individual was placed in the center of the arena, equidistant from the entrance of each arm. Individuals were allowed to walk about the arena for up to 10 minutes, and their choice was recorded. Bioassays were completed, and final positions were recorded when: 1) a beetle remained in a field boundary for at least one minute; 2) the 10-minute time limit was reached; or, 3) a beetle attempted to crawl into an arm. After bioassays, *Laricobius* specimens were identified as *L. nigrinus*, native *L. rubidus* or hybrids, via microsatellites as described in Havill *et al*. (2012) at the USDA Forest Service Northern Research Station, in Hamden, CT. Only *L. nigrinus* were included in the analysis so as to not confound the data. The 2011 and 2012 bioassays included 55 and 31 *L. nigrinus* beetles, respectively. Because methodology for live sexing of *Laricobius* was not published until after the completion of this study, and *Laricobius* does not orient using pheromones (Shepherd *et al*. unpublished data), beetles were not sexed as part of this study.

The proportions of beetles choosing various treatment fields for their final positions in the olfactometer were compared using the Cochran Q test. Analyses were made separately for each year (2011; 2012), as well as for pooled data from both years.

Laricobius nigrinus responded to odors in the olfactometer by walking in the arena and choosing a stimulus field over the centre field or blank chamber in both 2011 and 2012. Treatment stimulus had significant effect on the proportion of *L. nigrinus* choosing a field in 2011, and when the 2011 and 2012 data were pooled (Table 1). In the pooled data, a greater proportion of *L. nigrinus* chose the stimulus field containing eastern hemlock infested with hemlock woolly adelgid than the other fields (Table 1). The stimulus field containing the feeding beetle was consistently among the least preferred options (Table 1).

Laricobius nigrinus reliably responded to host foliage with hemlock woolly adelgid, but there was no significant difference in preference for infested versus uninfested foliage. As described in Wallin *et al.* (2011), the similarity in preference between these two host treatments may be due to the low detectability of the adelgid in the olfactometer without added information from visual and tactile stimuli. This phenomenon has been described as the reliability–detectability problem (Vet *et al.* 1991), where the magnitude and surface area available for release of olfactory cues is much greater for the foliage than for the prey. However, odors from hosts alone are not necessarily a reliable indication of prey availability, so predators can use a combination of these, as well as herbivore-induced volatile cues emitted by the host when foliage is wounded through feeding (Agrawal 1998; Dicke and Van Loon 2000; Havill and Raffa 2000; Radville *et al.* 2011). The data presented here support that *L. nigrinus* predators are attracted to hemlock woolly adelgid and host odors, and may use hemlock foliage as a proxy due to low detectability of prey.

In summary, this study presents evidence that *L. nigrinus* may use volatile cues to avoid feeding conspecifics when foraging for prey. Use of chemical cues may enhance dispersal of *L. nigrinus* populations across the *A. tsugae* prey resource on hemlock trees in the field. Additional research is needed to better understand how volatile cues may be integrated with visual, tactile, or other stimuli during the host-finding behavior of this important biological control agent in North American hemlock forests.

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