Orientation Behavior of the Predator *Laricobius nigrinus* (Coleoptera: Derodontidae) to Hemlock Woolly Adelgid and Host Tree Odors in a Multi-Chambered Olfactometer

KIMBERLY F. WALLIN,[1,2] TANYA M. LATTY,[3] AND DARRELL W. ROSS[4]

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ABSTRACT We studied the adult ambulatory response of the predator, *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), to odors from its prey, *Adelges tsugae* Annand, the hemlock woolly adelgid, and foliage of hemlock woolly adelgid, host hemlocks (*Tsuga* spp.), and other conifers. Both the predator and hemlock woolly adelgid are apparently native to western North America, but the predator is being released in the eastern United States, which has different hemlock species, for biological control of a lineage of hemlock woolly adelgid inadvertently introduced from Japan. *L. nigrinus* responded to odors from hemlock woolly adelgid host trees, but not to odors from hemlock woolly adelgid. *L. nigrinus* collected from hemlock woolly adelgid-infested western hemlock were more strongly attracted to odors from western hemlock [*Tsuga heterophylla* (Rafinesque) Sargent] than eastern hemlock [*Tsuga canadensis* (L.) Carrière] in most trials. Odors from western white pine (*Pinus monticola* Douglas ex D. Don) and white spruce [*Picea glauca* (Moench) Voss] were as attractive as western hemlock odors whereas odors from Douglas-fir [*Pseudotsuga menziesii* variety *menziesii* (Mirbel)] and ponderosa pine (*Pinus ponderosa* Douglas ex Lawson) were avoided. *L. nigrinus* reared on hemlock woolly adelgid-infested eastern hemlock in the laboratory were lethargic and were not attracted to either eastern or western hemlock odors. Predators collected in the field and tested monthly from December to March responded similarly each month, except February, when they flew rather than walked in the olfactometer, suggesting a period of dispersal or mate finding at that time of year. The implications of these results for programs to release *L. nigrinus* in the eastern United States for control of hemlock woolly adelgid are discussed.

KEY WORDS *Adelges tsugae*, biological control, host finding behavior, predator-prey interactions, *Tsuga* spp.

Chemical stimuli emanating from a plant-herbivore complex can originate from the herbivore, the plant, or result from the plant-herbivore interaction (Harmel et al. 2007). Stimuli generated by the herbivore prey are the most reliable source of information to a predator because they can inform the predator of the presence, identity, availability, and suitability of the prey (Whitman 1988, Gingras et al. 2002). Although these stimuli are reliable, they may not be apparent or available. Herbivore-derived information has two inherent constraints that limit its detectability and, therefore, its use as stimuli for prey location. Generally, herbivore prey are a small component of a complex environment and if they produce any information, it will be in small amounts. Secondly, there should be constant selection on the prey to be inconspicuous to avoid predation. Stimuli from the host plant of the herbivore prey are usually more readily available because of the plants comparatively large biomass, but are less reliable predictors of herbivore prey presence and suitability (Lima and Dill 1990, Dicke 1999, Cortesero et al. 2000). Understanding interactions among host plants, herbivore prey, and predator behavior may uncover important aspects of the biology of the predator that would otherwise be unnoticed, such as the influence of prerelease handling (e.g., laboratory rearing, level of satiation), and the response of predators to plant odors and herbivore-induced plant odors.

The hemlock woolly adelgid, *Adelges tsugae* Annand, is an introduced insect pest of eastern hemlock (*Tsuga canadensis* (L.) Carrière) and Carolina hemlock (*Tsuga caroliniana* Engelmann) (McClure 1996). Until recently, it was thought that the hemlock woolly adelgid also was introduced into western North America. However, genetic analyses have shown that there is no evidence of a recent introduction to western North America (Havill et al. 2007), and populations in the eastern United States were introduced from south-

[1] Corresponding author, e-mail: kwallin@uvm.edu.
[2] USDA Forest Service, Northern Research Station, Burlington, VT 05403.
[3] School of Biological Sciences, The University of Sydney, Macleay Building, Sydney, Australia.
[4] Department of Forest Science, 321 Richardson Hall, Oregon State University, Corvallis, OR 97331.

ern Japan (Havill et al. 2006). Hemlock species in North America vary in susceptibility to hemlock woolly adelgid. Eastern hemlocks are highly susceptible and usually die after being infested for a few years (McClure 1996). Western hemlock [*Tsuga heterophylla* (Rafinesque) Sargent] and mountain hemlock (*Tsuga mertensiana* (Bongard) Carrière.), species native to western North America are rarely injured by hemlock woolly adelgid in forests, although ornamental trees are sometimes weakened or killed (Furniss and Carolin 1977). Tree resistance and natural enemies likely contribute to keeping hemlock woolly adelgid populations below levels that cause mortality of hemlocks in the western United States (Montgomery and Lyon 1996, Kohler et al. 2008).

Research on biological control of hemlock woolly adelgid has been ongoing for over a decade and, recently, *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) has been shown to be a promising biological control agent. Based on museum specimens, *L. nigrinus* is known to occur in Alaska, Alberta, British Columbia, California, Idaho, Oregon, Washington, and Wyoming; with most records from western hemlock, but also western larch (*Larix occidentalis* Nuttall) and western white pine (*Pinus monticola* Douglas ex D. Don.) (Fender 1945, Lawrence 1989, Leschen 2011). It has been collected frequently on hemlock woody adelgid-infested western hemlocks near Victoria, British Columbia (Zilahi-Balogh et al. 2002; 2003a,b) and in Oregon and Washington (Kohler et al. 2008). In addition, *L. nigrinus* has been found in abundance on western white pine infested with *Pineus* spp. in Idaho (S. P. Cook, personal communication). *L. nigrinus* has a life cycle that is synchronous with hemlock woolly adelgid (Zilahi-Balogh et al. 2003a,b). Furthermore, in laboratory studies, its larvae only completed development in association with hemlock woolly adelgid, and not with *Adelges piceae* (Ratzeburg), *Pineus strobi* (Hartig), *Adelges abietis* (L.), *Cinara pilicornis* (Hartig), or *Chionapsis pinifoliae* (Fitch) (Zilahi-Balogh et al. 2002).

The family Derodontidae is poorly studied because it is a small family and, until recently, not considered of economic importance. All members of the family are apparently mycophagous except for the genus *Laricobius*, which has shifted to a close predatory association with Adelgidae (Lawrence and Hlavac 1979, Lawrence 1989, Zilahi-Balogh et al. 2002). Because of its potential as a biological control agent of hemlock woolly adelgid, *L. nigrinus* has received considerable attention in recent years (Zilahi-Balogh 2001; Zilahi-Balogh et al. 2002; 2003a,b; Broeckling and Salom 2003; Flowers et al. 2005). It is not known what *L. nigrinus* fed upon and developed on before the introduction of hemlock woolly adelgid into the Pacific Northwest. One possible explanation may be that hemlock woolly adelgid is native to the Pacific Northwest (Havill et al. 2007). In that case, *L. nigrinus* prey location behavior may have evolved with the herbivore host tree, western hemlock. Alternatively, before the introduction of hemlock woolly adelgid in the Pacific Northwest, *L. nigrinus* may have been associated with several adelgid species and the introduction of hemlock woolly adelgid provided a more abundant food resource. In that case, *L. nigrinus* prey location behavior would include additional tree species. Evaluation of a biological control agent requires an understanding of the mechanisms employed by the agent to recognize and orient toward the target organism (Cortesero et al. 2000), in this case, hemlock woolly adelgid and hemlock trees. However, other than the study conducted by Broeckling and Salom (2003), there has been little attention given to this specialist's prey location behavior.

Our objective was to study the role of prey and host tree odors in prey location by *L. nigrinus.* Here, we report the results of a series of laboratory experiments by using four-armed olfactometer behavioral bioassays. The first experiment characterized the response of *L. nigrinus* to its prey, hemlock woolly adelgid, in the absence of host plant material. Second, we evaluated responses of *L. nigrinus* to two host plants of hemlock woolly adelgid, eastern and western hemlock, with and without hemlock woolly adelgid present. Third, we evaluated responses of *L. nigrinus* to hemlock woolly adelgid host trees and host trees of other adelgid species. Lastly, we characterized the responses of *L. nigrinus* that developed on hemlock woolly adelgid feeding on eastern hemlock under laboratory conditions and those that were field collected from hemlock woolly adelgid-infested western hemlocks.

Materials and Methods

Behavioral bioassays were conducted to test the responses of adult beetles to hemlock woolly adelgid and several tree species. Bioassays were conducted in 2005 and 2006. We tested adult *L. nigrinus* collected from western hemlocks at 16 sites in Washington and Oregon (Kohler et al. 2008) or reared under laboratory conditions on eastern hemlock branches. Beetles were brought to the laboratory and held at 16°C with a photoperiod of 12:12 (L:D) h on hemlock woolly adelgid-infested western hemlock branches for 24–48 h. To standardize beetle condition we placed individual beetles into 2-cm petri dishes lined with moistened filter paper but without food for 24 h before conducting the bioassay. Depriving beetles of food for 24 h before assaying their searching behavior increased their movement toward host material versus remaining stationary in the olfactometer (K.F.W., unpublished data). All beetles were randomly chosen from a test group, tested once, and not used for further testing. Twenty beetles were tested each month in each experiment, except experiment 5, when 25 beetles were tested.

Olfactory Bioassay Procedure. The four-arm olfactometer (Analytical Research Systems, #OLFM-4-C-2440PE, Gainesville, FL) had the same shape as the one designed by Pettersson (Vet et al. 1983), but was enlarged to 30 by 30 by 3 cm. The olfactometer was made of opaque high-density polyethylene with a polymethyl methacrylate transparent lid. It consisted

of three parts: the base with the air output; the intermediate part that delimited the walking chamber (3 cm high, 1-l volume) with the four air inputs; and the lid (1 cm thick) with a 9-mm circular central opening to introduce insects. Two Neoprene (DuPont, Wilmington, DE) rings and four large thumb screws ensured a secure and airtight fit between the three parts. We used the same constant flow rates (0.12 Mpa) in all four arms. Air was brought to the olfactometer through Tygon 2275 plastic tubes (ID = 10 mm) that had high chemical inertness and good flexibility. Arms with chambers receiving air but without test material were regarded as 'blank' or control chambers. Four flow-meters (Brooks Instrument, Hatfield, PA) controlled airflow into the glass chambers containing the test material and carrying volatiles into the olfactometer. A diaphragm pump (Cole Parmer, Vernon Hills, IL) was used to draw air at constant flow rates (−0.1 Mpa), to the center and out of the arena thereby preventing the mixing of volatiles within the assay arena.

All ambulatory responses were tested at 20°C with standard laboratory lighting. The four-arm olfactometer was first tested without hemlock woolly adelgid or host tree material to ensure the laboratory environment did not bias ambulatory behavior. For each experimental run an individual beetle was introduced at the center of the olfactometer, equidistant from the entrance to all four olfactometer arms. Each test lasted 15 min. Two criteria were used to quantify behavior: 1) time spent in each field, and 2) the field the insect was in when the test ended (final position). When air passed through a chamber into the olfactometer stage, each of the airfields was considered to be a separate field. Each airfield was 3 cm high, 3 cm across, and 10 cm in length from the opening of each glass chamber receiving air as part of the experiment. However, there was an additional central field (CF) that corresponded to a 9-cm circular central zone where the airflows from the four arms exited the olfactometer. We followed the insects visually and recorded their entries into the different fields, then calculated mean time spent in each field. We considered that an insect entered a given field when its entire thorax crossed the field boundary and remained in the field for >1 min. A test was not retained when an insect remained motionless in the CF for >5 min.

A maximum of 30 bioassay trials could be conducted with the olfactometer in one day. We replaced plant material with fresh plant material cut from the distal end of branches every 2 h for experiments lasting longer than 2 h. Assignment of treatments to the olfactometer arms was rerandomized within each replication for each experiment. Cleaning the walking arena and the cover plate with ethanol and demineralized water between trials minimized contamination of the walking arena with sample odors or by possible pheromones.

Experiment 1: Does *L. nigrinus* Respond to Odors From its Prey, Hemlock Woolly Adelgid? Two-Way Choice. We observed and recorded the response of *L. nigrinus* collected from western hemlock in Oregon and Washington to hemlock woolly adelgid. We collected western hemlock branches infested with hemlock woolly adelgid from trees at the Weyerhaeuser Company's Eola Hills Seed Orchard ≈12 km northwest of Salem, OR. Branches were stored in an environmental chamber at 16°C with a photoperiod of 12:12 (L:D) h and with cut ends submerged in water. Within 24 h of field collection we removed 10 woolly masses from the western hemlock branch and placed them into one chamber of the olfactometer. One chamber was left empty to serve as the blank control. Each chamber was attached to a randomly chosen arm of the olfactometer. Airflow was limited to the arms containing hemlock woolly adelgid woolly masses and the blank control. This experiment was conducted in December 2005 and in January and March 2006.

Experiment 2: Does *L. nigrinus* Respond to Odors from Hemlock Woolly Adelgid Host Trees? Three-Way Choice. We observed and recorded the responses of *L. nigrinus* collected from western hemlock in Oregon and Washington to infested and uninfested eastern and western hemlocks. Branches of eastern and western hemlock with and without hemlock woolly adelgid present were collected and held in separate environmental chambers as described for experiment 1. Eastern hemlock branches were collected from trees at the Hoyt Arboretum in Portland, OR, and western hemlock branches were collected from trees at Weyerhaeuser Company's Eola Hills Seed Orchard. Behavioral assays were conducted within 24 h of collecting the hemlock branches. We tested the tree species in separate trials. These assays were conducted in December 2005 and February and March 2006.

Experiment 2a. One 5-cm-long branch of both hemlock woolly adelgid-infested and uninfested eastern hemlock was randomly placed into a separate chamber. The two treatments and blank control were attached to randomly chosen arms of the olfactometer.

Experiment 2b. This assay was repeated using hemlock woolly adelgid-infested and uninfested western hemlock.

Experiment 3: Does *L. nigrinus* Distinguish Between Odors Emitted From Eastern Versus Western Hemlock? Three-Way Choice. We observed and recorded the responses of *L. nigrinus* collected from western hemlock in Oregon and Washington to uninfested eastern versus uninfested western hemlock branches. We collected and stored eastern and western hemlock branches as described for experiments 1 and 2. 5-cm-long branches of eastern and western hemlock were randomly placed into separate chambers. The two treatments and blank control were randomly attached to arms of the olfactometer. This assay was conducted monthly from November 2005 to March 2006.

Experiment 4: Does *L. nigrinus* Respond to, Prefer Odors, or Both, From Host plants of Hemlock Woolly Adelgid, Other Adelgid Species, or Both? Four-Way Choice. We observed and recorded the responses of *L. nigrinus* collected from western hemlock in Oregon and Washington to western and eastern hemlock, and several other conifer species that are not currently

Table 1. Ambulatory response of adult *L. nigrinus* to odors from hemlock woolly adelgid (HWA) and host tree branches in laboratory olfactometer bioassays

Experiment no. and stimulus field in olfactometer	Month of bioassay and mean (± SE) time spent in field, min[a]			Month of bioassay and % final position in each field[b]		
Experiment 1	Dec.	Jan.	Mar.	Dec.	Jan.	Mar.
HWA	3.1 ± 0.6b	3.0 ± 1.3b	3.0 ± 1.7b	10b	20b	10b
Blank control	4.9 ± 1.5a	5.0 ± 1.2a	5.0 ± 1.3a	10b	10b	15b
Central field	7.0 ± 1.5a	7.0 ± 2.5a	6.8 ± 3.2a	80a	70a	75a
Experiment 2a	Dec.	Feb.[c]	Mar.	Dec.	Feb.[c]	Mar.
Eastern hemlock	5.5 ± 1.5a	-	5.8 ± 1.5a	30a	-	40a
Eastern hemlock + HWA	4.5 ± 1.2a	-	5.0 ± 2.7a	45a	-	35a
Blank control	3.0 ± 0.8b	-	2.2 ± 2.2b	10b	-	15b
Central field	2.0 ± 1.5b	-	2.0 ± 1.8b	15b	-	10b
Experiment 2b	Dec.	Feb.[c]	Mar.	Dec.	Feb.[c]	Mar.
Western hemlock	6.0 ± 1.5a	-	6.8 ± 3.2a	50a	-	45a
Western hemlock + HWA	7.0 ± 3.8a	-	6.0 ± 1.8a	35a	-	45a
Blank Control	1.0 ± 2.5b	-	1.0 ± 3.5b	10b	-	5b
Central field	1.0 ± 2.5b	-	1.2 ± 3.5b	5b	-	5b

[a] Means in each column within an exp followed by the same letter are not significantly different by multiple comparison test of ranked data based on Nemenyi test, $P > 0.05$. There were no significant differences among months, $P > 0.05$.

[b] Percentages in each column within an exp followed by the same letter indicate no significant difference in the numbers in final positions by multiple contrasts of data subjected to the Cochran Q test, $P > 0.05$.

[c] During all experiments conducted in Feb., *L. nigrinus* flew around the olfactometer rather than walking and, therefore, those data were not analyzed.

known to be hosts of hemlock woolly adelgid. Each bioassay included one blank control chamber, a chamber with one 5-cm-long branch of western hemlock, and a chamber with one 5-cm-long branch of eastern hemlock. In the fourth chamber, we placed either one 5-cm-long-branch of white spruce (*Picea glauca* (Moench) Voss), Douglas-fir [*Pseudotsuga menziesii* variety *menziesii* (Mirbel) Franco], ponderosa pine (*Pinus ponderosa* Douglas ex Lawson), or western white pine. All branches from both hemlock species were collected as described for experiments 1 and 2. Branches of all other species were collected from trees at the Oregon State University Peavy Arboretum located ≈13 km north of Corvallis, OR. This experiment was conducted monthly from December 2005 to March 2006.

Experiment 5: Does the Odor From Host Trees of Hemlock Woolly Adelgid (Eastern or Western Hemlock) in which *L. nigrinus* Developed on Affect its Response to Eastern or Western Hemlock Odors? Three-Way Choice. We observed and recorded the responses of *L. nigrinus* reared from egg to adult on hemlock woolly adelgid-infested eastern hemlock or collected in the field as adults from hemlock woolly adelgid-infested western hemlock. *L. nigrinus* reared for three generations on eastern hemlock infested with hemlock woolly adelgid were sent overnight from the Biological Rearing Facility at Virginia Polytechnic Institute and State University to the Oregon State University Integrated Forest Protection Laboratory. We collected and stored uninfested eastern and western hemlock branches as described above. Each bioassay included one blank control chamber, a chamber with one 5-cm-long branch of western hemlock, and a chamber with one 5-cm-long branch of eastern hemlock that were randomly attached to arms of the olfactometer. Populations of *L. nigrinus* were kept separate, but individuals were randomly selected for each trial. Therefore, the position of plant material, blank control, and population of *L. nigrinus* selected from were rerandomized for each trial. This experiment was conducted once in January 2006.

Statistical Analyses. As data for individual trials on the time spent walking in each odor field were not normally distributed in the bioassays with hemlock woolly adelgid and plant material, they were analyzed with a nonparametric Friedman test with a multiple comparison analysis for ranked data based on Nemenyi test (Zar 1999). For each experiment, the data were ranked and the Friedman test was conducted using the ranks. The numbers in final positions for each odor field were analyzed using the Cochran Q test; pair-wise comparisons of final positions were analyzed with a test for multiple contrasts of data subjected to the Cochran test (Zar 1999). Although the analyses were conducted on numbers in final positions, we present these data as percentages of total numbers tested in final positions for ease of comparing treatments. In each experiment, the combined data across months on the time spent walking in each odor field were normally distributed and subjected to analysis of variance (ANOVA) to test for the effect of month.

Results

Experiment 1: Does *L. nigrinus* Respond to Odors From its Prey, Hemlock Woolly Adelgid? Two-Way Choice. *L. nigrinus* spent significantly more time walking in the CF or the blank control field compared with the field with hemlock woolly adelgid woolly masses (Table 1, Friedman test, $\chi^2 = 7.54$; df = 2; $P = 0.01$). The percentages of final positions in each field followed a similar pattern among treatments to the time spent walking in fields, with *L. nigrinus* choosing to remain in the CF more than in the hemlock woolly adelgid woolly mass or blank control fields (Table 1, Cochran Q test, $\chi^2 = 9.59$; df = 2; $P = 0.02$). There

Table 2. Ambulatory response of adult *L. nigrinus* to odors from uninfested eastern and western hemlock branches in laboratory olfactometer bioassays

Simulus field in olfactometer	Month of bioassay and mean (± SE) time spent in field, min[a]				Month of bioassay and % final position in each field[b]			
	Nov.	Dec.	Jan.	Mar.	Nov.	Dec.	Jan.	Mar.
Western hemlock	7.5 ± 1.2a	10.5 ± 1.2a	6.0 ± 1.8a	9.8 ± 1.0a	45a	50a	45a	40a
Eastern hemlock	3.0 ± 0.8b	3.8 ± 0.8b	4.5 ± 0.8a	1.5 ± 0.8b	30a	20b	25b	30b
Blank control	2.2 ± 0.8b	0d	3.0 ± 1.0b	1.5 ± 0.8b	10b	20b	15c	20c
Central field	2.2 ± 1.2b	0.8 ± 0.2c	1.5 ± 0.5b	2.2 ± 0.5b	15b	15c	10d	10c

[a] Means in each column followed by the same letter are not significantly different by multiple comparison test of ranked data based on Nemenyi test, $P > 0.05$. There were no significant differences among months, $P > 0.05$.

[b] Percentages in each column followed by the same letter indicate no significant difference in the numbers in final positions by multiple contrasts of data subjected to the Cochran Q test, $P > 0.05$.

In Feb., *L. nigrinus* flew around the olfactometer rather than walking and, therefore, those data were not analyzed.

were no differences in the time *L. nigrinus* spent walking in the different odor fields among months ($F = 12.09$; df = 2; $P = 0.26$).

Experiment 2: Does *L. nigrinus* Respond to Odors From Hemlock Woolly Adelgid Host Trees? Three-Way Choice. *Experiment 2a.* The time *L. nigrinus* spent walking in fields with eastern hemlock volatiles was not significantly different with or without hemlock woolly adelgid on the branches, but it was significantly greater for fields with eastern hemlock volatiles compared with the CF or blank control (Table 1, Friedman test, $\chi^2 = 6.37$, df = 3, $P = 0.03$). The percentages of final positions in fields followed a similar pattern among treatments to the time spent walking in fields (Table 1, Cochran Q test, $\chi^2 = 6.96$, df = 3, $P = 0.01$). The time *L. nigrinus* spent walking in the different odor fields was similar in December and March ($F = 12.98$, df = 1, $P = 0.32$). However, *L. nigrinus* behaved differently during laboratory assays conducted in February. In February, each *L. nigrinus* flew, rather than walked, around the olfactometer. They were recorded as not making a choice and excluded from analyses.

Experiment 2b. Similar to experiment 2a, the time adult *L. nigrinus* spent walking in fields with western hemlock volatiles was not significantly different for chambers with or without hemlock woolly adelgid, but it was significantly greater for fields with western hemlock volatiles compared with the CF or blank control (Table 1, Friedman test, $\chi^2 = 9.61$, df = 3, $P = 0.02$). Also, the percentages of final positions in fields followed a similar pattern among treatments to the time spent walking in fields (Table 1, Cochran Q test: $\chi^2 = 13.87$, df = 3, $P = 0.001$). There were no differences in time spent walking in different odor fields between December and March ($F = 1.5$, df = 1, $P > 0.05$), but, in February, *L. nigrinus* flew around the olfactometer and those observations were excluded from analyses.

Experiment 3: Does *L. nigrinus* Distinguish Between Odors Emitted From Eastern Versus Western Hemlock? Three-Way Choice. *L. nigrinus* responded to the volatiles emanating from the branches of eastern and western hemlock trees. The percent of time *L. nigrinus* spent walking was significantly different among the treatments, with the insects staying longer in the field with western hemlock volatiles than in those with eastern hemlock volatiles, CF or the blank control (Table 2, Friedman test: $\chi^2 = 12.12$, df = 3, $P = 0.001$). Similarly, percentages of final positions in fields were significantly different among treatments with insects responding positively to volatiles from western hemlock over those from eastern hemlock, CF, or control (Table 2, Cochran Q test: $\chi^2 = 13.27$, df = 3, $P = 0.001$). There were no differences in the time *L. nigrinus* spent walking in the different odor fields among months ($F = 1.8$; df = 3; $P > 0.05$).

Experiment 4: Does *L. nigrinus* Respond to, Prefer, or both, Odors From Host Plants of Hemlock Woolly Adelgid, Other Adelgid Species, or Both? Four-Way Choice. In all four trials with different tree species, there were significant differences in the time *L. nigrinus* spent walking in the different fields, and the time spent walking in fields with western hemlock was consistently among the highest (Table 3). In all trials conducted in February, *L. nigrinus* flew around the olfactometer and those observations were excluded from analyses. In the trial with western white pine, *L. nigrinus* spent significantly more time walking in the fields with western hemlock or western white pine than in the fields with eastern hemlock, the CF, or the blank control (Table 3, Friedman test: $\chi^2 = 9.95$, df = 3, $P = 0.02$). There were no differences in the time spent walking in fields with western hemlock or western white pine in January and March, although *L. nigrinus* spent significantly more time walking in the field with western white pine than in the field with western hemlock in December. The percentages of final positions in fields followed a similar pattern among treatments to the time spent walking in fields (Table 3, Cochran Q test, $\chi^2 = 7.82$, df = 3, $P = 0.05$). There were no differences in the time *L. nigrinus* spent walking in the different odor fields among months ($F = 1.45$; df = 2; $P > 0.05$).

In the trial with white spruce, *L. nigrinus* spent significantly more time walking in the fields with western hemlock, eastern hemlock, or white spruce than in the CF or the blank control (Table 3, Friedman test: $\chi^2 = 6.60$, df = 3, $P = 0.03$). Furthermore, there were no differences in time spent walking in the fields among western hemlock, eastern hemlock, or white spruce. The percentages of final positions in fields followed a similar pattern among treatments to the

Table 3. Ambulatory response of adult *L. nigrinus* to odors from different adelgid host tree species in laboratory olfactometer bioassays

Stimulus field in olfactometer	Month of bioassay[a] and mean (± SE) time spent in field, min[b]			Month of bioassay[a] and % final position in each field[c]		
	Dec.	Jan.	Mar.	Dec.	Jan.	Mar.
Trial 1						
Western hemlock	4.0 ± 1.2b	4.5 ± 1.8a	5.0 ± 2.0a	35a	50a	35b
Eastern hemlock	2.0 ± 1.0c	3.0 ± 0.5b	1.8 ± 1.5b	20b	15c	10c
Western white pine	5.0 ± 0.5a	5.5 ± 1.0a	5.2 ± 1.5a	35a	25b	50a
Blank control	2.0 ± 1.0c	0.2 ± 0.2d	1.0 ± 0.8b	5c	5d	0d
Central field	2.0 ± 2.8c	1.8 ± 1.2c	2.0 ± 1.8b	5c	5d	5d
Trial 2						
Western hemlock	5.2 ± 1.5a	4.2 ± 1.2a	3.5 ± 1.2a	30a	35a	35a
Eastern hemlock	3.5 ± 2.0a	4.5 ± 1.8a	5.0 ± 2.0a	20b	20b	15b
White spruce	3.5 ± 2.0a	4.0 ± 1.5a	4.5 ± 2.0a	25b	25b	30a
Blank control	1.0 ± 1.0b	1.0 ± 0.2b	1.0 ± 0.8b	5c	10c	5c
Central field	1.8 ± 0.8b	1.2 ± 0.2b	1.0 ± 0.5b	20b	10c	15b
Trial 3						
Western hemlock	6.0 ± 2.2a	6.5 ± 1.2a	6.8 ± 1.8a	65a	55a	65a
Eastern hemlock	4.2 ± 1.0b	6.2 ± 1.0a	3.5 ± 1.0b	25b	25b	20b
Douglas-fir	0.0 ± 0.0d	0.2 ± 0.2b	0.0 ± 0.0d	0d	5c	0d
Blank Control	2.5 ± 0.8c	1.0 ± 0.2b	1.2 ± 1.8c	5c	5c	5d
Central field	2.2 ± 1.5c	1.0 ± 0.2b	3.5 ± 1.0b	5c	10c	10c
Trial 4						
Western hemlock	6.0 ± 1.5a	6.0 ± 1.5a	6.5 ± 2.8a	65a	60a	70a
Eastern hemlock	4.5 ± 1.8b	5.5 ± 1.0a	1.8 ± 1.5c	25b	20b	15b
Ponderosa pine	0.0 ± 0.0d	0.0 ± 0.0d	0.0 ± 0.0d	0d	0d	0d
Blank control	2.0 ± 1.0c	1.0 ± 0.2c	3.0 ± 1.5b	0d	5d	10c
Central field	2.5 ± 0.8c	3.0 ± 0.5b	3.8 ± 2.5b	10c	15c	5d

[a] During all experiments conducted in Feb., *L. nigrinus* flew around the olfactometer rather than walking and, therefore, those data were not analyzed.

[b] Means in each column within a trial followed by the same letter are not significantly different by multiple comparison test of ranked data based on Nemenyi test, $P > 0.05$. There were no significant differences among months, $P > 0.05$.

[c] Percentages in each column within an exp followed by the same letter indicate no significant difference in the numbers in final positions by multiple contrasts of data subjected to the Cochran Q test, $P > 0.05$.

time spent walking in fields, although the percentage of final positions in the western hemlock field was significantly higher than in the eastern hemlock or white spruce fields except in March when there was no difference between the western hemlock and white spruce fields (Table 3, Cochran Q test, $\chi^2 = 8.35$, df = 3, $P = 0.05$). There were no differences in the time *L. nigrinus* spent walking in the different odor fields among months ($F = 2.1$; df = 2; $P > 0.05$).

Results for the trials with Douglas-fir and ponderosa pine generally were similar. *L. nigrinus* spent significantly more time walking in fields with western hemlock and eastern hemlock than in fields with Douglas-fir (Table 3, Friedman test: $\chi^2 = 7.82$, df = 3, $P = 0.05$) or ponderosa pine (Table 3, Friedman test: $\chi^2 = 16.25$, df = 3, $P = 0.01$). The percentages of final positions in fields followed a similar pattern among treatments for Douglas-fir (Table 3, Cochran Q test, $\chi^2 = 8.69$, df = 3, $P = 0.05$) and ponderosa pine (Table 3, Cochran Q test, $\chi^2 = 6.95$, df = 3, $P = 0.05$). In both of these trials, *L. nigrinus* spent significantly more time walking in fields with western hemlock than in fields with eastern hemlock in December and March, but there was no difference between these treatments in January. The percentages of final positions in fields with western hemlock were significantly higher than in fields with eastern hemlock for all months in both trials. There were no differences in the time *L. nigrinus* spent walking in the different odor fields among months for Douglas-fir ($F = 1.8$; df = 2; $P > 0.05$) or ponderosa pine ($F = 1.72$; df = 2; $P > 0.05$).

Experiment 5: Does the Odor From Host Trees of Hemlock Woolly Adelgid (Eastern or Western Hemlock) in which *L. nigrinus* Developed on Affect its Response to Eastern or Western Hemlock Odors? Three-Way Choice. The behavior of *L. nigrinus* collected from hemlock woolly adelgid on western hemlock was different from those specimens reared under laboratory conditions on hemlock woolly aledgid from eastern hemlock (Table 4). *L. nigrinus* collected from hemlock woolly adelgid on western hemlock spent significantly more time walking in fields with western hemlock than in the field with eastern hemlock, CF, or blank control (Table 4, Friedman test: $\chi 2 = 14.26$, df = 1, $P = 0.004$). *L. nigrinus*, however, reared under laboratory conditions on hemlock woolly adelgid from eastern hemlock, spent significantly more time in the CF than the other fields. The same patterns also were observed in their final positions (Table 4, Cochran Q test, $\chi^2 = 9.21$, df = 1, $P = 0.01$). Eighty-four percent of *L. nigrinus* collected from hemlock woolly adelgid on western hemlock had a final position in a field with plant material present, whereas only 36% of the final positions of *L. nigrinus* reared from hemlock woolly adelgid on eastern hemlock were in fields with plant material (Table 4).

Discussion

L. nigrinus responded to odors from hemlock woolly adelgid's host trees, but not to odors associated with hemlock woolly adelgid. In the enclosed environment

Table 4. Ambulatory response of laboratory reared and field collected adult *L. nigrinus* to odors from uninfested eastern and western hemlock branches in laboratory olfactometer bioassays in Jan. 2006

Source of *L. nigrinus*	Stimulus field in olfactometer and mean (± SE) time spent in field, min[a]				Stimulus field in olfactometer and % final position in each field[b]			
	Center field	Blank	Eastern hemlock	Western hemlock	Center field	Blank	Eastern hemlock	Western hemlock
Laboratory reared on eastern hemlock	8.5 ± 2.2a	4.0 ± 2.8b	1.5 ± 0.5c	1.0 ± 1.0c	53a	10c	26b	10c
Field collected on western hemlock	1.5 ± 1.5c	2.0 ± 0.2c	4.2 ± 1.2b	7.2 ± 0.5a	10b	6b	16b	68a

[a] Means in each row followed by the same letter are not significantly different by multiple comparison test of ranked data based on Nemenyi test, $P > 0.05$.

[b] Percentages in each row followed by the same letter indicate no significant difference in the numbers in final positions by multiple contrasts of data subjected to the Cochran Q test, $P > 0.05$.

of the assay arena, the concentration of volatile chemicals likely was higher than would be present in the field. Yet even under these conditions, hemlock woolly adelgid remained inconspicuous to *L. nigrinus*, suggesting that hemlock woolly adelgid is extremely difficult to detect. Given the low detectability of hemlock woolly adelgid, it is not surprising that *L. nigrinus* responded to volatiles produced by hemlock woolly adelgid's host trees. Indeed, many predator and parasitoid species are thought to use odors of host plants for prey location, rather than odors of the prey themselves (Lima and Dill 1990, Vet et al. 1990, Gingras et al. 2002, Dicke 1999, Cortesero et al. 2000).

Although host odors may be more detectable than prey derived odors, they are also less reliable. The problem of low reliability can be solved in several ways. Predators may coevolve with plants to respond to particular volatiles that the plant releases only when damaged by herbivores (Vet et al. 1990, Harmel et al. 2007). Herbivore-induced volatiles provide specific information to the predator and greatly increase the reliability of the host derived odors (Harmel et al. 2007). Eastern hemlocks infested with hemlock woolly adelgid have increased monoterpene concentrations that might provide *L. nigrinus* with a specific signal of hemlock woolly adelgid feeding damage (Broeckling and Salom 2003). However, the presence of feeding hemlock woolly adelgid did not increase the attractiveness of hemlock branches, suggesting that *L. nigrinus* is responding to general hemlock volatiles produced through mechanical wounding, rather than to volatiles produced as a specific response to adelgid feeding.

The second way in which predators can overcome low reliability is via conditioning or associative learning (Vet et al. 1990, Cortesero et al. 2000). Several species of predators and parasitoids are conditioned during preadult development to respond to volatiles from the host plant they are raised on (Tamò et al. 2006). Other species have highly flexible learning that develops during adult feeding. We found that *L. nigrinus* collected in the field on western hemlock had a strong preference for western hemlock odors, whereas those specimens reared in the laboratory on eastern hemlock were unresponsive to odors from eastern or western hemlocks. This difference may suggest preadult conditioning, although it is not clear why adults raised on eastern hemlock did not show increased attraction to their natal hemlock species. Because one group of *L. nigrinus* was raised under laboratory conditions and the other was field collected, we cannot rule out an influence of developmental conditions on behavior. Indeed, the majority of beetles reared in the laboratory on eastern hemlock remained in the central field demonstrating a general lethargy and lack of response to odors. Multigenerational studies on the effect of laboratory rearing on *L. nigrinus* prey location behavior are needed, as are studies on associative learning.

L. nigrinus collected in the field from western hemlock were more strongly attracted to odors of western hemlock than those of eastern hemlock, whereas odors of western white pine and white spruce were as attractive to these beetles as western hemlock. Furthermore, odors of Douglas-fir and ponderosa pine were apparently repulsive. The preference for western hemlock, western white pine, and white spruce odors suggests that *L. nigrinus* originated in northwestern North America as a predator on hemlock woolly adelgid and possibly other adelgid species associated with these trees. Although *L. nigrinus* has been recorded from western white pine and larch in addition to western hemlock, there are no records of occurrence on white spruce (Fender 1945, Lawrence 1989, Leschen 2011 (S. P. Cook, personal communication). It should be noted, however, that our results apply to an enclosed environment where odors were highly concentrated and easily discernible. Whether or not *L. nigrinus* would demonstrate the same responses to odors in a field situation is unknown and requires further study.

In February, *L. nigrinus* consistently flew rather than walked around the arena and we did not observe any response to odors from hemlock woolly adelgid or conifers at that time. February coincides roughly with the beginning of the *L. nigrinus* oviposition period, reported to be late January–May for British Columbia, Canada (Zilahi-Balogh et al. 2003a). Consequently, the behavior observed in February may indicate the beginning of a dispersal or mate finding period. The change in *L. nigrinus* behavior should be taken into account when timing releases for biological control, because establishment may be less likely if *L. nigrinus* are released during or close to the dispersal, mating period., or both. However, Mausel et al. (2010) did not find an effect of season of release in establishment

success of laboratory released *L. nigrinus* released in the eastern United States, but that study only included February release dates for two of 22 sites and, *L. nigrinus* became established at only one of the two sites with February release dates. Seasonal variation in behavior also should be tested in the field.

Overall, our study suggests that *L. nigrinus* uses odors from hemlock woolly adelgid host trees to locate prey, but that these responses are flexible and depend on both season and on rearing environment. In particular, *L. nigrinus* reared under laboratory conditions on eastern hemlock, and taken from populations that were planned for field releases in the east, showed no response to odors from eastern or western hemlocks. Furthermore, our tests indicate that, in February, *L. nigrinus* adults may be physiologically predisposed to fly for dispersal or mate location purposes. The attractiveness of western white pine and white spruce suggests that additional conifer species from the eastern United States should be tested. If *L. nigrinus* is attracted to eastern white pine or spruces, that could impact the success of releases targeting hemlock woolly adelgid on eastern hemlock. Recently discovered hybridization between *L. nigrinus* and *L. rubidus* (N. P. Havill, personal communication), which is often found in association with *Pineus* spp. infestations on eastern white pine, supports this concern. All of these factors should be taken into consideration when planning future research and releases of *L. nigrinus* as a biological control agent in the eastern United States.

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