Trends in Entomology
Vol. 9, 2013

Original Communication

Attraction of bark beetle-associated Coleoptera to verbenone and 3-methylcyclohex-2-en-1-one

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ABSTRACT

In stands with ongoing infestations of tree-killing bark beetles (Coleoptera: Curculionidae: Scolytinae) such as mountain pine beetle (*Dendroctonus ponderosae* Hopkins) and Douglas-fir beetle (*D. pseudotsugae* Hopkins), anti-aggregation pheromones can act to divert potentially attacking beetles away from a tree that has already been fully colonized. By diverting additional beetles from attacking a fully colonized tree, competition among developing larvae for the within-tree nutrients should decrease. We placed traps baited with two known bark beetle anti-aggregation pheromones, verbenone and 3-methylcyclohex-2-en-1-one (MCH), in mixed conifer stands to determine if the compounds were attractive to individual species in five beetle families (Curculionidae, Buprestidae, Cerambycidae, Trogositidae and Cleridae) known to be associated with tree-killing bark beetles. Four species were caught in significantly higher numbers in traps baited with verbenone compared with non-baited traps or traps that were baited with MCH. No species were captured in significantly higher numbers in the MCH-baited traps. The species that were attracted to the verbenone-baited traps included a bark beetle (*Pityogenes fossifrons* (LeConte)), a buprestid (*Buprestis lyrata* Casey), one cerambycid (*Megasemum asperum* (LeConte)) and a trogositid (*Temnochila chlorodia* (Mannerheim)). Aside from being a beetle-produced anti-aggregation pheromone, verbenone is also produced by some conifers as the trees die. It is possible that insect species that depend on stressed or dying trees as host material may utilize verbenone as a cue for host location.

KEYWORDS: anti-aggregation pheromones, Buprestidae, Trogositidae, Curculionidae, Scolytinae

INTRODUCTION

Two of the more destructive bark beetle (Coleoptera: Curculionidae: Scolytinae) species in the forests of western North America are the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, and the Douglas-fir beetle, *D. pseudotsugae* Hopkins [1]. Both of these beetle species utilize semiochemicals that act to divert potentially attacking beetles away from a tree that has already been fully colonized, decreasing the competition among developing larvae for the within-tree space and nutrient resources. The identified compound used by *D. ponderosae* is verbenone [2, 3] while the compound used by *D. pseudotsugae* is 3-methylcyclohex-2-en-1-one (MCH) [4, 5]. One management technique that is used for protecting host trees from attack by these bark beetles is to place the appropriate semiochemical onto the tree's outer surface to repel/divert attacking populations [6, 7].

While some bark beetles may be the primary insect agent involved in killing their host trees during attack, they do not act alone, but instead make up one part of a community of insects that

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utilize these trees [8, 9, 10]. Among the families of beetles that are associated with the scolytids in utilizing the trees are other Curculionidae (including other scolytids) and species of wood-boring beetles in the families Buprestidae and Cerambycidae (all three families contain potential competitors for the tree resources). Other families of beetles typically associated with bark beetles include potential predators in the families Cleridae and Trogositidae.

Some of these bark beetle-associated species come to the trees coincidentally with the bark beetles and are attracted to bark beetle aggregation pheromones and/or host volatiles [6, 8]. Other associated species typically arrive at the hosts after the trees have been successfully attacked by bark beetles [11]. We hypothesized that one of the potential cues used by these later arriving species may be semiochemicals such as the anti-aggregation pheromones released by within-tree populations of bark beetles to stop colonization of the infested trees.

The objective of the current project was to determine if traps placed in a mixed conifer forest with low, but detectable levels of bark beetle activity and baited with either verbenone or MCH captured individual bark beetle-associated species in higher numbers than were captured in non-baited traps. We were specifically interested in examining the trap catch of five beetle families (Curculionidae, Buprestidae, Cerambycidae, Trogositidae and Cleridae).

MATERIALS AND METHODS

The study was conducted at six locations on the University of Idaho's Experimental Forest, located in Latah County, Idaho, USA. The experimental forest primarily consists of mixed conifer stands and our six sites were located in stands dominated by ponderosa pine, lodgepole pine, Douglas-fir, grand fir and Engelmann spruce. The Geographic Positioning System (GPS) coordinates for the six locations are: N46.86460, W116.72897; N46.85296, W116.74905; N46.8403, W116.75360; N46.84700, W116.76797; N46.83469, W116.77807 and N46.83357, W116.79330.

Three black cross-vane panel traps [12] were hung from black, metal shepherd's hooks at each site. The traps were placed in a straight line transect, 10 m apart and were suspended at a height of approximately 2 m. One of three treatments (non-baited controls, verbenone and MCH) was randomly assigned to each trap at individual locations. The verbenone and MCH baits were purchased from Contech Inc. (Burnaby, BC, Canada). Insecticidal strips (Hercon® Vaportape® II) were placed into the collection cups. Traps were placed in the field on 10 July 2013 and all captured insects were removed from the traps at 7-day intervals for four weeks. All beetles within the five families of interest were identified to species. The numbers of all specimens of a single species within the five families captured in an individual trap were combined for the statistical analyses.

Comparisons among treatments were made using analysis of variance tests conducted using the STATISTIX® software package [13] for individual species. Only sites that had the species captured at that site were used in the analysis and only species with more than ten individuals captured were analyzed. Using these two criteria, there was one scolytid (*Pityogenes fossifrons* (LeConte)), two buprestids (*Buprestis lyrata* Casey and *Chalcophora angulicollis* (LeConte)), one cerambycid (*Megasemum asperum* (LeConte)) and one trogositid (*Temnochila chlorodia* (Mannerheim)) that could be compared among treatments.

RESULTS

There were a total of four species of Curculionidae, five species of Buprestidae, four species of Cerambycidae, three species of Cleridae and one species of Trogositidae captured during the four weeks of this study (Table 1). Of the curculionid species caught during this study, one was a weevil, *Cossonus crenatus* Horn, and three were scolytids, *Scolytus piceae* (Swaine), *S. subscaber* LeConte and *Pityogenes fossifrons* (LeConte) (Table 1). Only *P. fossifrons* was captured in high enough numbers to warrant further analysis (Table 2). There were significantly more ($F = 6.30$; df = 2, 12; $[P > F] = 0.0135$) *P. fossifrons* captured in verbenone-baited traps than in non-baited traps or in traps baited with MCH. Indeed, all 29 of the *P. fossifrons* captured during this study were caught in verbenone-baited traps.

Table 1. The total number of specimens captured regardless of trap treatment (non-baited controls, or traps baited with either verbenone or MCH) for each species of five beetle families (Curculionidae, Buprestidae, Cerambycidae, Cleridae and Trogositidae) captured in black, cross-vaned panel traps during a four-week sample period.

Family	Species	Total captured
Curculionidae	*Cossonus crenatus* Horn	1
	Pityogenes fossifrons (LeConte)	29
	Scolytus piceae (Swaine)	1
	S. subscaber LeConte	1
Buprestidae	*Buprestis lyrata* Casey	25
	B. subornata (LeConte)	1
	Chalcophora angulicollis LeConte	18
	Chrysophana placida (LeConte)	1
	Dicera tenebrosa (Kirby)	1
Cerambycidae	*Megasemum asperum* (LeConte)	11
	Leptura obliterata Haldeman	1
	Pygoleptura nigrella (Say)	5
	Stictoleptura canadensis (Olivier)	7
Cleridae	*Enoclerus schafferi* Barr	1
	Thanasimus undatulus (Say)	3
	Trichodes ornatus Say	4
Trogositidae	*Temnochila chlorodia* (Mannerheim)	29

Table 2. Mean (± SEM) number of potential competitors in the beetle families Curculionidae, Buprestidae and Cerambycidae that were captured in traps baited with either 3-methylcyclohex-2-en-1-one (MCH) or verbenone during a four-week trapping period. Only species that were represented by a minimum of ten specimens were used in the analysis and only sites where at least one specimen of the species was captured were used.

	Curculionidae	Buprestidae		Cerambycidae
Treatment	*Pityogenes fossifrons*	*Buprestis lyrata*[a]	*Chalcophora angulicollis*[a]	*Megasemum asperum*[a]
Non-baited	0.0 ± 0.0 a	0.0 ± 0.0 a	3.7 ± 0.9 a	0.5 ± 0.3 a
MCH	0.0 ± 0.0 a	0.0 ± 0.0 a	1.7 ± 1.2 a	0.3 ± 0.3 a
Verbenone	5.8 ± 2.3 b	6.3 ± 2.1 b	0.0 ± 0.0 a	2.0 ± 0.4 b

a/ Within a column, letters denote significant differences among means.

Buprestis lyrata was the most common buprestid captured and *C. angulicollis* was the only other species of buprestid captured that met the minimum number of specimens captured for comparing among treatments (Table 2). Of these two buprestids, *B. lyrata* was captured significantly more often (F = 9.24; df = 2, 9; [P > F] = 0.0066) in traps baited with verbenone than in either the

Table 3. Mean (± SEM) number of *Temnochila chlorodia* (Coleoptera: Trogositidae), potential bark beetle predators in the beetle family Trogositidae, that were captured in traps baited with either 3-methylcyclohex-2-en-1-one (MCH) or verbenone during a four-week trapping period.

Treatment	*Temnochila chlorodia*[a]
Control	0.4 ± 0.4 a
MCH	0.4 ± 0.2 a
Verbenone	5.0 ± 1.3 b

a/ Within a column, letters denote significant differences among means.

non-baited control traps or the MCH-baited traps while there was no significant difference among treatments (F = 4.55; df = 2, 6; [P > F] = 0.0627) in trap catch of *C. angulicollis*. The only cerambycid that was caught in high enough numbers to compare among treatments was *M. asperum*. Similar to the results with *B. lyrata*, *M. asperum* was captured significantly more often (F = 8.60; df = 2, 9; [P > F] = 0.0082) in traps baited with verbenone than in either the non-baited control traps or the MCH-baited traps (Table 2).

While there were three species of Cleridae captured during the four-week trapping period, none were captured in high enough numbers to warrant a statistical comparison (Table 1). The other family of beetles captured that contains potential bark beetle predators was the Trogositidae. *Temnochila chlorodia* was the only species of trogositid caught during this study and it was captured significantly more often (F = 11.02; df = 2, 12; [P > F] = 0.0019) in traps baited with verbenone than in non-baited control traps or MCH-baited traps (Table 3).

DISCUSSION

In stands with ongoing infestations of bark beetles, anti-aggregation pheromones can act to divert potentially attacking beetles away from a tree that has already been fully colonized. By diverting additional beetles from attacking a tree that is full colonized, competition among developing larvae for the within-tree nutrients should decrease. The use of commercially available verbenone and MCH has been documented to successfully protect individually-treated trees or high-value areas from beetle attack [6, 7]. However, one bark beetle (*P. fossifrons)* and three wood borers (two buprestids and one cerambycid) were attracted in significantly higher numbers to verbenone-baited traps during the current study. One of the species, *B. lyrata*, had previously been reported as being attracted to verbenone [14]. None of the species captured during the current study were caught in higher numbers in MCH-baited traps.

In general, members of the Scolytinae genus *Pityogenes* are secondary attackers that primarily utilize pines as host material [1]. *Pityogenes* can be found in the tops and limbs of stressed or weakened trees and some have been reported to be capable of killing small trees. The species captured during this study, *P. fossifrons*, is typically found attacking pines but has also been recorded from Engelmann spruce [1]. Similarly, wood borers such as *B. lyrata*, *C. angulicollis* and *M. asperum* that were captured during the current study typically follow primary bark beetles when attacking a tree and come into trees that have already succumbed to bark beetle attack [1]. Temporally, the wood borers would be arriving at approximately the time at which the bark beetles would be releasing their anti-aggregation pheromone. However, many of the wood boring beetles can attack multiple host species and are not species (or genus) specific. For example, the buprestid *C. angulicollis* has a host list that includes pines (*Pinus*), true firs (*Abies*) and Douglas-fir (*Pseudotsuga*). Both true firs and Douglas-fir are listed as hosts for *M. asperum* [1]. Therefore, it may be reasonable to assume that these wood borers are being attracted to compounds being released from trees and not just the attacking bark beetles.

Unlike MCH, verbenone is also a tree-produced semiochemical that is released as a tree dies [15, 16]. Further, it has been previously proposed that verbenone acts as a compound that indicates host quality to attacking insects [17]. As an indicator of host quality, verbenone could act as either an attractant or deterrent, depending on the type of host material utilized by a particular species. Under this scenario, verbenone should act as a repellent for species that colonize fresh or living tree material (i.e. tree-killing species of bark beetles) but as an attractant for species that colonize recently dead trees (i.e. many secondary bark beetles and wood borers).

The trogositid *T. chlorodia* has been reported to be attracted to bark beetle aggregation pheromones [18]. Attraction to bark beetle aggregation pheromones would benefit *T. chlorodia* because one of the major prey items for the beetle are bark beetles [19] and attraction to aggregation pheromones would put them onto a tree at the same time as their prey. However, *T. chlorodia* are generalist predators, consuming prey species other than bark beetles [1]. Therefore, the attraction to trees even after they are fully colonized by bark beetles may continue to place *T. chlorodia* in a location where appropriate prey are available. During the current study, we caught *T. chlorodia* in significantly higher numbers in traps baited with verbenone compared with non-baited or MCH-baited traps. If verbenone is an indicator of tree health, it could also be a more reliable indicator of the presence of other prey items at these trees. A prior study reported significantly higher catches of *T. chlorodia* in traps when verbenone is added to various host monoterpenes compared with traps that are baited only with the monoterpenes [18]. However, our current results differ from [14] where *T. chlorodia* was not captured in significantly higher numbers in verbenone-baited traps compared with non-verbenone traps. These differences may be related to such parameters as the relative abundance of *T. chlorodia* on the different sites, timing of the tests or geographic locations.

CONCLUSION

Anti-aggregation pheromones can act to divert potentially attacking conspecific bark beetles away from a tree that has been fully colonized. By diverting additional beetles from attacking a fully colonized tree, competition among developing larvae for the within-tree nutrient and resources should decrease. Of the two compounds tested, one (MCH) is produced by Douglas-fir beetle and it did not attract any members of beetle families that are common associates of bark beetles. The second compound we tested (verbenone) attracted five species belonging to four different families (Curculionidae, Buprestidae, Cerambycidae and Trogositidae). Aside from being a beetle-produced anti-aggregation pheromone, verbenone is also produced by some conifers as the trees die. We hypothesize that for some species of beetles that depend upon stressed or dying trees as host material, verbenone may be used as a cue for host location. Similarly, predators whose prey develops in these stressed trees may also utilize the compound to locate habitats in which their prey lives.

ACKNOWLEDGEMENT

We thank the University of Idaho’s Experimental Forest for experimental sites. The identity of *Buprestis lyrata* was confirmed by Richard L. Wescott, Oregon Department of Agriculture. The work was supported in part by University of Idaho, Agricultural Experiment Station. Voucher specimens have been placed in the William Barr Entomological Museum at the University of Idaho.

CONFLICT OF INTEREST STATEMENT

The work reports the results of research and does not represent an endorsement of any of the compounds tested. None of the authors have personal or financial associations with companies or their products.

REFERENCES

1. Furniss, R. L. and Carolin, V. M. 1977, USDA For. Serv. Misc. Publ. No. 1339.
2. Amman, G. D., Their, R. W., McGregor, M. D. and Schmitz, R. F. 1989, Can. J. For. Res., 19, 60.
3. Lindgren, B. S., Borden, J. H., Cushon, G. H., Chong, L. J. and Higgins, C. J. 1989, Can. J. For. Res., 19, 65.
4. Rudinsky, J. A., Morgan, M. E., Libbey, L. M. and Putnam, T. B. 1974, J. Appl. Entomol., 76, 65.
5. Lindgren, B. S., McGregor, M. D., Oakes, R. D. and Meyer, H. E. 1988, J. Appl. Entomol., 105, 289.
6. Ross, D. W. and Dauterman, G. E. 1997, USDA For. Serv. Gen. Tech. Rep. NE-236, pp 135-145.

7. Lindgren, B. S. and Miller, D. R. 2002, Environ. Entomol., 31, 759.
8. Billings, R. F. 1985, J. Appl. Entomol., 99, 483.
9. Gara, R. I., Werner, R. A., Whitmore, M. C. and Holsten, E. H. 1995, J. Appl. Entomol., 119, 585.
10. Haberkern, K. E. and Raffa, K. F. 2003, J. Chem. Ecol., 29, 1651.
11. Stephen, F. M. and Dahlsten, D. L. 1976, Can. Entomol., 108, 283.
12. Czokajlo, D., McLaughlin, J., DeGroot, P., Warren, J. C., Teale, S. A. and Kirsch, P. 2001, J. For. Sci., 47, 34.
13. Analytical Software, 2009, Statistix 8 User's Manual, Tallahassee, Florida.
14. Macias-Samano, D., Wakarchuk, D. and Rattray, P. 2012, http://www.semiochemical.com/B_lyrata_verbenone.pdf (accessed 24 October 2013).
15. Bhattacharyya, P. K. and Prema, B. R. 1962, Appl. Microbiol., 10, 524.
16. Hunt, D. W. A., Borden, J. H., Lindgen, B. S. and Gries, G. 1989, Can. J. For. Res., 19, 1275.
17. Lindgren, B. S., Nordlander, G. and Birgersson, G. 1996, J. Appl. Entomol., 120, 397.
18. Fettig, C. J., McKelvey, S. R., Dabney, C. P. and Borys, R. R. 2007, Can. Entomol., 139, 141.
19. Stark, R. W. and Dahlsten, D. L. 1970, Univ. Calif. Div. Agricul. Sci. Publ., Berkeley, CA.