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RESPONSES OF *HYLASTES SALEBROSUS* TO TURPENTINE, ETHANOL, AND PHEROMONES OF *DENDROCTONUS* (COLEOPTERA: SCOLYTIDAE)

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ABSTRACT

Attraction of the pine bark beetle *Hylastes salebrosus* Eichhoff to the host chemicals turpentine and ethanol, and the *Dendroctonus* pheromones frontalin and *exo*-brevicomin, were assessed in four field trapping experiments. *H. salebrosus* were attracted to turpentine alone, and the addition of ethanol, whether mixed with the turpentine or deployed at any of three different release levels, elicited a significant increase in attraction. In another experiment incorportaing all semiochemicals tested, beetles were more attracted to traps baited with the combination of the turpentine:ethanol mix, frontalin, and *exo*-brevicomin, than to traps with the turpentine:ethanol mix only. A final pair of experiments determined that *exo*-brevicomin, and not frontalin, deployed with turpentine was important for eliciting attraction. *H. salebrosus* may produce and use *exo*-brevicomin as an attractive pheromone, but the data also suggest that *H. salebrosus* can exploit *exo*-brevicomin as a kairomone from other scolytid species that colonize the same pine resource.

RESUMEN

Atracción del escarabajo descortezador de pino *Hylastes salebrosus* Eichhoff a las químicas producidas por el huésped, aguarraz y etanol, y a las feromonas de *Dendroctonus* frontalina y *exo*-brevicomina, se ensayó en cuatro experimentos de trampeo en el campo. *H. salebrosus* se atrayeron a aguarraz solo, y la adición de etanol, ya sea mezclado

con el aguarraz o puesto en combinación a cualquier de 3 niveles de evaporación, elicitó un aumento significativo en la atracción. En otro experimento incorporando todas las semioquímicas probadas, los escarabajos fueron más atraidos a trampas encebadas con la combinación de mezcla aguarraz:etanol, frontalina, y *exo*-brevicomina, que a trampas con solamente la mezcla de aguarraz:entanol. Un par final de experimentos determinaron que *exo*-brevicomina, y no frontalina, mezclado con aguarraz fué importante en elicitar atracción. *H. salebrosus* puede producir y usar *exo*-brevicomina como feromona atrayente, pero los datos también sugieren que *H. salebrosus* puede explotar *exo*-brevicomina como una kairomona de otras especies de Scolyididae, las cuales también colonizan el mismo recurso de pinos.

The bark beetle genus *Hylastes* Erichson is comprised of several species in North America that breed in the lower boles and roots of diseased or moribund conifers (Wood 1982). Very little is known of the biologies and habits of these species, but their potential as pests of conifer reproduction (Ciesla 1988) and vectors of root pathogens (Witkosky et al. 1986) is substantial. While conducting research on the chemical ecology of *Dendroctonus terebrans* (Olivier) and other pine bark beetles (e.g., Phillips et al. 1988, 1989), I observed *H. salebrosus* Eichhoff responding to traps baited with various semiochemicals. Here I report results from several trapping experiments that clearly show *H. salebrosus* is attracted to pine turpentine, that the attraction to turpentine is enhanced by ethanol, and that these beetles also are attracted to a pheromone of *Dendroctonus* Erichson.

Materials and Methods

Four field experiments were conducted during 1988 in commercial slash pine, *Pinus elliottii* Engelm. Var. *elliottii*, plantations about 20 km east of Gainesville in Alachua Co., Florida. Traps used were 16-unit multiple funnel traps (Lindgren 1983) that were hung from steel support stands so that the collection jars were within 15 cm of the ground. Semiochemical release devices were hung on the outside of the third funnel from the bottom of each trap. Experiments were set up for four to seven days as completely randomized block designs, each with seven replicates. Treatments were randomly assigned to traps within each block. Traps within a block were spaced 20 m apart in a line and blocks of traps were spaced at least 40 m apart throughout the forest. Total numbers of trapped *H. salebrosus* were determined, transformed via $\sqrt{x+0.5}$, subjected to analysis of variance, and means were compared using the Student-Newman-Keuls test.

The first experiment investigated the responses of *H. salebrosus* to turpentine deployed with different release levels of 95% ethanol. The five treatments were: 1) turpentine only; 2) turpentine plus a low dose of ethanol; 3) turpentine plus a medium dose of ethanol; 4) turpentine plus a high dose of ethanol; and 5) turpentine and ethanol released from one container as a 1:1 mix. Ethanol doses were compared with the turpentine:ethanol mix because earlier work showed that some beetle species differed substantially in their responses to the mix and to undiluted turpentine with separately released ethanol (Phillips et al. 1988). Turpentine and the turpentine:ethanol mix were evaporated from 250-ml Nalgene bottles with 5 cm of a 15x1 cm cotton dental wick extending through a 1 cm hole in the cap. Undiluted turpentine evaporated at a rate of 4.70 g/d and the turpentine ethanol mix evaporated at 10.14 g/d; analysis of released volatiles determined that the turpentine components (monoterpene hydrocarbons) were released at similar levels from both bait types (see Phillips et al. 1988 for details). The turpentine was commercially distilled from local pines and composed primarily of *alpha-* and *beta-*pinenes (see Phillips et al. 1990). Ethanol release devices were: low ethanol, 10 ml in a sealed 16.3x15.0 cm x 1.12 mil polyethylene sandwich bag; medium ethanol, 10 ml in an

open 14.8 ml glass vial (1.1 m I.D. opening); and high ethanol, 200 ml in a 250 ml Nalgene bottle with 5 cm of a 15x1 cm cotton dental wick extending through the cap. Evaporation rates of ethanol from these devices were: low, 3.18 mg/hr; medium, 25.84 mg/hr; and high, 596.40 mg/hr.

The second experiment, designed initially to study the responses of *D. terebrans* (Phillips unpublished), examined the responses of *H. salebrosus* to the turpentine:ethanol mix and the bark beetle pheromones frontalin and *exo*-brevicomin. The five treatments were: 1) turpentine:ethanol only; 2) turpentine:ethanol plus frontalin released from three glass capillaries; 3) turpentine:ethanol plus three capillaries of frontalin and one capillary of *exo*-brevicomin; 4) turpentine:ethanol plus three capillaries of frontalin and three capillaries of *exo*-brevicomin; and 5) turpentine:ethanol plus three capillaries of frontalin and five capillaries of *exo*-brevicomin. Source, purities, release devices, and release rates for the synthetic, racemic pheromones are given in Phillips et al. 1990.

Because the second experiment found that the *Dendroctonus* pheromones were attractive to *H. salebrosus*, the third and fourth experiments were designed to determine the roles of frontalin and *exo*-brevicomin separately. Both experiments utilized a low release rate of undiluted turpentine so that any atractive effects of the synthetic pheromones would not be overwhelmed by the attraction to host odors. Turpentine was evaporated from an open 14.8 ml glass vial (1.1 cm I.D. opening) at a rate of about 180 mg/d, as determined in the laboratory at 30° C. The third experiment assessed the responses of *H. salebrosus* to traps baited with 1) turpentine only, and 2) turpentine plus three capillaries of frontalin. The fourth experiment compared responses to 1) turpentine only, and 2) turpentine plus three capillaries of *exo*-brevicomin.

Results and Discussion

Turpentine alone was attractive to *Hylastes salebrosus*, and the addition of ethanol at any release level markedly increased this attraction (Fig. 1). Earlier studies failed to catch any *H. salebrosus* or other phloem-feeding bark beetles at traps baited with ethanol only (Phillips et al. 1988) or at unbaited traps (unpublished). Therefore, ethanol acts synergistically to increase the attraction of *H. salebrosus* to turpentine. This synbergistic effect of ethanol is similar to that found for *D. terebrans* (Phillips et al. 1988), *Hylobius pales* Herbst (Curculionidae) (Fatzinger 1985), *H. abietis* (L.) (Tilles et al. 1986), *Monochamus titillator* (F.) (Cerambycidae) (Fatzinger et al. 1987), and other pine-infesting beetles (e.g., Klimetzek et al. 1986, Chénier & Philogène 1989). Varied doses of ethanol affected responses of other beetles to host odors and pheromones in other studies (e.g., Klimetzek et al. 1986), but different doses of ethanol had no significant effect on the responses of *H. salebrosus* in this study. I believe this is the first clear demonstration of a turpentine-ethanol synergism for a species of *Hylastes*. Witkosky et al. (1987) reported that *H. nigrinus* (Mannerheim) was more attracted to *alpha*-pinene than to ethanol, but they did not combine the two to test for synergism or enhancement. Chénier & Philogène (1989) caught very low numbers of *H. porculus* Erichson and did not detect a significant difference between responses to monoterpenes and ethanol, either singly or in combination.

Turpentine, which is comprised primarily of monoterpene hydrocarbons, is the volatile fraction of pine oleoresin and is representative of odors emanating from cut or injured pines. Ethanol is produced by stressed, injured, or decomposing conifers and other woody plants (Moeck 1970, Kimmerer & Kozlowski 1982) and is attractive alone to many scolytids and cerambycids (e.g., Roling & Kearby 1975, Montgomery & Wargo 1983). Turpentine, therefore, would be a general cue from a potential host for pine-breeding insects, but ethanol with host terpenes would present a more defined signal

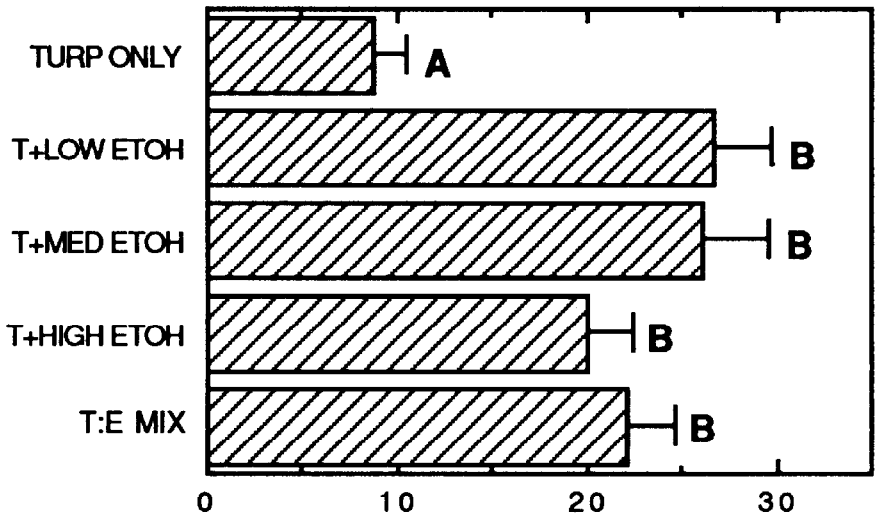

Fig. 1. Mean responses and standard errors of *Hylastes salebrosus* in the first experiment; TURP and T = Turpentine, ETOH = ethanol deployed at various doses (low, medium, high), T:E = a 1:1 mix of turpentine and ethanol. Means followed by the same letter are not significantly different ($P>0.05$, Student-Newman-Keuls test; $F=6.20$, $df=10, 24$, $P=0.001$).

regarding the physiological stress or state of decompostition of the host. This scenario of host selection, in which terpenes and ethanol signify a host of a particular condition, applies to *H. salebrosus*, which colonizes dead pine material or cut stumps and roots of recently killed trees (Wood 1982).

Traps baited with the combination of turpentine:ethanol, frontalin, and a high dose of *exo*-brevicomin in the second experiment caught significantly more *H. salebrosus* than traps baited with turpentine:ethanol only (Fig. 2). In the third experiment beetles did not respond differently to traps baited with turpentine or turpentine plus frontalin, but the fourth experiment demonstrated that turpentine plus *exo*-brevicomin was significantly more attractive than turpentine only (Fig. 3). The low number of beetles caught in the third and fourth experiments can be attributed to the relative lower level of turpentine released (and perhaps the lack of ethanol) compared with other experiments. Data from the third and fourth experiments suggest that responses of *H. salebrosus* in the second experiment (Fig. 2) were not strongly affected by frontalin, but that *exo*-brevicomin was primarily responsible for the significant attraction to the combination of turpentine:ethanol, frontalin, and the high dose of *exo*-brevicomin. Previous studies (Phillips et al. 1989, unpublished) failed to attract any *H. salebrosus* to traps baited with *exo*-brevicomin only, therefore I assume that the attraction observed here to turpentine with *exo*-brevicomin, like similar phenomena in other bark beetles (Borden 1982), resulted from a synergism of the two materials deployed together.

There are at least two explanations for why *H. salebrosus* was attractd to *exo*-brevicomin in my experiments. First, one or both sexes of *H. salebrosus* may produce and utilize *exo*-brevicomin as an attractant pheromone. *Exo*-brevicomin is a common pheromone in several species of the closely related genus of *Dendroctonus*, and also occurs in other scolytids (Borden 1982). Support of this hypothesis would require, among other data, experimental proof of intraspecific pheromone-based attraction in *H. salebrosus*, and subsequent chemical identification of *exo*-brevicomin from the pheromone-

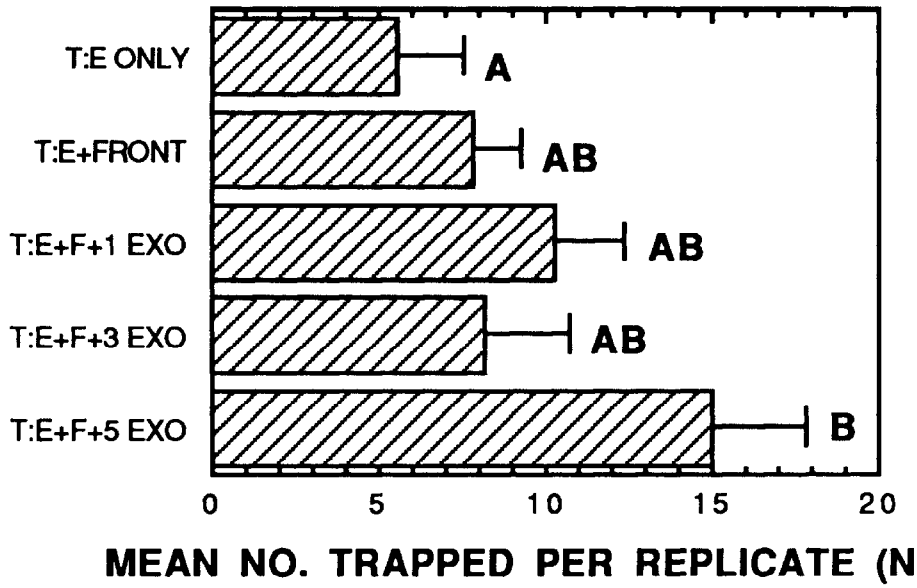

Fig. 2. Mean responses and standard errors of *Hylastes salebrosus* in the second experiment; T:E = a 1:1 mix of turpentine and ethanol, FRONT and F = frontalin, EXO = *exo*-brevicomin deployed at various doses (1x, 3x, 5x). Means followed by the same letter are not significantly different (P>0.05, Student-Newman-Keuls test; F = 2.81, df = 10, 24, P = 0.018).

producing beetles. A second, and more accessible, explanation for the attraction elicited by *exo*-brevicomin is that this compound represents a kairomone produced by co-attacking species of bark beetles. *H. salebrosus* may exploit the *exo*-brevicomin produced by other species, such as *D. terebrans* (Phillips et al. 1989), which would have made the initial effort and risk of locating and attacking a tree. *Exo*-brevicomin, therefore, would signify to *H. salebrosus* a suitable host for colonization (i.e., one dying or being killed by other bark beetles) and also a place for locating potential mates. Interspecific exploitation of chemical signals is common in bark beetles and their associates (Borden 1982).

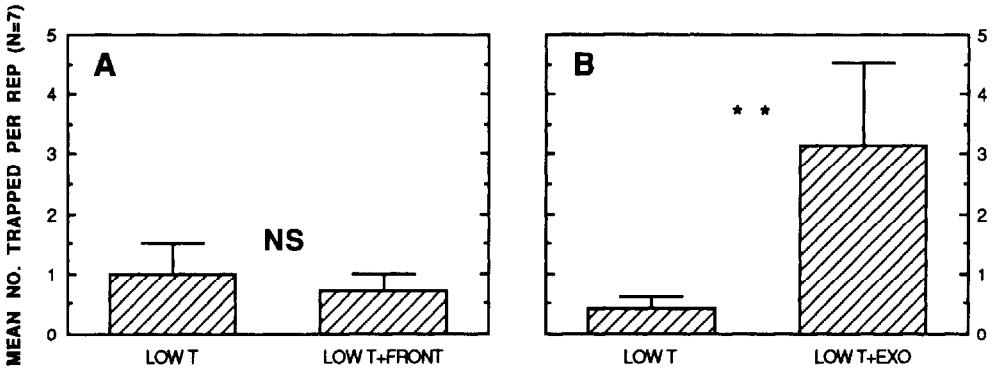

Fig. 3. Mean responses and standard errors of *Hylastes salebrosus* in the third (A) and fourth (B) experiments; LOW T = a low dose of undiluted turpentine, FRONT = frontalin, EXO = *exo*-brevicomin. Differences between means for each experiment were assessed by analysis of variance (NS, P = 0.776, F = 0.084, df = 1, 12; **, P = 0.032, F = 5.815, df = 1, 12).

It is possible, of course, that *H. salebrosus* both produces *exo*-brevicomin as a pheromone and also responds to it opportunistically as a kairomone. Such a pheromone-kairomone system has been suggested for *D. terebrans* and *D. frontalis* Zimmermann, both of which commonly co-attack the same host trees and produce and respond to frontalin (Payne et al. 1987).

ACKNOWLEDGMENTS

I am grateful to Tom Atkinson for initially identifying *H. salebrosus* from my samples, for writing the Spanish translation of the abstract, and for his review of the manuscript. I also thank John Foltz and Bob Wilkinson for their reviews of the manuscript. This research was supported by a grant from the USDA Competitive Research Grant Program, 87-CRCR-1-2491. This paper is Florida Agricultural Experiment Station Journal Series no. R-00282.

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FIELD RESPONSE OF FERAL MALE BANDED CUCUMBER BEETLES TO THE SEX PHEROMONE 6,12-DIMETHYLPENTADECAN-2-ONE

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ABSTRACT

Capture of male banded cucumber beetles *Diabrotica balteata* LaConte in sex pheromone-baited traps or in virgin female baited traps peaked at the beginning of the scotophase (2100-2200h) and declined thereafter. Virgin females (1 or 2) were not as effective as the racemic synthetic sex pheromone (0.5mg/septum) in attracting males. the most efficient design of capturing males was the Sentry wing trap and no differences in captures were found among traps placed 25.4, 50.8 or 101.6cm from the ground. Field tests showed that a rubber septum bait containing 0.5mg of the pheromone was attractive to males for a 12 month period and high captures occurred in August and September. Males were collected in pheromone traps from October through February in Charleston, SC.