Response of the bark beetle, *Hylesinus pruinosus*, to the male-produced pheromone, *endo*-brevicomin

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INTRODUCTION

The ash bark beetle, *Hylesinus pruinosus* Eichhoff (Curculionidae), infests recently cut or weakened trees in the genus *Fraxinus* in the eastern United States. Both monogyny and bigyny (one male simultaneously paired with one or two females, respectively) are reported in the genus *Hylesinus*, and either mating system may characterize geographically separate populations of a single species [e.g., *H. varius* (F.), Løyning and Kirkendall 1999]. Attractant pheromones have been isolated from both male and female *Hylesinus* spp., whereas females are always the pioneering (initially-arriving) sex (Schönherr 1970; Rudinsky and Vallo 1979). During field studies on the chemical ecology of *Dendroctonus frontalis* Zimmermann, we inadvertently captured large numbers of *H. pruinosus* in traps baited with (+)-*endo*-brevicomin. We performed the following studies to (1) determine whether *endo*-brevicomin is an aggregation pheromone for this species and (2) identify other possibly attractive semiochemicals for *H. pruinosus*.

Experiment 1: Response of *H. pruinosus* antennae to extracts of juvenile hormone-treated conspecifics

PROCEDURES: Male and female *H. pruinosus* beetles were live-trapped in the Homochitto National Forest in Mississippi and treated with 20 µg of juvenile hormone III to stimulate pheromone production. After 24 h, hindguts of either sex were extracted into pentane and concentrated. Coupled gas chromatography-electroantennographic detection (GC-EAD; apparatus pictured right) was then used to assay the sensitivity of male and female antennae to either pooled male or pooled female extract (0.05 beetle equivalents). Compounds that elicited antennal responses were subsequently identified using capillary gas chromatography-mass spectrometry (GC-MS).

RESULTS: Three compounds present in one or both sexes of JHIII-treated *H. pruinosus* elicited consistent electrophysiological responses from both sexes: *exo*-brevicomin, *endo*-brevicomin, and a third compound that could not be identified by its mass spectrum (Fig. 1). These three compounds were not detected in acetone-treated control insects. *exo*-Brevicomin was isolated predominantly from female beetles, whereas *endo*-brevicomin and the unknown compound were isolated in greatest quantities from males. All three compounds were found in at least trace amounts in either sex. The quantity of the unknown compound detected in individual beetles was closely correlated to the quantity of *endo*-brevicomin, suggesting that the two compounds might share the same synthetic pathway or regulatory mechanism. The EAD data suggested that antennae of both sexes were more sensitive to *endo*-brevicomin than the other two stimulants.

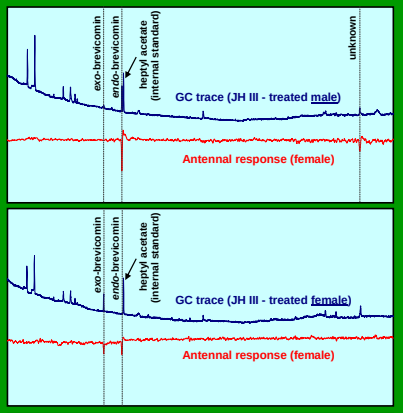
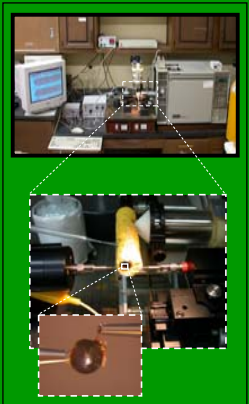


Figure 1: Female *H. pruinosus* antennal responses to concentrated hindgut extracts from male and female *H. pruinosus* beetles, which had been previously treated with juvenile hormone III. Male antennal responses (not shown) were similar. Test compounds were introduced to *H. pruinosus* antennae using a GC-EAD.



GC-EAD apparatus and antennal preparation

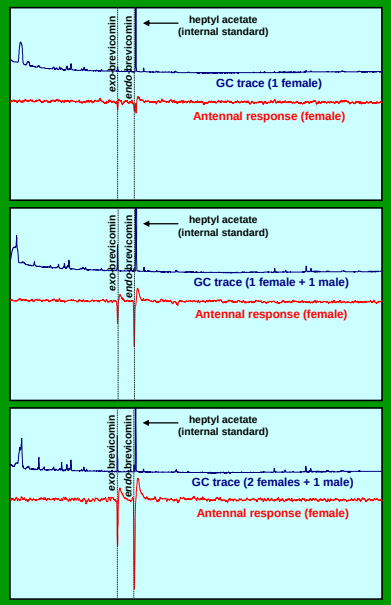


Figure 2: Female *H. pruinosus* antennal responses to aerations of green ash bark, which had been infested with females only, one female and one male pairs, or two females and one male. Male antennal responses (not shown) were similar. Test compounds were introduced to *H. pruinosus* antennae using a GC-EAD.

Experiment 2: Response of *H. pruinosus* antennae to volatiles from conspecific-infested ash logs

PROCEDURES: 25 female *H. pruinosus* were enclosed inside zippered pillow covers for 1 d with bolts from a recently-felled green ash tree. The female-infested bolts were then transferred to PTFE bags, and the volatiles inside the bags were collected for 6 hrs on cartridges containing the adsorbent Porpak Q® (0.1 g 50/80 mesh). Immediately following the aerations, 25 male *H. pruinosus* were added to each PTFE bag to allow pairing with the females, and after 1 d the volatiles inside the bags were sampled again. A further 25 females then were added to each bag, and volatiles were collected a third and final time the following day. The cartridges were extracted with pentane, and the resulting extracts were each assayed by GC-EAD with the antennae of five male and five female *H. pruinosus*.

RESULTS: *endo*- and *exo*-Brevicomin were the only electrophysiologically-active compounds in volatiles from *H. pruinosus*-infested ash logs (Fig. 2). Male and female responses were similar. The lack of antennal responses to other compounds in the volatile collections suggests that *endo*- and *exo*-brevicomin are the major semiochemicals released by infested hosts. *exo*-Brevicomin appeared to be the predominant EAD-active compound produced by pioneer females. *endo*-Brevicomin was present in almost undetectable (by GC-MS) concentrations in the aerations from bolts infested with females only, but increased in concentration after the addition of males and further females. Addition of the second cohort of females was associated with a further increase in concentration of both brevicomins.

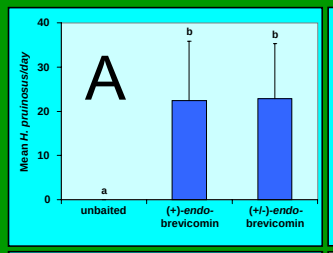
CITATIONS

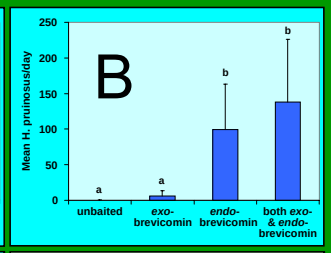
Løyning, M.K. and L.R. Kirkendall. 1999. J. Appl. Ent. 123: 77-82.
Rudinsky, J.A. and V. Vallo. 1979. Z. Ang. Ent. 87: 417-429.
Schönherr, J. 1970. Contrib. Boyce Thompson Inst. 24: 305-307.

Experiments 3A,B,C: Responses of *H. pruinosus* to traps baited with candidate semiochemicals

PROCEDURES: Three trapping experiments were conducted in hardwood stands in the Homochitto National Forest in Mississippi. Multiple-funnel traps were arranged in randomized complete blocks and assigned the following bait treatments: Experiment A (April 2006) -- (+)-*endo*-brevicomin or racemic *endo*-brevicomin; Experiment B (March 2008) -- racemic *exo*-brevicomin, racemic *endo*-brevicomin, or both racemic *exo*- and *endo*-brevicomin; Experiment C (March/April 2008) -- split green ash branches, racemic *exo*-brevicomin, or ash branches and racemic *exo*-brevicomin together. Unbaited control traps also were used in each experiment. Beetles captured in the traps were collected weekly or semi-weekly. Data from each experiment were analyzed using 2-way ANOVA with Tukey's mean separation.

RESULTS:
Exp. A: *H. pruinosus* catches were not significantly different in (+)- and racemic *endo*-brevicomin-baited traps, and both were greater than in the unbaited control (Fig. 3a). Thus, the presence of the (-)-enantiomer apparently does not inhibit *H. pruinosus* responses to (+)-*endo*-brevicomin, and suggests that, from a practical standpoint, the (+)- and racemic compounds are interchangeable. Since the racemate is much less costly, it was used in subsequent experiments.
Exp. B: Traps baited with *endo*-brevicomin or both *exo*- and *endo*-brevicomin caught significantly more *H. pruinosus* than traps baited with either *exo*-brevicomin alone or nothing (Fig. 3b). *exo*-Brevicomin was not attractive by itself, nor did it significantly enhance attraction of *H. pruinosus* to *endo*-brevicomin. More males than females were captured in all bait treatments; however, no significant differences in sex ratio among treatments were revealed by a Chi-square analysis.
Exp. C: Significantly more *H. pruinosus* were caught in traps baited with either *exo*-brevicomin alone or both *exo*-brevicomin and ash branches than unbaited traps (Fig. 3c). Traps baited only with ash branches (a presumed source of host odors) were not significantly different from the other treatments, and the addition of ash branches did not significantly increase the attractiveness of *exo*-brevicomin-baited traps.





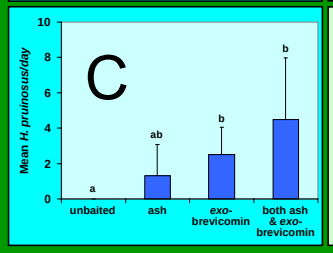


Figure 3: Mean number of *H. pruinosus* captured per trap per day (+ SE) in three experiments (A,B,C) using traps baited with the following treatments:

A: unbaited; (+)-*endo*-brevicomin (0.15 mg/d); and racemic *endo*-brevicomin (0.15 mg/d)
B: unbaited; racemic *exo*-brevicomin (0.15 mg/d); racemic *endo*-brevicomin (0.15 mg/d); and both racemic *exo*- & *endo*-brevicomin (each 0.15 mg/d)
C: unbaited; split green ash branches; racemic *exo*-brevicomin (0.15 mg/d); and both split green ash branches & racemic *exo*-brevicomin (0.15 mg/d).

Different lower-case letters indicate significantly different mean catch numbers.

Summary

Our studies with *H. pruinosus* suggest that the secondarily-arriving males produce greater quantities of the more potent attractant pheromone (i.e., *endo*-brevicomin) than pioneering females. Both sexes were attracted merely weakly or inconsistently to *exo*-brevicomin, which was the major olfactory stimulant produced by pioneering females. There was no evidence that the male and female compounds were synergistic in combination. Our finding runs counter to the bulk of previous studies on bark beetle chemical ecology which have shown that the attack-initiating sex is predominantly responsible for production of the aggregation attractant. It is possible that bigynous male *H. pruinosus* paired with single females release *endo*-brevicomin in order to attract a second female to the gallery; simultaneously flying males may respond to *endo*-brevicomin from other males as a cue to the presence of susceptible host material and arriving females. The ways in which variation in beetle mating systems have shaped the chemical ecology of individual bark beetle taxa are clearly a potentially rich area of research.