Semiochemicals for management of the southern pine beetle (Coleoptera: Curculionidae: Scolytinae): successes, failures, and obstacles to progress

Supplementary Materials 3

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Methods, results, and interpretation for experiment in Fig. 5: Effect of *endo*-brevicomin background on southern pine beetle response to *endo*-brevicomin devices associated with a source of attractant

Six pairs of traps were established in the same areas as in Supplementary Materials 1 with approximately 300 m between traps within pairs and more than 300 m distance between traps in adjacent pairs. All traps were baited uniformly with frontalin and *alpha*-pinene release devices. One trap in each pair was randomly assigned an artificial *endo*-brevicomin "background" produced by three *endo*-brevicomin releasers attached to the tops of 1.5-m poles arranged in an equilateral triangle with each pole located 20 m from the trap. The other trap of the pair lacked satellite *endo*-brevicomin releasers. Half of the pairs (selected at random) had an *endo*-brevicomin device attached directly to the traps. After 6–7 days, catches were collected from all traps, and the *endo*-brevicomin devices on traps were moved to the traps that lacked them. Catches were collected again after 6–7 days. Thus, there were four treatments in a split-plot design: factor *endo*-brevicomin present or absent on trap nested within factor *endo*-brevicomin background present or absent in the environment. All other details of the traps themselves and their deployment are as described in Supplementary Materials 1.

Two iterations of the experiment were run sequentially. In the first iteration (19 April–8 May 2013), the *endo*-brevicomin release device attached to the trap was a low density polyethylene microcentrifuge tube with approximately 250 mg racemic *endo*-brevicomin (Synergy Semiochemicals, Delta, British Columbia, Canada; product 3148; >95% purity; 2–5 mg/day release at ~26 °C); in the second (8 May–28 May 2013), the *endo*-brevicomin on the trap was reduced in rate (0.3–0.7 mg/day at ~26 mg/day) and was a polyethylene sachet containing 50 mg racemic *endo*-brevicomin (ChemTica, San Jose, Costa Rica; product IP049; >95% purity). For both iterations, the *endo*-brevicomin devices on the poles were the same as those placed on the traps in iteration (A). The attractive lure attached to all traps was a single high-release *alpha*-pinene "sock" (75% (–)-enantiomer; Synergy 3069; ~2 g/day at ~26 °C) and two polyethylene microcentrifuge tubes containing frontalin (Synergy 3065; racemic, total release 12 mg/day at ~26 °C). The frontalin and *endo*-brevicomin devices were attached at the centre of the trap, and the *alpha*-pinene device was attached at the top.

For statistical analyses, catches were cube-root transformed to meet distributional assumptions and analysed by a mixed-model analysis of variance, with main and interaction effects for four fixed factors: beetle sex, presence of *endo*-brevicomin background (factor "background"; two levels: present or absent), presence of an *endo*-brevicomin device on the trap (factor "brevicomin"; two levels: present or absent), and iteration (first or second). The model had four random effects: trap pair, pair × iteration, background × pair (iteration), and brevicomin × pair (background × iteration). No significant interactions with sex were detected, therefore summed catches of the sexes (log transformed) were analysed with the same model but with sex removed from the fixed effects and with three random effects: pair, pair × iteration, and background × pair (iteration). Pairwise comparisons of beetle responses with and without *endo*-brevicomin on the trap were performed with a SLICE statement (contrast of least square means) and a Bonferroni correction. For all tests, $\alpha = 0.05$.

Results

No significant interactions were detected between beetle sex and other fixed factors in the experiment; however, a marginal interaction was detected between sex and presence of background ($F = 3.531$; $df = 1{,}40$; $P = 0.068$). With sexes summed, there was a significant main effect for iteration of the experiment ($F = 7.05$; $df = 1{,}5$; $P = 0.045$), a significant interaction between brevicomin and background ($F = 54.0$; $df = 1{,}20$; $P < 0.001$), and a three-way interaction among brevicomin, background, and iteration ($F = 4.67$; $df = 1{,}20$; $P = 0.043$). For the first experimental iteration (which used *endo*-brevicomin devices with a high release rate on traps; Fig. 5A), addition of the *endo*-brevicomin device to the trap significantly increased catches if background was absent ($F = 7.65$; $df = 1{,}20$; $P = 0.048$) but not when background was present ($F = 5.87$; $df = 1{,}20$; $P = 0.10$). For the second iteration (which used *endo*-brevicomin devices with a low release rate on traps; Fig. 5B), addition of the *endo*-brevicomin device to the trap likewise significantly increased catches if background was absent ($F = 37.4$; df = 1,20; $P < 0.001$); however, catches were reduced in the presence of background ($F = 11.6$; $df = 1{,}20$; $P = 0.011$; Fig 5B).

Interpretation

The southern pine beetle pheromone *endo*-brevicomin has a peaked dose–response curve, with synergistic effects reaching a maximum at a specific concentration level and then declining and reversing to inhibition as the concentration increases further (Sullivan 2016). *endo*-Brevicomin releasers can have synergistic effects on sources of attractant located tens of metres away (Sullivan and Mori 2009), with the level of synergism at the distant point governed by the release rate of the *endo*-brevicomin device and the distance (BTS, unpublished data). Therefore, we had expected the observed increase in catches in response to presence of the *endo*-brevicomin background produced by the trio of releasers. Our results support the hypothesis that ambient *endo*-brevicomin may conceal effects of *endo*-brevicomin devices co-located with an attractant by rendering their presence redundant (Fig. 5A). Furthermore, our data show that it is possible for an *endo*-brevicomin device to "reverse" its effects in response to background: in iteration B, attraction was increased in the absence of *endo*-brevicomin background but decreased in its presence (Fig. 5B). We believe this should have occurred if the background concentration was closer to the concentration of maximum response (*i.e.*, the dose associated with the peak of the *endo*-brevicomin dose–response curve) than was the concentration resulting from the sum of the background and the contribution of the device placed on the trap. Because the release rate of the *endo*-brevicomin device was evidently within the synergistic range (*i.e.*, it increased attraction with background absent), the result was catch enhancement without, but catch reduction with, a background of *endo*-brevicomin. If this interpretation is correct, it suggests that point sources of semiochemicals with a peaked dose–response curve might display different effects depending on prevalence of natural sources of the semiochemical in the environment. Since beetle abundances should govern pheromone concentrations in the environment, we infer that effects of these semiochemicals (*i.e.*, their being either attractive or inhibitory) could be determined by beetle population densities. Evidence that this occurs is the observation that *endo*-brevicomin devices reduce responses to attractant inside southern pine beetle infestations (where beetle attack densities and presumably pheromone concentrations are high) but increase attraction outside (Sullivan *et al.* 2011).

A caveat to this interpretation is that statistically significant inhibitory effects were observed (with the presence of background) for the lower-rate *endo*-brevicomin device but not the higher-rate device. Inhibitory effects should have been stronger for the higher-rate device. However, the difference in both the dose level and resulting effects (Fig. 5) was not large for the two devices, and low insect catches decreased the power of the statistical tests.

References

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