

Semiochemicals for management of the southern pine beetle

(Coleoptera: Curculionidae: Scolytinae): successes, failures, and obstacles to progress

Supplementary Materials 1

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Methods for experiment in Fig. 2: Effects of spatial displacement of a verbenone device on its anti-attractant activity with southern pine beetle

The experiment compared the capacity of verbenone devices to reduce southern pine beetle response to a source of attractant when either co-located with it or displaced four metres away. Traps were placed >150 m apart within mixed pine and hardwood stands of the Homochitto National Forest, Mississippi, United States of America (31.4 °N, 91.0 °W). Traps were invariably baited with frontalinal released at ~6 mg/day and turpentine released at 0.6–3 g/day. All traps had a (+)-*endo*-brevicomin device (~0.2 mg/day) attached to the top of a 1.5-m plastic pole that was located four metres away. Devices releasing a mix of 33% (+)-verbenone to 67% (–)-verbenone at approximately 80 mg/day were located in each of the following ways: (1) they were attached directly to the trap; (2) suspended from a pole as used for the *endo*-brevicomin lure but located four metres away in the opposite direction from the trap; (3) suspended adjacent to the *endo*-brevicomin device on the same pole; or (4) absent (check). The *endo*-brevicomin device was displaced four metres from the trap to maximise its synergistic effects (Sullivan and Mori 2009). The purpose for having the displaced verbenone device being either co-located with or

located opposite to the *endo*-brevicommin lure was to detect possible spatial interactions between the two semiochemicals that might influence the experimental outcome (none were detected; see Fig. 1).

The *endo*-brevicommin device consisted of a single glass capillary (1.17 mm diameter and 32 mm long), one-third full, with one end sealed, that was secured open-end-up inside an inverted one-dram screw-top (uncapped) phial. The (+)-*endo*-brevicommin was synthesised as described in Sullivan *et al.* (2007). Turpentine was steam distilled from *Pinus taeda* Linnaeus (Pinaceae), and the baits consisted of either a heat-sealed 5 × 5 cm high-density polyethylene sachet filled with 18 mL liquid (device 1) or a 250-mL screw-cap bottle with 2–3 cm of cotton dental wick (1.3 cm diameter) protruding through the cap (device 2). These two device types were interchanged midway through the experiment because of low trap catches with device 1. However, the balancing of the experiment should have accommodated this change for statistical analysis. The frontalin devices consisted of two capped polyethylene microcentrifuge tubes with ~250 µL contents (ChemTica Inc., San Jose, Costa Rica). The verbenone lure was a polyethylene pouch type (ChemTica), equivalent to a Beetle Block™ (Synergy Semiochemicals, Delta, British Columbia, Canada) device.

The traps (12-unit funnel type) were suspended from standards consisting of 2-cm-diameter steel electrical conduit, with the bottommost funnel located approximately one metre above the ground. Trap cups were filled with a few centimetres of propylene glycol (as preservative) and water (1:3). On the traps, the frontalin bait was suspended on the inside of the fourth funnel from the bottom, and the turpentine bait was suspended inside the first funnel below the trap top. Five lines of four traps each were established, and within each line, the four treatments were assigned randomly to the traps. Trap catch was collected every 5–9 days, and at

that time, treatment positions were re-randomised (without replacement to any previous position) until every treatment had been at every trap position within the line once (treatments were rotated completely a second time). The experiment was performed from 10 July to 29 September 2006. In the lab, trap catches were sorted, and numbers of male and female southern pine beetles were recorded.

Counts (sexes summed) of southern pine beetle that were trapped were cube-root transformed to meet test assumptions of homoscedasticity and normality, then analysed with PROC MIXED in SAS 9.4 with fixed-effect lure treatment and random effects trapline, treatment \times trapline, collection date nested in trapline, and trap nested in trapline. Post-hoc all-pairwise comparisons were performed with a Tukey test ($\alpha = 0.05$).

References

- Sullivan, B.T., Shepherd, W.P., Pureswaran, D.S., Tashiro, T., and Mori, K. 2007. Evidence that (+)-*endo*-brevicommin is a male-produced component of the southern pine beetle aggregation pheromone. *Journal of Chemical Ecology*, **33**: 1510–1527.
- Sullivan, B.T. and Mori, K. 2009. Spatial displacement of release point can enhance activity of an attractant pheromone synergist of a bark beetle. *Journal of Chemical Ecology* **35**: 1222–1233.