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

Supplementary Materials 2

Brian T. Sullivan and Stephen R. Clarke

Methods for experiment in Fig. 3: Relative olfactory sensitivity of southern pine beetle to the enantiomers of verbenone

Dose–response curves of the electrophysiological response amplitudes of southern pine beetle antenna to each enantiomer of verbenone were constructed. This study was performed on a gas chromatograph–electroantennographic detector, with apparatus described in Asaro *et al.* (2004) and antennal preparation methods described in Sullivan (2005). Briefly, the antennal preparation consisted of a pair of sharpened glass capillary electrodes filled with Beadle–Ephrussi Ringers solution. One electrode was inserted into the foramen of the excised head, and the saline meniscus of the other made contact with one entire side of an antennal club. The electrodes were attached to a high-impedance input preamplifier, an analogue–digital converter, and a personal computer–based data recorder. The antennal preparation was mounted in the centre of a flow of charcoal-filtered, humidified air into which one half of the effluent of the capillary gas chromatograph column was shunted; the remainder of the effluent was directed to a flame ionisation detector (FID). Two separate dilution series were made with either (+)-verbenone (85% purity, ee +97, PheroTech, Delta, British Columbia, Canada) or (–)-verbenone

(95% purity, ee -94, Pherotech) at approximately 0.0004, 0.004, 0.04, 0.4, and 4 µg/µL in redistilled hexane. To each dilution was added the olfactory stimulant *endo*-brevicomin at 0.4 µg/µL to provide an internal reference for normalising response amplitudes to verbenone among antennal preparations and analyses. Because runs were performed in split mode with a split ratio of 1/20 and an effluent split of 1:1 between EAD and FID, the approximate quantity delivered to the antenna was 0.00001, 0.0001, 0.001, 0.01, and 0.1 µg. An enantioselective capillary column (Gamma-Dex 225, Supelco Inc., Bellefonte, Pennsylvania, United States of America; 30 m × 0.25 mm × 0.25 µm film thickness) was installed in the gas chromatograph and operated with the temperature program 120–175 °C at 5°/min, with injector at 200 °C, detector 220 °C, and 30-psi inlet pressure. For each individual antennal preparation, two runs were performed in sequence (each 12.5 min long); each used a different enantiomer and dilution. The order of presentation of enantiomers and dilutions was randomised. At the beginning and end of each run, the antenna was exposed to a two-second, 30-mL/min puff of air through a Pasteur pipette containing a filter paper treated with 10 µL of a 1/1000 mineral oil solution of *endo*-brevicomin. The antennal response at the beginning and end of the run was used to correct antennal responses for the effects of decline in preparation vigour over the duration of the runs (Sullivan *et al.* 2007).

References

Asaro, C., Sullivan, B.T., Dalusky, M.J., and Berisford, C.W. 2004. Volatiles associated with preferred and nonpreferred hosts of the Nantucket pine tip moth, *Rhyacionia frustrana*. Journal of Chemical Ecology, **30**: 977–990.

Sullivan, B.T. 2005. Electrophysiological and behavioral responses of *Dendroctonus frontalis* (Coleoptera: Curculionidae) to volatiles isolated from conspecifics. Journal of Economic Entomology **98**: 2067–2078.

Sullivan, B.T., Shepherd, W.P., Pureswaran, D.S., Tashiro, T., and Mori, K. 2007. Evidence that (+)-*endo*-brevicomin is a male-produced component of the southern pine beetle aggregation pheromone. Journal of Chemical Ecology, **33**: 1510–1527.