

New Techniques for Capturing and Analyzing Semiochemicals for Scolytid Beetles (Coleoptera: Scolytidae)

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ABSTRACT Techniques are described for capturing and analyzing volatiles that emanate from newly established galleries of scolytid beetles in on-tree or phloem sandwich preparations. They include an air input system that collects volatiles from the gallery entrance, a micro-Porapak Q trap, and gas-liquid chromatography through a 60-m, fused-silica capillary column. In studies on *Dendroctonus ponderosae* Hopkins, *D. rufipennis* Hopkins, and *Ips pini* (Say), the techniques have proven to be useful in determining simultaneously the quantity and quality of all semiochemicals produced by host tissue and individual beetles; demonstrating how pheromone production can vary in relation to a beetle's sex, behavior, mating status, and stage of gallery development; and establishing behaviorally optimal and environmentally acceptable release rates for semiochemicals used in pest management programs.

KEY WORDS Insecta, Scolytidae, semiochemicals, pheromones

BEFORE SEMIOCHEMICALS are used in pest management programs, it is necessary to identify their structure, to determine the rates of their production and emission by the insects or their hosts, and to develop controlled release systems (Golub & Weatherston 1984). The first efforts at pheromone collection from insects were mainly directed at structural elucidation, and often required thousands of insects (Silverstein 1970, Jacobsen 1972). Whole insects or parts of them, such as producing glands, abdominal tips or hindguts, or their frass, were solvent-extracted and processed. With advances in analytical chemistry, particularly in capillary gas-liquid chromatography (GLC), and mass and nuclear magnetic resonance (NMR) spectrometry, detection methods have become so sensitive that pheromone analysis often can be done on extracts from a single insect (Slessor et al. 1985). However, solvent extraction can lose key volatile components, as well as those that are rapidly released without being stored by the insect. Moreover, contents of pheromone-producing glands or pheromone-laden frass and the composition of their emitted volatiles are not necessarily identical (Peacock et al. 1975; Silk et al. 1980, 1982). Therefore, the extraction method is now often replaced by methods of capturing volatiles from live insects in adsorbents (Rudinsky et al. 1973, Byrne et al. 1975) or cryogenic traps (Browne et al. 1974).

Bark beetles characteristically produce pheromones when feeding (Wood & Bushing 1963), or

when exposed to host volatiles (Vité et al. 1972). Identification and quantification of semiochemicals produced by bark beetles ideally requires trapping of airborne volatiles from beetles attacking hosts under natural or near-natural conditions. Browne et al. (1979) captured volatiles from standing ponderosa pines under attack by western pine beetles, *Dendroctonus brevicornis* Le Conte, and G. Birgersson³ (personal communication) has succeeded in capturing the volatiles from individual galleries of *Ips typographus* L.

Our objectives were to develop simple, reliable, and adaptable techniques to determine simultaneously the quantity and quality of all semiochemicals produced by host tissue and single bark beetles during the attack of new hosts; to demonstrate that these new techniques can be used to determine how pheromone production can vary in relation to a beetle's sex, behavior, mating status, and stage of gallery development; and to show how the quantification of semiochemicals produced by individual beetles under near-natural conditions can be used to establish behaviorally optimal and environmentally acceptable release rates for semiochemicals in pest management programs.

Materials and Methods

Collection and Handling of Experimental Insects and Host Material. Experiments were conducted on spruce beetles, *Dendroctonus rufipennis*

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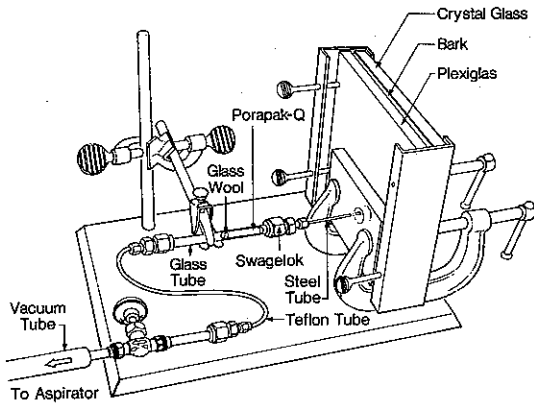


Fig. 1. Phloem sandwich with trap containing approximately 0.13 g of Porapak Q. Glass and large Plexiglas plates measure 12 cm². Steel and glass tubes are connected by a Swagelok (reducing union, 6 mm to 2 mm) fitted with Teflon back and front ferrules.

Kirby, mountain pine beetles, *Dendroctonus ponderosae* Hopkins, and pine engravers, *Ips pini* (Say). *D. rufipennis* was obtained from Engelmann spruce, *Picea engelmannii* Parry Engelmann, near Princeton and Kelowna, British Columbia, and *D. ponderosae* and *I. pini* were obtained from lodgepole pine, *Pinus contorta* var. *latifolia* Engelmann, near Squamish and Princeton, British Columbia. All beetles were allowed to emerge in screen cages at approximately 26°C and a photoperiod of 16:8 (L:D). Before use, the insects were held for 4 d at room temperature in layers of filter paper in glass jars. After 5 mo storage, Engelmann spruce bolts

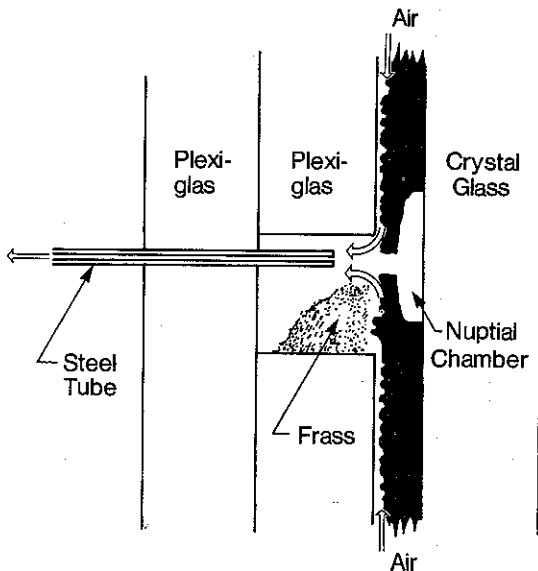


Fig. 2. Sectional cut through the phloem sandwich.

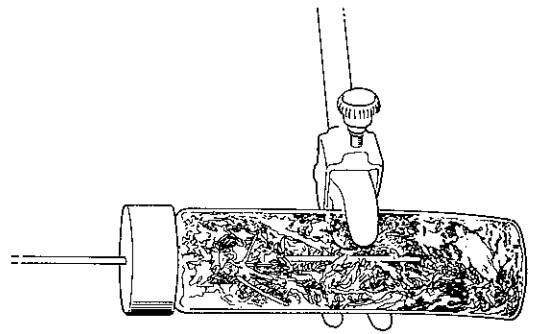


Fig. 3. Device for aerating shredded phloem tissue placed in a 30-ml vial. Air enters the vial through the 2-mm opening in the lid that is slightly wider than the steel tube, which passes through the lid and draws volatiles from deep inside the vial.

were infested with beetles; lodgepole pine bolts were infested within 2 wk of felling the tree.

Systems for Volatile Collection. Three kinds of apparatus were used—a modified phloem sandwich (Reid 1958, Hopping 1961) (Fig. 1 and 2), a device for aerating shredded phloem (Fig. 3), and an on-tree device (Fig. 4).

For phloem sandwich preparations, squares of phloem (with cortex) were peeled from logs (May–September) and placed between Plexiglas and glass plates (Fig. 1 and 2). To protect the exposed phloem from oxidization in air, a lower plate of rigid crystal glass was used, and the phloem was firmly pressed onto it by a plate of Plexiglas under continuous pressure exerted by thumb screws threaded through strong aluminum brackets. The Plexiglas plate had a hole in which a single beetle of the first attacking sex was placed. The hole then was covered with a smaller plate, through which was inserted a steel tube connected to a Porapak Q trap. The smaller plate was secured in place with C-clamps that applied further pressure on the phloem preparation (Fig. 1). A constant aspirator-driven, valve-controlled air flow was maintained at 0.7 liter/min between the rough bark surface and the Plexiglas plate toward the opening of the steel tube (Fig. 2). Volatiles released from the entrance hole and from the frass expelled from the gallery were continuously collected in the air stream and trapped in Porapak Q (50–80 mesh, Applied Sciences Division, Milton Roy Laboratory Group, State College, Pa.). Following addition of a mate (*Dendroctonus* spp.) or mates (*I. pini*) after 30 h, the volatiles were captured in a second Porapak Q trap, again for 30 h. Progress of gallery construction and behavior of the beetles could be observed through the crystal glass plate.

To compare volatiles produced by hosts and beetles, strips of phloem from the same log used for preparing the sandwiches were placed into a vial (Fig. 3) and the volatiles were trapped as described above.

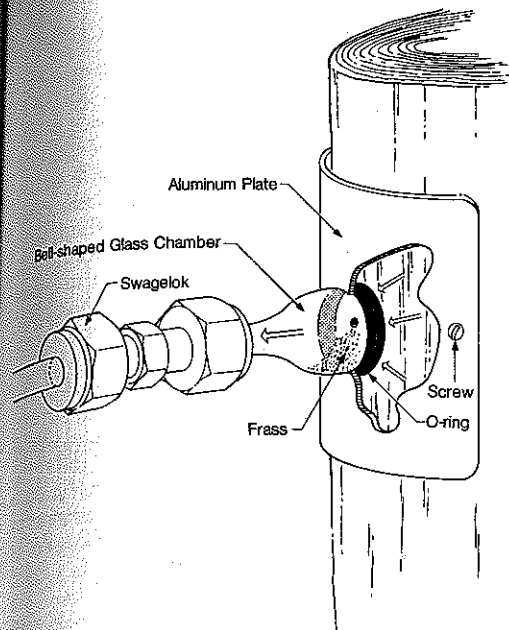


Fig. 4. On-tree device. An expanded glass tube (1.0 cm. o.d.) is inserted into two rubber O-rings and pressed against the log by a flexible aluminum plate (14.5 by 8.5 cm). A Swagelok (union, 6 mm) fitted with back and front Teflon ferrules provides the connection to the Porapak Q trap.

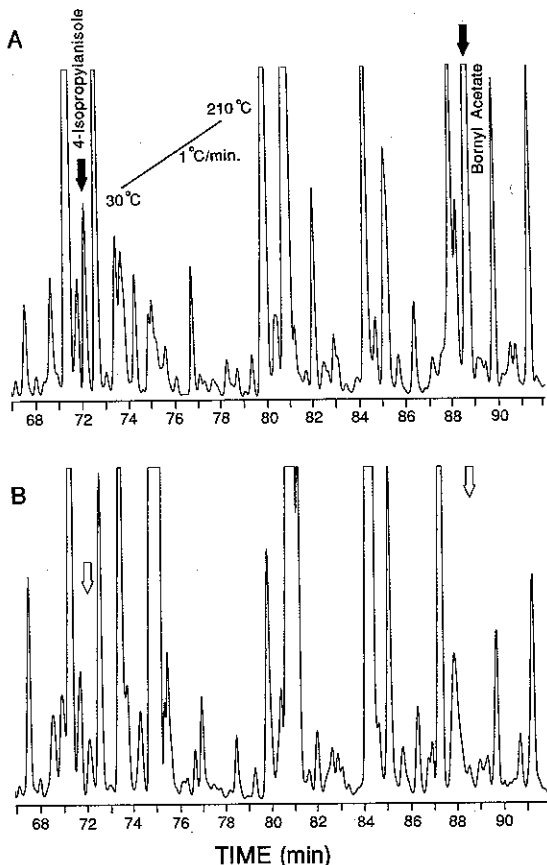
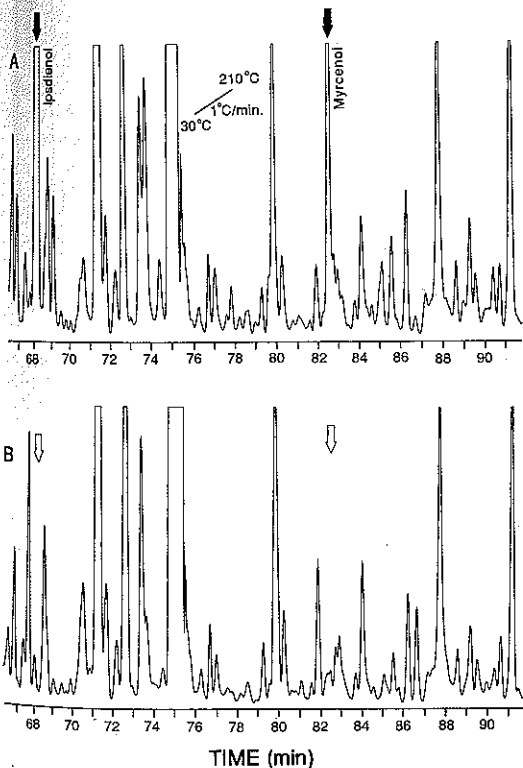


Fig. 6. Chromatogram of volatiles captured for 30 h from a female *Dendroctonus ponderosae* feeding in lodgepole pine phloem tissue in phloem sandwich preparation (A), compared with chromatogram of volatiles captured for 30 h from phloem tissue of the same lodgepole tree (B).

To aerate bark beetle galleries in living trees or intact logs, or to aerate galleries from ambrosia beetles, for which a "sapwood sandwich" has not yet been developed, a third volatile collector was designed (Fig. 4). A glass tube expanded toward one end was connected to a Porapak Q trap. The expanded end was inserted into two rubber O-rings. To secure the tubing over the entrance hole of the gallery, the O-rings were pressed against the bark with a flexible aluminum plate. The air

Fig. 5. Chromatogram of volatiles captured for 30 h from a male *Ips pini* feeding in lodgepole pine phloem tissue in on-tree preparation (A), compared with chromatogram of volatiles captured for 30 h from phloem tissue of the same lodgepole pine tree (B).

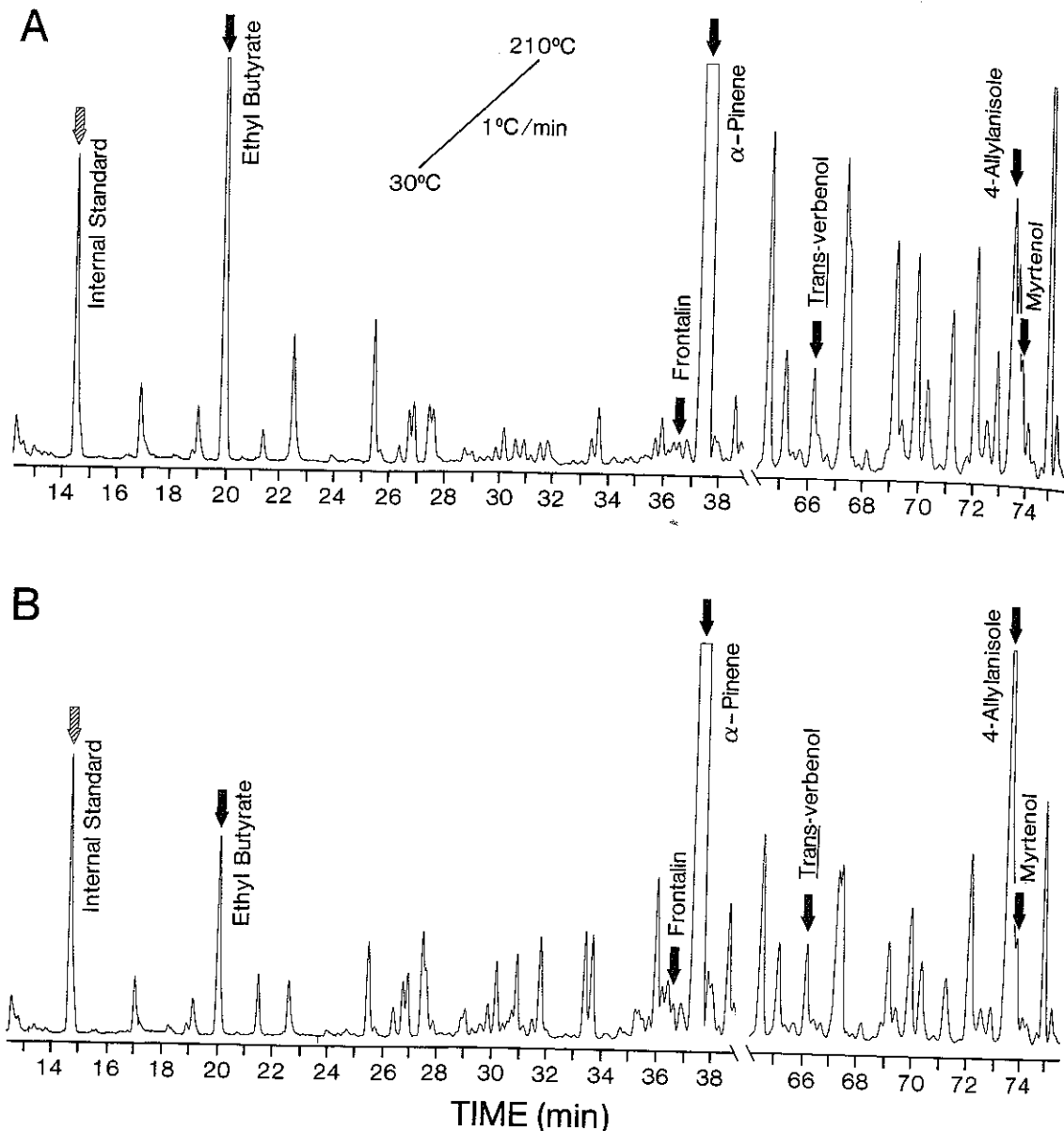


Fig. 7. Chromatogram of volatiles captured for 30 h from a female *Dendroctonus rufipennis* feeding in Engelmann spruce phloem tissue in phloem sandwich preparation (A), compared with chromatogram of volatiles for 30 h from the same female following the addition of the male (B).

inlet occurred between the tubing and the rough bark. Otherwise, volatile capture was as described for the phloem sandwich technique.

Analysis of Trapped Volatiles. Volatiles trapped in Porapak Q were desorbed with 0.5 ml double-distilled pentane: ether (95:5), concentrated to 10% of the former volume and analyzed by GLC or coupled gas-chromatography-mass spectrometry. To separate the components efficiently, a DB-1

column (60 m by 0.32 mm inner diameter) with an appropriate temperature program and the lowest possible pressure of the carrier gas was used. To identify compounds produced by beetles, chromatograms of volatiles captured from galleries containing beetles of the first attacking sex were compared with chromatograms of host volatiles (Fig. 5 and 6) and with those following the addition of the mate(s) (Fig. 7).

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