NOTE

A Phloem Sandwich Allowing Attack and Colonization by Bark Beetles (Coleoptera: Scolytidae) and Associates[1, 2]

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Much of the life cycles of bark beetles and their associates are spent under the bark of the host tree and are impossible to observe under completely natural conditions. To observe the behavior and development of insects in the phloem layer, phloem sandwiches have been developed, in which a piece of bark and phloem is removed from a live tree and pressed against a glass plate (Bedard, 1933, J. Econ. Entomol. 26: 1128-1134; Borden, 1969, Can. Entomol. 101: 870-878; Schmitz, 1972, Can. Entomol. 104: 1723-1728; Salom et al., 1986, J. Entomol. Sci. 21: 43-51; Langor and Raske, 1987, Can. Entomol. 119: 965-992). Previous designs (e.g., Kinn and Miller, 1981, USDA-FS Res. Note 50-239) required that the insects be placed into the sandwich by the researchers. This has precluded studies of the attack process (e.g., response to aggregation pheromones) and of the interactions among naturally-created combinations and densities of species. We report on a modification which allows insects to freely and naturally colonize the phloem sandwich.

Sandwich design and construction. Sandwiches of several sites have been used successfully, however, a standardized size 12.7 cm × 40.6 cm was used for the tests described below. This is large enough to allow apparently normal s-shaped gallery construction, without being so large that problems arise in obtaining intact bark pieces, or from warping of the sandwich.

Front pieces were made from 2.5 cm thick plywood. Holes 1.9 cm in diameter were drilled through the plywood, roughly on 2.5 cm centers, in the area to be covered by the phloem and glass (Fig. 1a). In a sandwich of standard size, 39 holes were drilled, exposing ca. 30% of the bark surface. Alternatively, 2.0 cm thick polyvinylchloride (PVC) board can be used for the front pieces. This material is stronger than plywood, allowing the holes to be larger: 2.0 cm wide by 10.2 cm long ovals, 1.0 cm apart in an offset pattern, exposing 60% of the bark surface. The back piece was 0.6 cm thick glass. Clamps made of 16-gauge steel shelf standards (Bushing 1967 PhD Dissertation, University of California, Berkeley) hold these pieces together.

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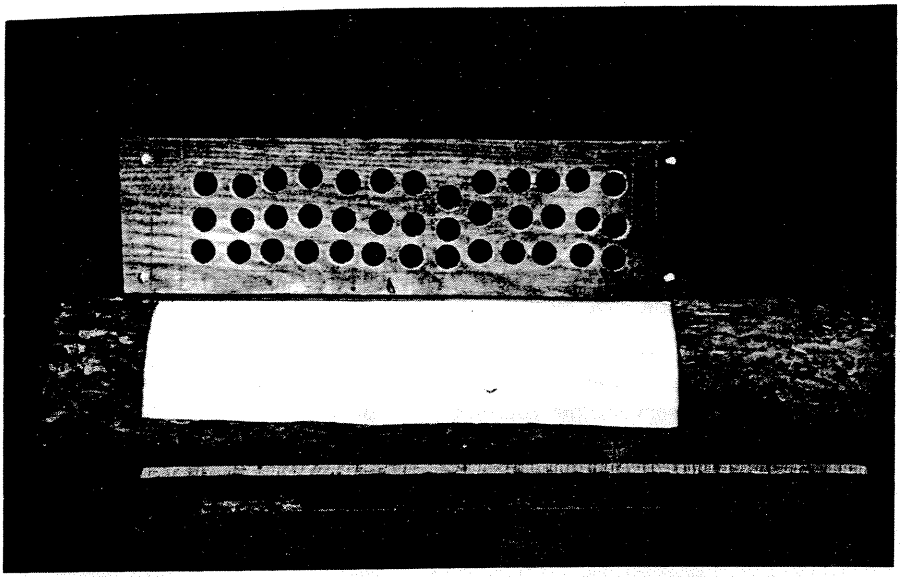

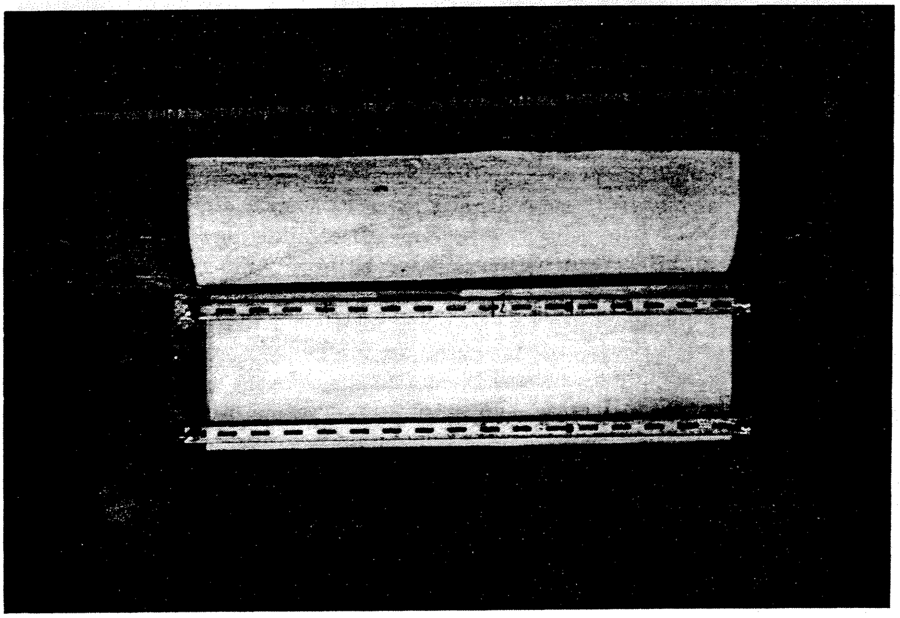

Fig. 1. a. Plywood front piece of sandwich showing pattern of holes, and b. reverse side with phloem under glass beside bolt from which section of bark and phloem has been stripped.

Bark and phloem were removed from bolts of an appropriate tree species; for southern pine beetle, loblolly pine (*Pinus taeda* L.) was typically used, although longleaf pine (*P. palustris* Mill.) can be substituted. Bolts should be ca. 20-35 cm in diameter and free of knots, limbs, or other protrusions. The outer bark was smoothed with a high-speed sander or drawknife. A piece of bark/phloem of the desired size was then cut and peeled from the xylem (Fig. 1b), with the intact phloem remaining attached to the bark. The bark/phloem section was placed bark-side down on the plywood or PVC front piece. A glass back piece and a pair of clamps were bolted tightly to the front piece. Finally, the edges of the sandwich were sealed with paraffin.

The effectiveness of these sandwiches in allowing infestation by SPB and associates was tested in two laboratory studies (May 1988) and two field studies (April 1988, May 1989).

(1) **Laboratory infestation by SPB.** Five sandwiches were placed on one side of a 2.44 m × 3.04 m screened room. Two loblolly pine bolts, 90 cm long and ca. 26 cm in diameter, from which SPB brood adults were emerging, were placed on the opposite side of the room. A plastic vial containing 1 ml of a 2:1 mixture of α-pinene and frontalin (frontalure) was placed in the middle hole of each sandwich to instigate SPB attack by simulating attractants released by pioneer female beetles. Pheromone vials were removed the following day, after beetle attack was observed. Sandwiches were exposed to the SPB-infested bolts for 10 days, then removed to a separate screened room and observed daily. Screened rooms were outdoors under an aluminum roof, so temperatures and humidity were similar to natural conditions.

All five sandwiches were quickly attacked. Attacks were first observed one day after the sandwiches were exposed to emerging SPB. Final numbers of attacks were 15, 16, 19, 20 and 21 (on 516 cm^2 of bark surface). These attack densities (3-4/100 cm^2) were within the highly variable range reported for attack densities on individual trees (Fargo, et al., 1979 Environ. Entomol. 8: 624-628). Larval mines were usually longer and more winding than observed in intact trees (Fig. 2a), perhaps indicating nutritional inadequacy due to invasion by fungi (Barras, 1970, Ann. Entomol. Soc. Amer. 63: 1187-1190). Several SPB reached the mid-larval stage, but only one developed past the late-larval stage.

(2) **Laboratory attack by a predator.** Adult *Thanasimus dubius* (F.) (Coleoptera: Cleridae), a predator of SPB, were collected in the field in Lindgren funnel traps baited with frontalure. Five sandwiches were placed individually in screen cages 50 cm × 50 cm × 95 cm. Frontalure was placed on each sandwich as described above, and 25 unsexed SPB (reared from loblolly pine bolts placed in rearing cans) were released into each cage. On day 5, when SPB galleries were observed in the sandwiches, 50 more SPB were released into each cage. One hour later, 50 unsexed adult *T. dubius* were released into each cage. On day 10, the sandwiches were removed from the cages, placed in a screened room (described above) and observed daily.

SPB successfully colonized the five sandwiches with successful attacks numbering 13, 15, 17, 20, and 21 respectively. Clerids (*T. dubius*) also successfully colonized all five sandwiches. Clerid adults were seen preying on SPB adults that were attempting to colonize the sandwiches. Clerid larvae were observed in the sandwiches within 8 days of clerid adult release, and on several occasions were seen preying on SPB larvae inside the sandwiches (Fig. 2b). However, no clerid larvae survived beyond the second instar.

(3) **Field infestation by associates.** Six sandwiches were infested by SPB under laboratory conditions, as in (1) above. When each sandwich had at least three successful SPB attacks, the sandwiches were placed in an active SPB infestation in the field. This infestation was naturally initiated with the colonization of a lightning-struck tree and consisted of ca. 45 successfully attacked loblolly pines (x = 40 cm dbh, diameter at breast height) at the time of this study. Three uninfested loblolly pines closest to the active front of the infestation were selected and on each tree two sandwiches were hung 50 cm apart, with the lower sandwich 3 m above the ground. Sandwiches were observed daily, and attacks by any associates were recorded, along with the developmental status of the SPB at that time.

Three species of SPB associates were observed in these sandwiches *Ips calligraphus* (Germar) were observed on three sandwiches in which SPB were in the attack or eggs stages. *Pityophthorus confusus* Eichhoff was found in one sandwich, 3 days post-attack with SPB in the egg stage. *T. dubius* was found in one sandwich, 7 days post-attack with SPB in the early/mid larval stage. Immatures of these species did not develop beyond early larval instars.

(4) **Field infestation by SPB and associates.** Three sandwiches were hung on each of three uninfested trees at the front of an active SPB infestation. This infestation was naturally initiated and consisted of 15-18 loblolly pines (x = 20 cm dbh). Sandwiches were placed at 180 cm, 260 cm, and 340 cm above the ground on one tree (day 1), and at 260 cm, 340 cm, and 420 cm on the other trees day 6 and 13). Each sandwich was baited as described above. Sandwiches were left on trees for 9 to 13 days, and observed daily during this time. Approximately 1.6 km from this infestation, six sandwiches were hung on hardwood trees at the site of a SPB-related salvage operation.

Seven of the nine sandwiches were attacked by SPB, but attack densities were considerably lower than those in laboratory-infested sandwiches. Sandwiches at all heights were attacked (180-420 cm; $\bar{x}$ = 303 cm). Attacks occurred from 1 to 8 days ($\bar{x}$ = 2.6 d) after sandwich placement, with 1-10 attacks ($\bar{x}$ = 6.1) per sandwich. Two associate species, *Ips avulsus* (Eichoff) and an unidentified staphylinid, were observed in sandwiches. Development of SPB was poor in all sandwiches, in part because heavy rains caused them to become water-logged. None of the sandwiches hung on hardwood trees were attacked by SPB, but all six were attacked by a cerambycid beetle, three locally abundant *Ips* species and *Monochamus* sp. Successful development was accomplished by each of these *Ips* species and a *Monochamus* larva was found alive in a sandwich after 12 months.

Although work is still needed to perfect the sandwich as a rearing tool, by allowing attack to occur more naturally, the modified sandwich offers several immediate advantages. First, the densities of insects are determined by the insects themselves and are likely to be more similar to those in nature than if they were artificially imposed. Any density dependent phenomena, such as predation rates or competition, therefore can be studied more realistically. Similarly, these sandwiches will allow detection and study of the associate species active at a given place and time, rather than only selected species. For instance, we

Fig. 2. a. SPB gallery construction in a phloem sandwich showing long winding pattern, and, b. clerid larva in SPB gallery inside sandwich.

have observed two species of SPB predators, *Platysoma cylindrica* (Pa; and *P. parallelum* Say (Coleoptera: Histeridae), in sandwiches exposed to infested bolts. The predatory behavior of these insects indicates that the; be more important than previously realized, and certainly warrant fu study (Hayes, unpublished data). The modified sandwich can be used for mining whether particular species respond to bark beetle pheromones or natural attack cues. This could be relevant in screening candidate species fc logical control programs. The sandwiches also could be used for collecting o susing species which are otherwise difficult to trap but are attracted by pheromones.

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