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Effects of Feeding and Mating on Pheromone Release in the Southern Pine Beetle

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ABSTRACT

Response of field populations of *Dendroctonus frontalis* Zimmermann to their aggregating pheromone was correlated with pheromone content of dissected hindgut tissues of adult beetles as determined by the gas-liquid chromatography (GLC) technique. Adult beetles in various stages of feeding activity and reproductive states were used for this purpose.

Two major components of the aggregating pheromone, frontalin and trans-verbenol, were found in the largest quantities in emergent unfed females. After 48 hours of feeding, frontalin content of the hindguts was 29% and trans-verbenol content was only 5% of that of emergent females.

Continuous bioassays of females feeding in host material showed increasing response of field populations to the beetles until 24-48 hours after feeding had begun, then response declined. The fact that pheromone content declined steadily following beetle attack while field response increased was thought to be due to capture of the pheromone in frass particles, thus slowing its rate of release.

The decline of pheromone components in hindguts of mated females was not precipitous or abrupt as suggested by behavioral studies on other species of *Dendroctonus*. Field bioassays confirmed the GLC studies and showed that in both virgin and mated females peak attractiveness occurred 24-48 hours after the tree was attacked by the beetles.

PROCEDURES

Field Bioassay.—Beetles were introduced into loblolly pine, *Pinus taeda* L., posts (13 cm × 2.3 m) in a manner previously described (Coster 1970). For bioassay, these posts were placed in tree-trunk-simulating olfactometers (Gara et al. 1965) at forest sites adjacent to natural infestations of the beetles in Hardin County, Tex.

GLC Analysis.—Gas-liquid chromatography (GLC) analyses were performed on extracts of dissected hindguts. Instrumentation included a Varian Aerograph equipped with flame ionization detector and 4%in. × 5-ft stainless-steel column. The columns were packed with 5% DEGS on Chromosorb W, 3% SE-30 on Varaport 30, or 2% FFAP on Chromosorb G. Operating temperatures for the DEGS column were: at the injector 120°C, on the column 105°C, and at the detector 120°C. The SE-30 and FFAP columns were both used at a column temperature that was programmed from an initial temperature of 70°C.
In this way, each of the feeding times was replicate 5 times at each of the 5 olfactometer positions. The feeding virgin pine beetles showed increasing attractiveness to flying beetles until 48 hr after “a tick” of the host material (Fig. 1). On the 1st da of attack (less than 6 hr) the beetles slowly became attractive. Response to the beetles increased sharply 24 hr after attack, reaching a maximum after 48 h of boring activity. Response declined on the 3rd an 4th days of attack.

GLC using the DEGS column was used to determine changes in the amounts of trans-verbenol an, frontalin in the hindguts of feeding virgin females. Each extract sample consisted of 10 hindguts. The effect of feeding on trans-verbenol decreased until less than 5% of the original amount remained at 4 hr (Fig. 2).

Detection of frontalin in the feeding females was more difficult than in unfed females because of smaller amounts present and because of the presence of terpenes from the ingested phloem. Two of these terpenes, alpha- and beta-pinene, had retention time similar to that of frontalin. For these reasons, frontalin in the feeding females was verified by comparing sample retention times to retention times of synthetic frontalin and pinenes on both a polar (FFAP) and nonpolar (SE-30) column.

Fig. 3 shows the effect of feeding on frontalin content of the female southern pine beetles. Data for the comparisons were obtained from analyses using the SE-30 column. The newly emerged unfed virgil females contained 3 times more frontalin than the 2-day-old feeding females. The GLC analyses show

![Graph](image)

**Fig. 2.—Change in relative trans-verbenol content of hindguts of feeding unmated D. frontalis females as determined by GLC. Average GLC peak height of unfed beetles = 100%. The number of 10 hindgut samples for each feeding period was: 0 hr, 7; ½ hr, 3; 1 hr, 7; 2 hr. 7; 4 hr, 7; 8 hr, 5; 16 hr, 6; 24 hr, 5; 48 hr, 4.**
that feeding, independent of mating activity, can result in a decrease of the pheromone content of female southern pine beetles.

**Field Attractiveness and Pheromone Content of Feeding Mated Beetles.**—The field attractiveness of virgin feeding females was tested in the previous experiments. To provide comparison with mated females, an identical procedure was used except that the females were introduced into the posts, and the entrance holes were covered with gelatin capsules. About 2 hr later the capsules were removed, and a single male was placed in each entrance hole. A plastic screen was then stapled over the hole to prevent escape of the beetles.

Fig. 1 shows results of this test. Although the general response pattern is similar, the χ² criterion showed the frequency distribution of beetles responding to the mated females over the entire test period was significantly different from that of beetles responding to the virgin females (χ² = 22.7, 4 df). Thus, the greater attractiveness of mated beetles during the 1st 48 hr of attack is significant, as is the greater attractiveness of virgin beetles thereafter.

Influence of mating on frontalin and trans-verbenol content of the hindguts of the beetles also was investigated using GLC techniques. One half of a group of virgin emergent females from rearing cages was introduced into pines and allowed to feed for 48 hr. The remaining beetles were placed in pines, but males were added to each gallery 4–6 hr after introduction of females. The 2nd lot of females was allowed to feed for 48 hr in company with males. At the end of the feeding period all beetles were removed from the logs, hindguts of the females were dissected, and 2 extract samples (20 hindguts each) were prepared for each class of females (mated and virgin).

The peak areas of both frontalin and trans-verbenol were less in the mated beetles (Table 1). Feeding virgin females contained 2.8–3.5 times as much frontalin and 1.4–3.4 times as much trans-verbenol as fed mated females.

**Observations on Feeding Behavior.**—The length of time required for passage of phloem tissue to the hindgut after the beetle bores through the outer bark also was checked. Squares of phloem tissue (15x15 cm × 3 mm) were dyed with an aqueous solution of acid fuchsin and placed firmly between 2 sheets of Plexiglas®. Female beetles were placed in contact with the phloem by insertion into 2-mm-diam holes bored through the Plexiglas. After 1 hr of continuous feeding, 25 beetles that had produced the largest quantities of frass were removed and their hindguts were dissected. Under magnification the red of the dye was plainly visible in particles in the ileum and rectum of all the beetles. Faint coloring was noted in a few midguts during the dissections. The red of the hindguts began to fade soon after dissection, until after 45 min it was undetectable.

**DISCUSSION**

Emergent unfed females contained the greatest amount of frontalin and trans-verbenol (Fig. 2, 3). These pheromone materials decreased in amount with time as the beetles fed. The results confirm the observations of others that infested host material is most attractive during the early stages of attack (Vité and Pitman 1968); however, trans-verbenol was not entirely depleted from the hindguts within 18–24 hr of initial attack as was reported previously (Vité and Crozier 1968). The substance was still present at a low level 48 hr after feeding.

There is an apparent anomaly between the GLC results showing highest pheromone content in unfed beetles and the response of field populations to feeding beetles. Pheromone content in the hindgut declined steadily after initial feeding began, while field response to feeding beetles increased for 24–48 hr.

**Table 1.**—Relative amounts (peak areas, mm²) of frontalin and trans-verbenol found in the hindguts of virgin and mated *D. frontalis* females fed for 48 hours. Compounds determined by GLC.

<table>
<thead>
<tr>
<th>Sexual condition of females</th>
<th>Virgin</th>
<th>Mated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of GLC column¹</td>
<td>Frontalin</td>
<td>trans-Verbenol</td>
</tr>
<tr>
<td>SE-30</td>
<td>118</td>
<td>507</td>
</tr>
<tr>
<td>FFAP</td>
<td>42</td>
<td>452</td>
</tr>
</tbody>
</table>

* Data obtained from both column materials are presented to show differences in efficiency.
after attack. Evidence has been found in work on *Ips confusus* (LeConte) that some portions of the attractant are bound more strongly to the feces than others (Wood et al. 1966). It was necessary to crush and heat the pellets to extract the maximum amounts of *I. confusus* attractant. Such an adherence of the pheromone to fecal pellets may explain the differences in field attraction and hindgut pheromone content. Although southern pine beetles eject the larger part of their attractant in the 1st day of attack, its slow evaporation from the fecal pellets may reduce the amount that is immediately exposed to the atmosphere. The observations with fuchsin-dyed phloem showed that ingestion does occur upon contact with the inner bark so that feces could become impregnated with hindgut liquids.

Hindguts of feeding mated females contain less pheromone than those of unmated females fed for the same length of time (Table 1). In field tests, however, the differences in attractiveness between virgin and mated beetles were not so great (Fig. 1). The filtering effect of the boring dust and fecal pellets as previously discussed may have lessened differences in attractant release at the test posts. In addition, the greater gallery construction activity of mated beetle pairs as opposed to single females (Yu and Tsao 1967) produces more frass which, in turn, may expose more pheromone to the atmosphere. Very little frass is produced by the males (Frank 1947, Tsao and Yu 1967). This increased boring activity of mated pairs may also explain the greater attractiveness of mated beetles in the 1st 48 hr after attack of the tree (Fig. 1).

Pheromone-masking substances have been implicated in the attack behavior of *D. pseudotsugae* (Rudinsky 1969) and *T. lineatum* (Nijholt 1970), in which species males are thought to stimulate release of the masks from females. Although critical tests for such a substance were not performed with the southern pine beetle, no abrupt cessation of attraction indicating release of such compound(s) was observed following introduction of males. However, male southern pine beetles do play a role in attack behavior. Verbenone, a prominent component of male hindgut liquids, has been suggested to be the compound responsible for regulation of the sex ratio of beetles responding to attractive sources (Renzin and Vité 1969, Coster 1970). The action does not appear to be as a mask of the female pheromone but rather as an additional chemical messenger to which male and female southern pine beetles respond differently.

**ACKNOWLEDGMENT**

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