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II. At sites in the Eastern Hemisphere and Pacific area. Ibid. 61: 1306–14.
1971. Distribution and relative abundance of dipterous pupae and their parasitoids in accumulations of domestic animal manure in the southwestern United States. Hilgardia 40(14): 505–35.
Manwell, C., and C. M. A. Baker. 1970. Molecular Biology and the Origin of Species. University of Washington Press, Seattle. 394 p.
Moore, J. A. 1963. Genetics today. Proc. 11th Int. Congr. Genet. Pergamon Press, Oxford, p. 431–5.
Povolny, D. 1971. Synanthropy. Definition, evolution and classification, p. 17–54. *In* B. Greenberg [ed.] Flies and Disease. Princeton Univ. Press, Princeton, N. J.
Rivnay, E. 1968. Biological control of pests in Israel. J. Entomol. Soc. Israel. 3: 1–156.
van den Bosch, R., B. D. Frazier, C. S. Davis, P. S. Messenger, and R. Hom. 1970. *Trioxys pallidus* . . . an effective new walnut aphid parasite from Iran. Calif. Agr. 24(11): 8–10.
Wiener, G. 1957. The significance of grading-up in the Ayrshire cattle population of Great Britain. J. Agr. Sci. 49(3): 313–8.

Effects of Feeding and Mating on Pheromone Release in the Southern Pine Beetle[1,2]

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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.

The aggregating pheromone of the southern pine beetle, *Dendroctonus frontalis* Zimmerman, is contained in the hindguts of emergent females and is released to the outside where, along with certain host odors, it brings about aggregation of both sexes at trees undergoing attack (Vité and Renwick 1968). Infested host material declines in attractiveness after the beetles have begun to feed extensively (Vité and Pitman 1968). A similar pattern was shown with *D. pseudotsugae* Hopkins where greatest attractiveness of the frass occurred in the 1st few hours of attack, but it declined thereafter (Borden et al. 1968).

The relationship between mating and cessation of pheromone release is unclear in the mongamous scolytids. Rudinsky (1963, 1966) and Jantz and Rudinsky (1966) reported that the attraction decreased rapidly when *D. pseudotsugae* males were introduced to their females. Rudinsky (1969) suggested a female-released pheromone mask in this species to account for the rapid decline in attractiveness. For the same insect, McMullen and Atkins (1962) reported only a tendency for attraction to decline after mating. Mating of *Trypodendron lineatum* (Olivier) reduced attractiveness, but this effect was neither immediate (Chapman 1966) nor thought to be entirely dependent on mating (Nijholt 1970). Tsao and Yu (1967) found in laboratory bioassays of southern pine beetle pheromones that extracts of mated females were only slightly less attractive than those of virgin females.

The following studies were performed to elucidate the effects of feeding and mating on the subsequent attractiveness of female southern pine 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 ⅛-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

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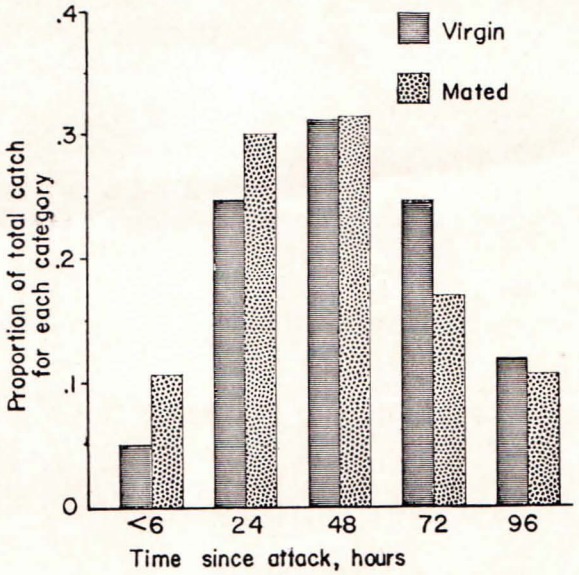

FIG. 1.—Field response of *D. frontalis* to olfactometers baited with loblolly pine posts containing either 200 feeding virgin females or 200 feeding females and 200 ♂ (mated). Each time period was replicated with 5 posts. Total response at posts containing virgin beetles = 202; at posts containing mated beetles = 206.

to a final 140°C at a rate of 4°/min. Injector and detector temperatures were both held at 190°C. Nitrogen- and hydrogen-flow rates were 25 ml/min.

Excised hindguts were placed in a 1-ml centrifuge tube held at approximately 0°C. Water was added at the rate of 2 μliters per hindgut, then the tissue was triturated with a small amount of purified sand and centrifuged. Samples were withdrawn immediately and injected into the gas chromatograph.

The 2 major volatiles in female hindguts are *trans*-verbenol (Renwick 1967) and frontalin (Kinzer et al. 1969). Hindgut compounds were verified by comparison of retention times with those of synthetic frontalin and *trans*-verbenol. Relative amounts of the compounds were determined by computing peak areas (mm^2) from the chromatograms.

EXPERIMENTS AND RESULTS

Field Attractiveness and Pheromone Content of Feeding Virgin Females.—To test the field attractiveness of feeding unmated females, loblolly pine posts were each artificially infested with 200 emergent unmated females collected from rearing cages. The experiment was designed to simultaneously test the infested posts containing virgin females fed for time intervals from 0 to 96 hr. To accomplish this, a post was prepared each day for 5 consecutive days. Immediately after the 5th post was prepared, all were taken to the field bioassay site and placed in olfactometers and simultaneously bioassayed. Responding beetles were captured and counted. On days 6 through 10, a post was infested in the laboratory with 200 ♀ and immediately taken to the test site, where it replaced the post with females fed for more than 96 hr. 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 increasin attractiveness to flying beetles until 48 hr after "a tack" of the host material (Fig. 1). On the 1st da of attack (less than 6 hr) the beetles slowly becam attractive. Response to the beetles increased sharpl 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 deter mine changes in the amounts of *trans*-verbenol an frontalin in the hindguts of feeding virgin female Each extract sample consisted of 10 hindguts. Th effect of feeding on *trans*-verbenol decreased unti less than 5% of the original amount remained at 4 hr (Fig. 2).

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

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

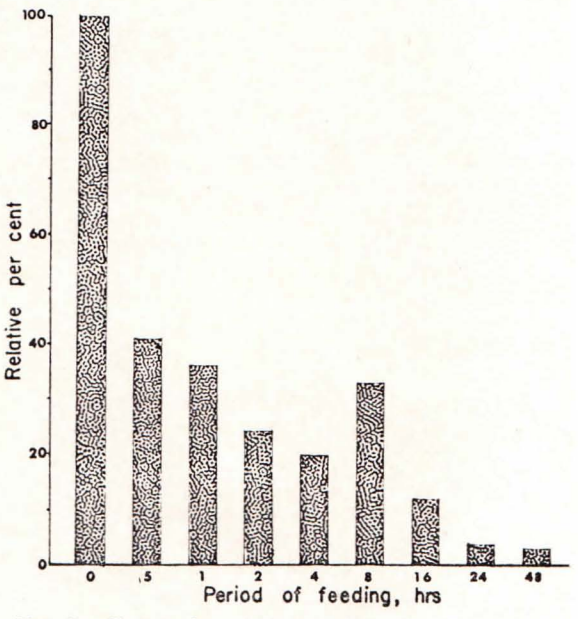

FIG. 2.—Change in relative *trans*-verbenol content of hindguts of feeding unmated *D. frontalis* females as determined by GLC. Average GLC peak height of unfed beetles = 100%. The number of 10 hindgut samples for each feeding period was: 0 hr, 7; ½ hr, 3; 1 hr, 7; 2 hr, 7; 4 hr, 7; 8 hr, 5; 16 hr, 6; 24 hr, 5; 48 hr, 4.

hat feeding, independent of mating activity, can result in a decrease of the pheromone content of female outhern 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 he 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 χ^2 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 ($\chi^2 = 22.7$, 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

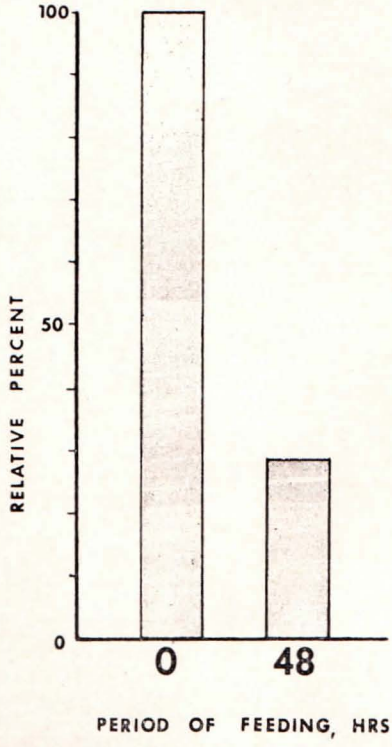

FIG. 3.—Change in relative frontalin content of hindguts of feeding, unmated *D. frontalis* females as determined by GLC. Avg GLC peak area of unfed beetles = 100%. The number of 10-hindgut samples for each period was 5.

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.

	Sexual condition of females			
	Virgin		Mated	
Type of GLC column[a]	Frontalin	*trans*-Verbenol	Frontalin	*trans*-Verbenol
SE-30	118	507	42	370
FFAP	42	452	12	130

[a] Data obtained from both column materials are presented to show differences in efficiency.

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 (15×15 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 fontalin 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

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 attractancy 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 (Fronk 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 (Renwick and Vité 1969, Coster 1970[5]). 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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[5] J. E. Coster. 1970. Certain aspects of pheromone release and aggregation behavior in the southern pine beetle (Coleoptera: Scolytidae). Ph.D. dissertation, Texas A&M University. 129. p.

REFERENCES CITED

Borden, J. H., R. M. Silverstein, and R. G. Brownle 1968. Sex pheromone of *Dendroctonus pseudotsi gae:* Production, bioassay, and partial isolation. Ca Entomol. 100: 597–603.

Chapman, J. A. 1966. The effect of attack by the an brosia beetle *Trypodendron lineatum* (Oliv.) on lo attractiveness. Ibid. 98: 50–59.

Coster, J. E. 1970. Production of aggregating pher mones in re-emerged parent females of the souther pine beetle. Ann. Entomol. Soc. Amer. 63: 1186–

Dyer, E. D. A., and D. W. Taylor. 1968. Attractiv ness of logs containing female spruce beetles, *Der droctonus obesus.* Can. Entomol. 100: 769–76.

Fronk, W. D. 1947. The southern pine beetle—its li history. Va. Agr. Exp. Sta. Tech. Bull. 108.

Gara, R. I., J. P. Vité, and H. H. Cramer. 1965. Man pulation of *Dendroctonus frontalis* by use of a popu lation aggregating pheromone. Contrib. Boyc Thompson Inst. Plant Res. 23: 55–66.

Jantz, O. K., and J. A. Rudinsky. 1966. Studies on th olfactory behavior of the Douglas-fir beetle, *Dendroc tonus pseudotsugae* Hopkins. Oreg. Agr. Exp. St Tech. Bull. 94.

Kinzer, G. W., A. F. Fentiman, Jr., T. F. Page, R. I Foltz, J. P. Vité, and G. B. Pitman. 1969. Bar beetle attractants: Identification, synthesis, and fiel bioassay of a new compound isolated from *Der droctonus.* Nature (London) 221: 447–8.

McMullen, L. H., and M. D. Atkins. 1962. On th flight and host selection of the Douglas-fir beetl *Dendroctonus pseudotsugae* Hopk. Can. Entomol. 94 1309–25.

Nijholt, W. W. 1970. The effect of mating and th presence of the male ambrosia beetle, *Trypodendro lineatum,* on "secondary" attraction. Ibid. 102: 894–

Renwick, J. A. A. 1967. Identification of two oxy genated terpenes from the bark beetles *Dendroctonu frontalis* and *Dendroctonus brevicomis.* Contril Boyce Thompson Inst. Plant Res. 23: 355–60.

Renwick, J. A. A., and J. P. Vité. 1969. Bark beetl attractants: Mechanism of colonization by *Dendroc tonus frontalis.* Nature (London) 224: 1222–3.

Rudinsky, J. A. 1963. Response of *Dendroctonus pseu dotsugae* Hopkins to volatile attractants. Contril Boyce Thompson Inst. Plant Res. 22: 23–38.

1966. Observations of olfactory behavior of scolyti beetles associated with Douglas-fir forests. Z Angew. Entomol. 58: 356–61.

1969. Masking of the aggregation pheromone in *Der droctonus pseudotsugae* Hopk. Science (Washing ton) 166: 884–5.

Tsao, C. H., and C. C. Yu. 1967. Sex pheromones c the southern pine beetle, *Dendroctonus frontali* Zimm. J. Ga. Entomol. Soc. 2: 13–20.

Vité, J. P., and R. G. Crozier. 1968. Studies on the at tack behavior of the southern pine beetle. IV. In fluence of host conditions on aggregation patter Contrib. Boyce Thompson Inst. Plant Res. 24: 87–9

Vité, J. P., and G. B. Pitman. 1968. Bark beetle aggre gation: Effects of feeding on the release of pher mones in *Dendroctonus* and *Ips.* Nature (London 218: 169–70.

Vité, J. P., and J. A. A. Renwick. 1968. Insect an host factors in the aggregation of the southern pin beetle. Contrib. Boyce Thompson Inst. Plant Re 24: 61–63.

Wood, D. L., L. E. Browne, and S. O. Rodin. 196 Sex pheromones of bark beetles. I. Mass productio bioassay, source, and isolation of the sex pheromon of *Ips confusus* (LeC.). J. Insect Physiol. 12: 523–3

Yu, C. C., and C. H. Tsao. 1967. Gallery constructio and sexual behavior in the southern pine beetl *Dendroctonus frontalis* Zimm. J. Ga. Entomol. So 2: 95–98.