midal nerve cells contralateral to the used paw.

These data merit only the conclusion that there is a correlation between the increased neuronal protein synthesis and the increased neural activity during the experimental conditions. It seems clear that the electrical activity changes significantly in the hippocampus, at the consolidation of learning. In addition, in his experiments on visual discrimination, Adey (*2*) found spatial as well as temporal changes of the electrical activity within the hippocampus. In early training the phase pattern was reversed to that found in fully trained animals.

The two protein fractions we investigated move near the anode front at electrophoresis. They immediately follow the protein fractions constituting the acidic protein of brain cell, S100 (*13*). This protein is of interest because of its specificity for the central nervous system, its acidic character, and its localization to the nuclei of the neurons. The basic histones of the nucleus are considered to regulate gene activities and the presence of acidic proteins may involve also regulatory effects on the histones.

The results, for example, of Flexner (*6*) in mice, or Agranoff (*14*) in goldfish, and of Albert (*15*) in rats, seem to link protein synthesis to the neural processes responsible for learning and storage of information. Barondes (*16*), on the other hand, has not found experimental evidence for the view that synthesis of a protein is required for memory storage. Our findings, however, are in agreement with those of Flexner and Agranoff. Neither type of finding gives any information about the specificity of proteins.

The trend to lateralization of the highest degree of protein synthesis to the learning side of the hippocampus is interesting but surprising with respect to the wealth of activities reaching and cross-reacting with the limbic area.

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## Response of Ips confusus to Synthetic Sex Pheromones in Nature

Abstract. *The first flight response of bark beetles to synthetic sex pheromones under natural conditions is reported. Two insect species predaceous on this bark beetle also responded. The synthetic compounds delivered in an airstream from a substrate of Carbowax 20M on Chromosorb A appear to elicit the same response as the natural attractant from male-infested bolts of ponderosa pine elicits.*

We report the first flight response of bark beetles to synthetic sex pheromones (*1*) under natural field conditions. These pheromones are three terpene alcohols—compound I: (−)-2-methyl-6-methylene-7-octen-4-ol; compound II: (+)-*cis*-verbenol; compound III: (+)-2-methyl-6-methylene-2,7-octadien-4-ol—which were isolated from frass from male *Ips confusus* (LeConte) (Coleoptera: Scolytidae) (*2*), identified, and synthesized (*3*). These compounds appear to elicit the same response as male-infested bolts of ponderosa pine do; therefore, they are probably the chemical messengers that evoke the concentration flight and subsequent mass attack of *I. confusus*.

Experiments were performed in a gently rolling, treeless brush field to avoid unknown, competing sources of attraction and unpredictable air movements. The site (20 hectares) was located near the south fork of Willow Creek, Madera County, California, in a drainage with a relatively consistent up- and down-valley wind system. Insect traps were placed on 20-m centers in two lines, 400 m apart, perpendicular to the prevailing winds. Each treatment was replicated once in each line, and the trap positions were randomly assigned at the beginning of each experiment. The endemic flying populations were probably emerging from naturally infested, ponderosa pine logging slash within 1 km of the experimental area. All tests were begun when the prevailing winds were moving at 6 to 13 km/hr up the valley toward the infestation. The temperature fluctuated between 20° and 31°C.

The traps consisted of hardware-cloth (mesh of 0.64 to 0.96 cm) cylinders, 30 by 12 cm in diameter, each fastened to the end of a 1.5-m rod driven into the ground. The cylinders were dipped in warm "Stickem Special," so that most of the squares remained open. Air was metered from a portable pressurized tank through a charcoal filter and then through nylon tubing (0.32 cm in outside diameter) to an aluminum tube containing the attractants. We maintained a flow rate of 50 $cm^3$/min with a regulator and a capillary (0.02 cm in inside diameter by 5 cm) in series. The exit port was placed in the center of the trap. One male-infested bolt in each line was the standard source of natural attractant. Each bolt held 20 male beetles confined within preformed entrance tunnels beneath wire screen. The logs were exposed 48 hours after confinement.

The synthetic attractants were confined to an aeration tube consisting of aluminum tubing which had an outside diameter of 1 cm. A solution of 0.5 g of Carbowax 20M in methanol was added to 9.5 g of Chromosorb A, and most of the methanol was evaporated. A solution of 1.5 mg of compound I in pentane was added to 7 ml of the above substrate. The excess pentane was evaporated, and the mixture was poured into a 15-cm length of tubing (section A). A second 15-cm tube (section B) was filled with the same substrate to which a solution of 1.5 mg of compound I, 1 mg of compound II, and 1 mg of compound III in pentane was applied in the same way. Glass-wool plugs were inserted into the ends of the tubes. Then the tubes were joined together with a short piece of rubber tubing. In the

Table 1. Numbers of *I. confusus* trapped in response to extract of male frass, male-infested bolts, and synthetic pheromones, Madera County, California, September 1967. Number trapped at each time period represents the total of two replications. A, 6 hours after start; B, 12 hours after start.

| 13 September | | 14 September | |
|---|---|---|---|
| A | B | A | B |
| *Compounds I, II, and III* | | | |
| 57 | 58 | 40 | 41 |
| *Extract of male frass (2 g)* | | | |
| 13 | 28 | 15 | 21 |
| *Male-infested bolts* | | | |
| 72 | 108 | 59 | 83 |
| *Blanks* | | | |
| 0 | 0 | 0 | 0 |

same manner, extract of 2 g of male frass was applied to the substrate in a 15-cm tube.

Air was passed through the tube from section A to section B at the rate of 50 $cm^3$/min at 40°C (an extreme field temperature). The effluent was collected in v-shaped, glass tubes cooled in a bath of dry ice and acetone. The glass tubes were changed at intervals and rinsed with pentane; the pentane solutions were analyzed by gas chromatography. The analysis was performed by an Aerograph 204 equipped with a hydrogen-flame detector and a Carbowax 20M column (10 percent on Chromosorb W, aluminum tubing with an outside diameter of 1.52 by 3.2 mm) at a column temperature of 120°C and a flow rate of 20 $cm^3$ of helium per minute. Retention times were 2½, 3¾, and 4¼ minutes for compounds I, II, and III, respectively. Linalool was used in laboratory tests as a reference compound. Because compound I traveled through this substrate faster than compounds II and III, it was distributed over twice the length (sections A and B). After 3 hours, the percentage of recovery of compounds I, II, III, and linalool was 28, 30, 20, and 19, respectively; after 6 hours, it was 61, 68, 50, and 52. Apiezon L, silicone oil (DC550), and silicone rubber (SE30) on Chromosorb A and DC550 on washed sea sand gave low recoveries (approximately 5 percent) of compounds II and III. Good recoveries were obtained with uncoated sand, Carbowax 20M on sand, and uncoated cellulose powder, but the retention time was much shorter. Nitrogen used as a carrier gas provided no improvement. The good results with Carbowax on Chromosorb A may be due to inactivation of the support.

Table 2. Numbers of *E. lecontei* trapped in response to synthetic pheromones presented individually and in combination. Number trapped each day represents the total of two replications. A, 20 September; B, 26 September; C, 21 September; D, 25 September.

| Treatment | Duplicate runs | | | |
|---|---|---|---|---|
| | A | B | C | D |
| I, II, and III | 10 | 2 | 4 | 2 |
| I | 3 | 3 | | |
| II | 0 | 0 | | |
| III | 3 | 5 | | |
| I and II | | | 0 | 1 |
| I and III | | | 2 | 1 |
| II and III | | | 0 | 1 |
| Infested bolts | 2 | 6 | 0 | 5 |
| Blanks | 0 | 0 | 0 | 0 |

In the first successful field experiment, *I. confusus* was attracted to the ternary synthetic mixture and to the male-infested bolt on 2 successive days (Table 1). The individual components and all of their combinations were then exposed under the same conditions. The beetles were again highly responsive to the ternary mixture on 4 days—20, 21, 25, and 26 September. The total number of beetles trapped in the two replications each day by the synthetic attractants was 80, 102, 16, and 52. During the same test period, the infested bolts attracted 70, 151, 27, and 80 beetles. The sex ratio (males to females) for the total 4-day catch was 0.43 and 0.42 for the infested bolts and the synthetic attractants, respectively. This mixture appeared to be as effective as the male-infested bolts. A mixture of compounds I and III attracted only three beetles. No other compound or combination was active. These results support those from an earlier study in which isolated compounds I and III and synthetic compound II were exposed in the field (*4*).

In earlier trials, solutions of compounds I, II, and III in hexane or heptane evaporated from cotton or paper wicks elicited a very weak response. We do not understand how the solvent interfered with the attractant response; nor do we understand why extracts of male frass always remained attractive for a longer period than the synthetic compounds did (Table 1).

The response of both male and female *Enoclerus lecontei* (Wolcott) (Coleoptera: Cleridae) to the synthetic attractants was a unique aspect of this study. Only compound II failed to elicit a response from this predator (Table 2). Although few predators were caught, the ternary mixture appeared to be as attractive as the male-infested bolt. The arrival of adult predators on the host tree has been observed to coincide with the bark beetle mass attack (*5*). Hence, these predators may use the chemical messenger produced by the bark beetle to find high-prey densities. The ternary mixture also attracted four (three males and one female) *Temnochila virescens chlorodia* (Mannerheim) (Coleoptera: Ostomidae).

These studies demonstrate that the chemoklinotaxis exhibited by pedestrian beetles to the sex pheromone in the laboratory olfactometer reflects the flight response of the insects to the same olfactory stimuli. A direct correlation between the walking and flight response is crucial to isolation and identification of sex pheromones, in which a laboratory bioassay is used. The field response to these attractants also indicates the dominance of olfactory stimuli during the aggregation of this bark beetle. The elution of 1.5 mg of compound I, 1 mg of II, and 0.5 mg of III [compounds I and III are mixtures of both (+) and (−) isomers] throughout the 6-hour test period makes available to the flying insect extraordinarily low concentrations of attractant. There are now exciting possibilities for investigating flight biology and manipulation of populations.

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### References and Notes

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