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RESPONSE OF THE EUROPEAN ELM BARK BEETLE, *Scolytus multistriatus* (Coleoptera: Scolytidae), TO ISOMERS AND COMPONENTS OF ITS PHEROMONE

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Abstract—Laboratory bioassays (two methods) and field tests demonstrated synergistic action of the three components [(−)-4-methyl-3-heptanol (I); (−)-2,4-dimethyl-5-ethyl-6,8-dioxabicylo[3.2.1]octane (α-multistriatin) (II); and (−)-α-cubebene (III)] of the pheromone bouquet of *Scolytus multistriatus*. Individually and in pairs the components were slightly attractive; I+II was clearly the most active doublet. Indirect evidence indicates that only one of the four enantiomers of I is active. Of the α, β, γ and δ isomers of II, only the α is active. With the addition of compound I, slightly attractive extract from mated females became nearly as active as extract from virgin females.

Key Words—*Scolytus multistriatus*, bioassay, aggregating pheromone, isomers, enantiomers, 4-methyl-3-heptanol, α-multistriatin, α-cubebene.

INTRODUCTION

The aggregating pheromone of the European elm bark beetle, *Scolytus multistriatus* (Marsham), has been identified as a combination of 3 compounds (−)-4-methyl-3-heptanol (I), (−)-α-multistriatin (II), and (−)-α-cubebene (III) (Pearce et al., 1975). Laboratory bioassays of the three compounds, singly and in all combinations, showed that the compounds act synergistically. Field tests demonstrated that a mixture of synthetic I and II, plus III obtained

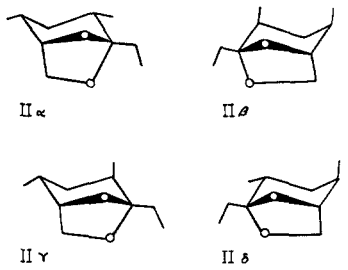

FIG. 1. Isomers of multistriatin: α—natural active; β—natural inactive; γ and δ not known in nature.

from cubeb oil, was as attractive to both sexes of *S. multiastiatus* as virgin females boring in elm logs (Pearce et al., 1975).

Synthetic 4-methyl-3-heptanol (I) consists of a pair of diastereomers, each with two enantiomers; only the (−) enantiomer is produced by virgin female beetles. Multistriatin (II) occurs as four diastereomers (α, β, γ, and δ, Figure 1), each consisting of two enantiomers. Pearce et al. (1975) found α- and β-multistriatin in extracts from aeration of beetle-infested logs, but only the α isomer was clearly attractive in the laboratory.

We present the results of further laboratory and field tests of the relative attractiveness of I, II, and III, their isomers, and various combinations of these compounds.

METHODS AND MATERIALS

Chemicals

Natural (−)-4-methyl-3-heptanol (In), (−)-α-multistriatin (IIn), and (−)-α-cubebene (IIIn) were isolated from the air surrounding virgin female *S. multistriatus* boring in elm logs. The logs were aerated, volatile compounds were collected with Porapak Q®, and crude extracts were obtained by solvent extraction of the Porapak (Byrne et al., 1975; Peacock et al., 1975). The pheromone components were isolated as pure compounds from the Porapak extracts by preparative GLC (Pearce et al., 1975). Porapak extracts of mixed sexes boring in elm logs were prepared in the same manner as the virgin female extracts.

Synthetic 4-methyl-3-heptanol (Is) was obtained from Aldrich Chem. Co., purified by preparative GLC (Carbowax 20M, 5% on AW DMCS Chromosorb G, 6 mm × 6 m silylated glass), and tested as a mixture of diastereomers (approximately 50:50).

Synthetic multistriatin isomers (IIα-δ) were prepared by two routes, and the individual isomers were purified by preparative GLC (Gore et al., 1975; Pearce et al., 1976). The relative stereochemistry of the multistriatin isomers with respect to the C-2 and C-4 methyl groups has been assigned: IIα is 2 *endo*, 4 *endo*; IIβ is 2 *exo*, 4 *exo*; IIγ is 2 *endo*, 4 *exo*; and IIδ is 2 *exo*, 4 *endo* (Gore et al., 1975, Gore and Armitage, 1976).

(−)-α-cubebene (IIIc) was obtained in sufficient quantities for field testing by distillation from cubeb oil (Pearce et al., 1975) and subsequent preparative GLC (SE-30 5% on AW DMCS Chromosorb).

Lab Bioassays

Two laboratory bioassays were used in this study. Bioassay A, conducted by JWP at Delaware, Ohio, employed an olfactometer in which beetles were induced by a weak light source to walk down a walkway covered with filter

TABLE 1. INDICES OF ATTRACTION[a] OF *Scolytus multistriatus* TO COMPONENTS OF ITS PHEROMONE

	Bioassay A[c,d]		Bioassay B		Field[e]	
Material tested[b]	No. of trials	Index	No. of trials	Index	No. of trials	Index
I	3	3	4	13	2	6
II	3	3	4	7	2	4
III	3	6	4	20	2	5
I+II	3	49	4	41	13	72
I+III	3	38	3	33	13	14
II+III	3	6	3	30	13	15
I+II+III	6	100	6	100	15	100
Extract	6	99	6	118		

[a] Index of attraction = No. responding to test/No. responding to standard tripartite mixture. Standard is index 100.

[b] I (4-methyl-3-heptanol) and II (α-multistriatin) were synthetic; III (α-cubebene) was distilled from cubeb oil.

[c] Dosage = the amount of the components produced by 50 female beetles in one hour (beetle-hour equivalents or BH) for lab bioassay A, 10 BH for bioassay B and 1500 BH for field bioassay. A 50-BH aliquot contained absolute amounts of the components as follows: I = 25 ng, II = 1.9 ng, III = 50 ng.

[d] Lab trials consisted of 25 beetles each.

[e] Field tests comparing compounds singly and I+II+III caught an aggregate of 704 beetles; test with doublets and I+II+III caught 4751 beetles. Quantities used were 14 μg I, 7 μg II, and 25 μg III per 4-day test.

paper. A petri dish recessed beneath the paper near the end of the walkway contained materials being tested. Perforations in the paper over the area of the petri dish allowed passage of the odorants. Beetles that made at least two 180° turns or four 90° changes in direction at the perforated area were considered to have responded to the test material (Peacock et al., 1973).

Bioassay B, conducted at Syracuse by GNL, employed the forced-air olfactometer described by Moeck (1970), except that the arena on which the beetles were placed was open rather than glass-covered. An air stream of 60 μl/min through a 3-mm ID glass tube carried odorant being tested. Beetles released at one side of the arena encountered the odorant when they walked toward the source of a light beam intersecting the air stream 10 cm from its outlet. Beetles that interrupted their path toward the light source and walked at least 2 cm up the air stream or that made at least one 360° turn within the air stream were considered to have responded to the odorant being tested. Beetles that did not respond the first time they passed through the air stream were retested. The number of positively responding beetles was the sum of those responding in both trials.

Each replicate in both bioassays used 25 male beetles that had been conditioned under a fluorescent lamp at 23°C for 18–24 hr. A 10-cm petri dish with a dry filter paper floor was used to hold 50–100 beetles during conditioning. Beetles used for bioassay A were from a laboratory colony; those used for bioassay B emerged from naturally infested elm wood. Dosages of the materials tested are given in Table 1.

Field Bioassays

For field tests, four blower olfactometers, described by Vité et al. (1963), were positioned 10 m apart in a row in a residential area in Syracuse, New York. Materials to be tested were dispensed from polyethylene vial caps (Kimble, 60975L) at the bottom of the blower. Beetles responding to the odorants were captured on a vertical plywood sheet, 60 cm^2, covered with polyethylene, and coated with Stikem Special® (Michael & Pelton Co., Emeryville, California 94608). Tests were conducted during the afternoons of warm days in July and August 1974. Positions of the odorants were rotated systematically so that each material would be at each olfactometer during one day of a 4-day replication. Between tests, vial caps containing odorants were held ca. −30°C.

RESULTS AND DISCUSSION

Attractiveness of I, II, and III Individually and in Combinations

Individual components of the pheromone were only slightly attractive

in the laboratory and in the field (Table 1). Any combination of two components was more attractive than single chemicals. Of the doublets, I+II was the most attractive by a slight margin in lab bioassays and clearly superior in the field. In 4 of 13 field tests, the olfactometer containing I+II caught more beetles than that containing I+II+III.

Although positions of the treatments in this comparison were rotated systematically, each doublet was necessarily adjacent to at least one treatment containing the missing component of the tripartite mixture. It was thought that the promity of III might have inflated the catch on the olfactometer containing I+II. The combinations I+II vs. I+II+III were therefore further compared by positioning the two treatments at opposite ends of the line of four blowers and leaving the intermediate positions blank. The obtained indices of 100, 8, 9, and 69 for the triplet, the two blanks, and the doublet, respectively, verify that the I+II doublet is almost as attractive as the triplet, at the concentrations tested. Subsequently, we have learned that increasing the concentration of III relative to I and II results in a corresponding increase in attractiveness (Cuthbert and Peacock, 1977).

Isomers of 4-Methyl-3-heptanol

Initial field tests of the synthetic heptanol (Is) in combination with IIn and IIIn indicated that it was less attractive than an equivalent amount of natural I (In); a fourfold increase in amount of Is resulted in attractiveness equal to that of the mixture containing In (Table 2). These data suggest that, of the 4 enantiomers in synthetic heptanol, only the naturally occurring (−) enantiomer is active.

TABLE 2. ATTRACTION OF SYNTHETIC AND NATURAL 4-METHYL-3-HEPTANOL (I) IN BLOWER OLFACTOMETERS IN THE FIELD[a]

Material tested[b]	No. of trials	Index	Material tested	No. of trials	Index
FI+IIn+IIIn	8	51	Is+IIn+IIIn	4	66
Is+IIn+FIII	8	24	In+IIn+IIIn	4	148
Is+IIn+IIIn	8	34	(4×) Is+IIn+IIIn	4	103
FI+FII+FIII	8	100	FI+IIn+IIIn	4	100

[a] Syracuse, New York, August 1973.

[b] FI, FII, and FIII indicate fractions of Porapak extract (of virgin females boring in elm logs) containing natural 4-methyl-3-heptanol, α-multistriatin and α-cubebene, respectively; Is and In indicate synthetic and natural 4-methyl-3-heptanol, respectively.

TABLE 3. INDICES OF ATTRACTION OF *Scolytus multistriatus* TO THE ISOMERS OF MULTISTRIATIN (II) IN COMBINATION WITH I AND III

Material tested	Lab bioassay A[a]		Lab bioassay B		Field bioassay[b]	
	No. of trials	Index	No. of trials	Index	No. of trials	Index
Is+IIIc	1	24	3	17		
Is+IIIc+IIβ	1	24	3	24	5	4
Is+IIIc+IIγ	1	16	3	12	4	2
Is+IIIc+IIδ	1	24	3	7	4	6
Is+IIIc+IIα	3	114	3	107	5	100
Is+IIIc+IIα (n)[c]	3	100	3	100		
Is+IIIc+IIα	3	105	3	122		
IIβ						
IIγ						
IIδ						

[a] Dosages same as those for the respective assays in Table 1.
[b] Field tests caught an aggregate of 5371 beetles.
[c] IIα (n) is natural; all other isomers of II are synthetic. Is (4-methy -3-heptanol) is synthetic and IIIc (α-cubebene) was distilled from cubeb oil.

Multistriatin

Both α- and β-multistriatin (II) were isolated from Porapak extract, but only the former was attractive in our initial laboratory bioassays (Pearce et al., 1975). Subsequent laboratory and field bioassays of synthetic pure α, β, γ, and δ isomers of II showed that only the α form increased the attractiveness of a mixture of Is+IIIc (Table 3). The β, γ, and δ isomers did not affect the activity of the mixture of Is+IIα+IIIc.

The individual isomers tested in blower olfactometers without I and III attracted relatively few beetles (838 in three heavy-flight days), but the α isomer was clearly superior (indices 100, 8, 26, and 15 for the α, β, γ, and δ forms, respectively).

The individual enantiomers of α-multistriatin were not available for comparison. However, the apparent equality of the attractiveness of the synthetic mixture of enantiomers with an equal amount of natural (−) enantiomer suggests that the (+) enantiomer might also be attractive (Table 3).

Role of 4-Methyl-3-heptanol in Termination of Attraction

After tunneling female *S. multistriatus* are mated by males, the rate

TABLE 4. IMPORTANCE OF 4-METHYL-3-HEPTANOL (I) IN THE PHEROMONE BOUQUET OF *Scolytus multistriatus*

Material tested[a]	Bioassay A		Bioassay B	
	No. of trials	Index	No. of trials	Index
Is	3	0	3	0
Extract mated females	3	59	3	31
Extract mated females + Is	3	82	3	86
Extract virgin females	6	100	3	100

[a] Dosage = 50 beetle-hour equivalents (BH) for bioassay A and 10 BH for bioassay B.

of beetles arriving at the attractive source declines precipitously (Peacock et al., 1971). Elliott et al. (1975) showed that the drop in the attractiveness of females after mating resulted from a decline in the release of the attractant rather than the production of an antiattractant by the mated females or the male. Gore et al. (1977) found that females cease the production of I shortly after mating, but the release of II by the female and III by the host material continues.

In the laboratory bioassays, we found that Porapak extract of boring virgin females was always more attractive than the extract from an equal number of mated females (Table 4). However, extract of mated females to which we added the heptanol was nearly as attractive as material from virgin females. Cuthbert and Peacock (1977) have recently demonstrated reduction in attractiveness of a mixture as the release rate of I declines relative to that of II.

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