

they behave as if stimulated sexually by the flower labellum. Analyses of volatiles from *O. litigiosa* flowers<sup>20</sup> indicate the presence of citronellyl and farnesyl esters. These are biochemically closely related to geranyl esters and thus might be responsible for the attraction of male elaterid beetles to various *Ophrys* flowers.

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## Accumulation of phenylpropanoids in the rectal glands of males of the Oriental fruit fly, *Dacus dorsalis*

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**Summary.** Two phenylpropanoid compounds, 2-allyl-4,5-dimethoxyphenol(II) and coniferyl alcohol(III), were characterized from body tissue of wild males of the Oriental fruit fly, *Dacus dorsalis*. These compounds accumulated in the rectal glands only when laboratory-reared males were fed with methyl eugenol. Compound II was released into the air during dusk, which coincides with the fly courtship period. Pheromonal and allomonal effects of the phenylpropanoids were examined.

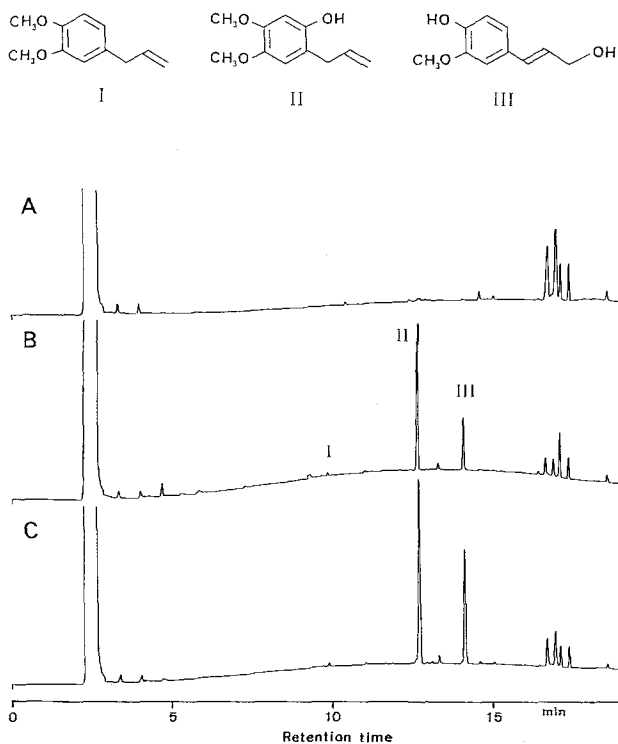
**Key words.** Oriental fruit fly; *Dacus dorsalis*; methyl eugenol; 2-allyl-4,5-dimethoxyphenol; coniferyl alcohol; phenylpropanoid; pheromone; allomone; sequestration.

Methyl eugenol(I) is a highly potent attractant for the Oriental fruit fly, *Dacus dorsalis*, and several other species in the family Tephritidae (Diptera). It has been successfully used as a trapping agent in eradication programs for these pests in many countries<sup>1</sup>; and in capturing native males for population estimation<sup>2</sup>. However, the biological significance of such specific attraction of the lure for the male tephritid flies has not yet been clarified. We have found two phenylpropanoid compounds (II and III), which are closely related to methyl eugenol, in the body tissue of wild *D. dorsalis* males. We describe here the identification and possible ecological functions of these compounds.

Adult *D. dorsalis* males were collected at various field sites in West Malaysia, and immediately used for extraction with ethanol. As shown in figure B, the capillary gas-liquid chromatogram exhibited two major volatile substances, II and

III, in unusually large quantities. In contrast, ethanolic extracts of the sexually mature males of a laboratory-reared culture (raised from star-fruits, *Averrhoa carambola*, and fed with water and a honey-yeast mixture during the adult stage) entirely lacked these compounds (fig. A).

Compounds II and III were isolated from wild males by means of silica gel column chromatography (Wako gel C-200) followed by high performance liquid chromatography (Nucleosil 100-5, 300 mm × 8 mm i.d., eluted with a mixture of 20–42% ethyl acetate in hexane, yield: 12 µg and 10 µg per male, respectively). Compound II was identified as 2-allyl-4,5-dimethoxyphenol from its mass (MS), proton and carbon-13 nuclear magnetic resonance (PMR and CMR) spectra: MS (70 eV) *m/z* (%) 194 (M<sup>+</sup>, 100), 179 (68), 151 (13), 123 (22), 69 (20). PMR (CDCl<sub>3</sub>): δ6.62 (1H, singlet), 6.46 (1H, singlet), 5.98 (1H, multiplet, J = 17.5, 8.0



Gas chromatograms of volatiles in the extracts of *Dacus dorsalis* males (25 m × 0.25 mm fused silica capillary coated with DB-1 0.25 μm thick, programmed from 100° (2 min holding) to 240°C at a rate of 10°C/min). A Laboratory-reared male fly (13 days old). B Wild male fly captured at Penang, Malaysia (August 27, 1987). C Laboratory-reared male fly fed with methyl eugenol (I) on the 8th day and extracted on the 13th day after adult eclosion. Retention times of I, II, and III are 9.7, 12.7 and 14.1 min, respectively.

and 6.3 Hz), 5.15 (2H, multiplet), 4.68 (1H, singlet), 3.83 (3H, singlet), 3.82 (3H, singlet), 3.34 (2H, double triplet,  $J = 6.2$  and 1.4 Hz). CMR (CDCl<sub>3</sub>): δ 148.7, 148.2, 143.4, 136.6, 116.4, 115.9, 114.4, 101.6, 56.8, 56.1, 34.9. The positions of the hydroxy and two methoxy groups in II were unequivocally verified by selective measurements of nuclear Overhauser effects between the neighboring protons<sup>3</sup>. Compound III was identified as 4-(3-hydroxy-1-propenyl)-2-methoxyphenol (coniferyl alcohol) by comparing its spectral data with that of an authentic specimen (Aldrich Chemical Co.). MS (70eV):  $m/z$  (%) 180 (M<sup>+</sup>, 79), 137 (100). CMR (CDCl<sub>3</sub>): δ 146.8, 145.8, 131.5, 129.4, 126.3, 120.4, 114.6, 108.6, 63.9, 56.0.

When the laboratory-reared males (8 days after adult eclosion) were individually fed with methyl eugenol for several minutes, the compound was quickly consumed, and II and III were markedly built up in their body tissue as shown in figure C, exhibiting the same profile as that of the wild males. Thus the wild males must have consumed I from plant sources during foraging, and sequestered the metabolites (II and III) in their body in varying quantities. The quantitative variation may be dependent on the availability of I in their natural habitat, and the physiological age of flies.

Dissection of the male insect body revealed that a large portion of the metabolites II and III accumulated in the rectal glands ( $9.5 \pm 1.3$  μg of compound II per gland was obtained from males 6 days after feeding with methyl eugenol). The gland complex of the rectal sac has been suspected to be the source of an olfactory pheromone<sup>4</sup>. Moreover, compound II was detected from the aeration extract in porapak Q of the methyl eugenol-fed males (selected ion monitoring at  $m/z$

194, 179 and 123 using a capillary GC-MS system). The porapak-Q trap was replaced 2 hourly starting from noon until midnight. Compound II was recovered from the trap only during the time around sunset (17:00–19:00 pm) which coincides with the period when *D. dorsalis* males congregate and emit a smoke-like substance from their anal opening prior to mating<sup>4,5</sup>. The emanations from the rectal glands have been shown to be attractive to virgin females<sup>5</sup>. This evidence suggests a possible role of the phenylpropanoids as a pheromone in courtship behavior, including male-to-male interaction.

In order to examine the behavioral role of these metabolites, an initial experiment was conducted in the field using sticky traps with the chemical samples (table 1). Compound II attracted male flies as strong as I. Compound III (0.5 mg) alone was found to be inactive in preliminary field tests. No synergistic action was observed between II and III. Males attracted to II extended their proboscis and licked the chemical source, but virgin females paid no attention to II (pure) in the laboratory bioassay. Further behavioral study is now in progress using these chemicals together with other ingredients found in the rectal secretion<sup>5,6</sup>.

On the other hand, an allomonal effect of the metabolites has been suggested, since an extract of methyl eugenol-fed males of *D. dorsalis* deterred feeding of the Japanese tree-sparrow, *Passer montanus*. The deterrent effects of compounds I, II and III were examined in the rice grain feeding test (table 2). Compound II was shown to act as a potent deterrent. Compound I was less active than II, and III was inactive at the given dosage. Since males fed with I usually store more than 10 μg of II in their body, the sequestration is very likely to provide an ecological advantage against predation.

Methyl eugenol is known to be present in various plants such as *Cassia fistula* (Leguminosae)<sup>7</sup>, *Zieria smithii* (Rutaceae)<sup>8</sup> and *Ocimum sanctum* (Labiatae)<sup>9</sup>. Some plants may produce a powerful chemical attractant to invite flies for the purpose of pollination; such interactions may have arisen in a coevolutionary pathway. It has been suggested that the plant chemical may directly provide a rendezvous site for certain tephritid species, to bring the sexes together in the environment of suitable host plants<sup>10</sup>. It has also been suggested that male fruit flies are attracted to these substances because of fortuitous chemical resemblance to male aggregation or female sex pheromones<sup>8</sup>. However, our observations rather

Table 1. Mean number of fruit flies caught by sticky traps with methyl eugenol (I), 2-allyl-4,5-dimethoxyphenol (II) and coniferyl alcohol (III)\*.

Sample	Mean ± SD
I (0.5 mg)	36.3 ± 11.5
II (0.5 mg)	22.7 ± 4.6
II + III (0.5 mg:0.3 mg)	24.8 ± 5.4
Blank	0

\* The test was conducted at Minden, Penang, Malaysia using a Latin square design<sup>2</sup> (3 days in May, 1987).

Table 2. Deterrent effect of the phenylpropanoids against sparrows in the rice grain feeding test

Compound (dose/grain)	Sample*	Control*
I (10 μg)	22.3 ± 5.0	0
I (3 μg)	6.3 ± 6.0	0
II (3 μg)	22.0 ± 4.8	0
III (10 μg)	0	0

\* Average number of grains remaining from an initial number of 30 grains after exposure to sparrow feeding in the field (Kyoto, Japan; April, 1987).

imply that secondarily formed metabolites II and/or III might play a behavioral role as a part of a male pheromone in their courtship sequence, and also potentially function as an allomone against predators. Rigorous work will be needed to clarify the ecological significance of the phenylpropanoid – fruit fly association.

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## The clerid beetle, *Thanasimus formicarius*, is attracted to the pheromone of the ambrosia beetle, *Trypodendron lineatum*

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**Summary.** Sticky traps containing (+)-lineatin, the pheromone of the ambrosia beetle, *Trypodendron lineatum*, attracted the predator *Thanasimus formicarius* to about the same extent as traps baited with ipsilure, the pheromone blend used for mass-trapping *Ips typographus*. The results indicate that *T. lineatum* is an important prey for *T. formicarius* early in the season before the main prey becomes active. Addition of *exo*-brevicommin to ipsilure and ethanol and/or  $\alpha$ -pinene to (+)-lineatin did not significantly influence the catches of the predator.

**Key words.** Predator/prey relationship; bark beetles; *Thanasimus formicarius*; *Trypodendron lineatum*; kairomone; (+)-lineatin; ipsilure.

In the extensive review by Gauss<sup>1</sup>, it is reported that the clerid beetle *Thanasimus formicarius* (Coleoptera: Cleridae) is a predator on at least twenty species of bark beetles which belong to the genera *Ips*, *Pityogenes*, *Tomicus*, *Polygraphus*, *Hylesinus*, *Hylastes* and *Scolytus*. However, no species of *Trypodendron* was considered as a prey for *T. formicarius*. In a later review<sup>2</sup> it is suggested that *T. lineatum* might be a prey for this clerid predator. It was therefore interesting to find by electrophysiological studies that *T. formicarius* has numerous olfactory receptor cells specifically responding to (+)-lineatin, the pheromone of *T. lineatum*<sup>3</sup>. It was proposed that *T. lineatum* might be a prey for *T. formicarius*, especially early in the spring before more important species of prey (e.g. *Ips typographus*) become active.

The present field study was made in order to find out whether or not (+)-lineatin is attractive to *T. formicarius*. Since ipsilure\*, the pheromone blend of *I. typographus*, is a well-known attractant to this predator<sup>4</sup>, the present field experiments were carried out in a manner that made it possible to compare the attraction of (+)-lineatin with that of ipsilure. Furthermore, the influence of ethanol and  $\alpha$ -pinene on the attraction of *T. formicarius* to (+)-lineatin was tested as well as the effect of *exo*-brevicommin on the attraction to ipsilure. These mixtures were studied since ethanol and  $\alpha$ -pinene synergize the attraction of *T. lineatum* to (+)-lineatin<sup>5</sup> and *exo*-brevicommin influence the attraction of *I. typographus* to ipsilure<sup>6,7</sup>.

As test areas six fields of Norwegian spruce were used (in Malvik, Klæbu and Trondheim), four of which had been clearcut the preceding winter. The same type of sticky traps as previously reported<sup>6</sup> were used. These were baited with

the following test compounds; (+)-lineatin (L), (+)-lineatin plus  $\alpha$ -pinene (L +  $\alpha$ -p), (+)-lineatin plus both  $\alpha$ -pinene and ethanol ( $\alpha$  +  $\alpha$ -p + et), ipsilure (I) and ipsilure plus *exo*-brevicommin (I + *exo*-B). In some replicates (carried out in 1985) two concentration levels of ipsilure (I) and ipsilure plus *exo*-brevicommin (I + *exo*-B) were tested. Here I (10 cm) and I (50 cm) mean 10 respectively 50 cm of the 100 cm plastic strip dispenser used for mass-trapping of *I. typographus*. In these replicates the *exo*-brevicommin dispenser (a polyethylene cap) contained respectively 9 mg (B (9 mg)) and 60 mg (B (60 mg)) of *exo*-brevicommin. Each replicate included one control trap (C) without dispenser. The traps were placed in a line, 40 m from the edge of a forest or from lumber of spruce logs. The distance between each trap was 15 m, except for the traps containing the high amount of compounds (I (50 cm) and I (50 cm) + *exo*-B (60 mg)) where the distance between the traps as well as the distance to the edge of the forest was 50 m. In 1984 the mounting of the traps was completed on May 5, in 1985 on May 15 and in 1986 on May 12. The traps were emptied four times about every 10th day and the total number of *T. formicarius* caught in each trap was counted.

The results are summarized in the table, showing the mean number of catches for each type of trap. For all traps the number of beetles was low. This is, however, due to the low population of *T. formicarius* in these areas which was observed in parallel during the years 1983–86, using the standard ipsilure traps for mass-trapping *I. typographus*. Meanwhile, in the replicates of the present study the (+)-lineatin sticky traps caught as many beetles of *T. formicarius* as the ipsilure traps. By further comparison of trap catches it was