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Transport of methyl eugenol-derived sex pheromonal components in the male fruit fly, *Bactrocera dorsalis*

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Abstract

Males of *Bactrocera dorsalis* (Diptera: Tephritidae) are attracted strongly to and feed compulsively on methyl eugenol (1,2-dimethoxy- 4 -(2-propenyl)benzene), a highly potent male attractant. Pharmacophagy of methyl eugenol results in the production of phenylpropanoids 2-allyl-4,5-dimethoxyphenol and (*E*)-coniferyl alcohol that are sequestered and stored in the rectal gland prior to release as sex pheromonal components during mating at dusk. While these pheromonal components have also been detected in the hemolymph and crop of methyl eugenol-fed males, there is currently little information on the transport of these compounds from the crop to rectal gland in male *B. dorsalis*. Therefore, using physiological techniques such as parabiosis, rectal gland transplantation and hemolymph transfusion coupled with gas chromatography–mass spectrometry (GC-MS) analyses, we were able to ascertain and confirm the role of the hemolymph in the transport of these sex pheromonal components from the crop to the rectal gland. Further, the temporal profile of these methyl eugenol-derived bioactive compounds in the hemolymph also shows an increase with time post-methyl eugenol-feeding, i.e., 2-allyl-4,5-dimethoxyphenol attaining maximum amounts 15 min after ME consumption and decreasing thereafter, while for (*E*)-coniferyl alcohol—the increase and decrease are more gradual. These results further demonstrate the ability of insect hemolymph to transport many diverse forms of bioactive molecules including attractant-derived sex pheromonal components.

Keywords: 2-Allyl-4,5-dimethoxyphenol; *Bactrocera dorsalis*; Crop; (*E*)-Coniferyl alcohol; Hemolymph; Methyl eugenol; Rectal gland; Sex pheromone transport

1. Introduction

The strong attraction to and compulsive feeding of *Bactrocera dorsalis* (Diptera: Tephritidae) on methyl eugenol (ME) is a unique characteristic of tephritid fruit flies. While the attraction was first observed in 1915 (Howlett, 1915), the actual reason for such a phenomenon has only recently been understood. ME plays a central role in the chemical ecology of the ME-attracted species, especially in understanding the complex interrelationships between fruit flies and plants plus predators (Tan, 1993, 2000; Tan and Nishida, 1998, 2000).

Males of *B. dorsalis* and *B. papayae* (not a distinct species from the former; Naeole and Haymer, 2003; Tan, 2003) were found to enhance their mating competitiveness after consumption of ME (Shelly and Dewire, 1994; Tan and Nishida, 1996, 1998; Hee and Tan, 1998; Shelly and Nishida, 2004). ME is a sex pheromone precursor as chemical analyses further showed that it is converted to 2-allyl-4,5-dimethoxyphenol and (*E*)-coniferyl alcohol in *B. dorsalis* male (Fig. 1) (Nishida et al., 1988; Tan and Nishida, 1996, 1998; Shelly and Nishida, 2004). These metabolites are then sequestered into the male rectal gland before release during courtship at dusk. Behavioral studies have also demonstrated that both of these phenylpropanoids function as male sex and aggregation pheromones in *B. dorsalis* (Tan and Nishida, 1998; Hee and Tan, 1998).

Therefore, in light of further understanding where ME is converted to pheromonal components, and how these ME-derived components are then transported to the male fruit fly

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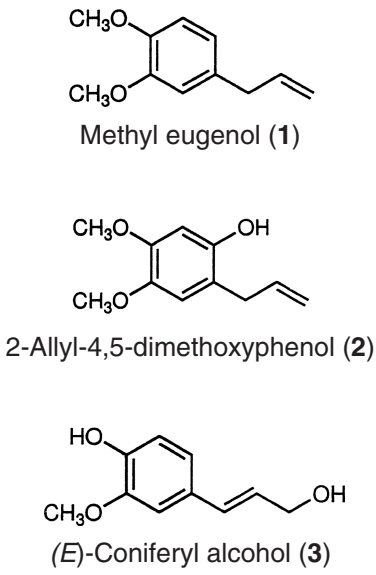

Fig. 1. Phenylpropanoids involved in the hemolymph transport of methyl eugenol-derived sex pheromonal components in male *B. dorsalis* from the crop to the rectal gland. **1**: Methyl eugenol, **2**: 2-ally-4,5-dimethoxyphenol, **3**: (*E*)-coniferyl alcohol.

rectal gland, subsequent studies were undertaken to ascertain the role of the male hemolymph and digestive system in this phenomenon.

We have recently demonstrated that 2-allyl-4,5-dimethoxyphenol and (*E*)-coniferyl alcohol, henceforth, collectively referred to as sex pheromonal components, were derived from ingested ME and detected in the hemolymph and crop, but not in midgut, of ME-fed males (Hee and Tan, 2004). Further, the hemolymph fractions containing these pheromonal components have been isolated and identified (Hee and Tan, 2005). However, little is known on the ME-derived pheromone transport in male *B. dorsalis.* Thus, our recent results led us to hypothesize that the hemolymph is involved in the attractant-derived pheromone transport from the crop to the rectal gland. Here we report the transport of sex pheromonal components via hemolymph in male *B. dorsalis* using a series of physiological experiments.

2. Materials and methods

2.1. Insects

Laboratory-reared *B. dorsalis* Hendel (Diptera: Tephritidae) were originally collected from infested starfruits, *Averrhoa carambola* Linnaeus in Penang Island, Malaysia, 1995, and cultured using an artificial diet as described by Hee and Tan (1998). Males and females were sexed and kept separately within 3 days of emergence. The flies were maintained under ambient conditions in an insectary with a photoperiod of L12: D12 and 83–90% r.h. at 25–29 °C. Sexually mature virgin males (14–20 days after adult eclosion) that responded maximally to ME (Tan et al., 1987) were used for experimentation. All experiments on the males were performed in the morning during the peak period, 0830–1100 h, of response to ME (Tan, 1985).

2.2. Chemicals

Methyl eugenol (ME) (1-allyl-3,4-dimethoxybenzene; >98 % purity) was obtained from Merck-Schuchardt, Germany. Authentic compounds of 2-allyl-4,5-dimethoxyphenol (DMP) (>96% purity) and (*E*)-coniferyl alcohol (CF) (96% *trans*) were synthesized and provided generously by R. Nishida (Kyoto University, Japan). ME to be used for feeding male flies was prepared by dilution to 210.4 μg/μL as an aqueous emulsion containing 1% Tween® 80 (polyoxyethylene sorbitan monooleate) (Merck-Schuchardt, Germany).

2.3. ME-fed male flies

Each male was held individually in its dorsal position onto plastic netting by plasticine moulds and fed directly with 0.5 μL (containing 105 μg) of ME from a capillary micropipette, to avoid bodily contamination. Male *B. dorsalis* flies used in all the physiological experiments were kept in high relative humidity (>90%) environment (over a saturated NaCl solution) to prevent dehydration.

2.4. Gas chromatography

DMP and CF were separately identified using methods based on those used by Nishida et al. (1988). Gas chromatography–mass spectrometry (GC-MS) analyses were performed on a HP 5989B mass spectrometer (Hewlett Packard, Palo Alto, CA) (electron impact, at 70 eV) connected to a non-polar GC column (30 m × 0.25 mm fused silica column coated with cross-linked 5% phenyl-methylpolysiloxane HP-5MS, 0.33 μm film thickness) (J&W Scientific, Folsom, CA, USA) programmed from 80 °C (1-min holding) to 240 °C at a rate of 10 °C/min. Chemical identification was performed by comparison with the retention time and mass spectra fragmentation pattern of authentic standards.

Sex pheromonal components were quantified on a Shimadzu GC-14A gas chromatograph (Shimadzu, Japan) using a HP Ultra-1 capillary column (25 m × 0.2 mm fused silica column coated with cross-linked methyl siloxane, 0.33 μm film thickness) (J&W Scientific) and under the same program conditions as above by comparing the flame ionization intensities with those of the authentic standards of known concentrations by using a C-R6A integrator (Shimadzu, Japan). For all the physiological experiments, 1-μL aliquots of the extract were used for GC-MS and GC analyses after extraction in each experiment.

2.5. Physiological experiments

2.5.1. Parabiosis between ME-fed and ME-deprived males.

The objective of this experiment was to demonstrate that when both ME-fed and ME-deprived males are joined together (i.e., having a common circulatory system), the sex pheromonal components from the ME-fed male would be transported by the hemolymph to the rectal gland of the ME-deprived male.