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PHEROMONE SHARING: BLENDS BASED ON THE SAME COMPOUNDS FOR *Euschistus heros* AND *Piezodorus guildinii*

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Abstract—Pheromone compounds shared between two species of pentatomids from the soybean stink bug complex are reported. Two male-specific compounds (R_t 27.47 and 29.62 min) were detected in the airborne extract of *Piezodorus guildinii* by gas chromatography and mass spectrometry analysis. The compounds were identified as methyl 2,6,10-trimethyldodecanoate and methyl 2,6,10-trimethyltridecanoate.

Key Words—Soybean, methyl 2,6,10-trimethyldodecanoate, methyl 2,6,10-trimethyltridecanoate, stinkbugs, pheromones, *Piezodorus guildinii*.

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**In memoriam.

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INTRODUCTION

Soybean, *Glycine max* (L.) Merryl, is severely attacked by complexes of stink bugs around the world. In Brazil, the most important members of the complex are *Nezara viridula* (L.), *Piezodorus guildinii* (westwood), and *Euschistus heros* (F.) (Panizzi and Rossi, 1991). This attack has led to the use of more than four million liters of chemical insecticides to control these pests (Corrêa-Ferreira and Moscardi, 1996). The use of semiochemicals in biological control of these pests would be a more ecologically benign approach to their integrated pest management.

The first attractant pheromone identified for a member of the soybean stink bug complex was from the southern green stink bug, *N. viridula* (Baker et al., 1987; Aldrich et al., 1987). Field tests of synthetic blends have been conducted on a small scale, but very few bugs or tachinid parasites were attracted (Aldrich et al., 1993).

Recently, the chemical communication system of the Neotropical brown stink bug, *E. heros*, was elucidated during trapping studies of its Nearctic relative *E. obscurus* (Borges and Aldrich, 1994; Aldrich et al., 1994). The synthesis of a racemic mixture of methyl 2,6,10-trimethyltridecanoate and stereoisomeric mixture of methyl 2,6,10-trimethyldodecanoate, two components of the male-produced pheromone of *E. heros*, was conducted by Mori and Murata (1994) and Ferreira and Zarbin (1996), respectively. In spite of the identification and synthesis process, those compounds were never tested against *E. heros*. Recently, confirmation that methyl 2,6,10-trimethyltridecanoate is a male-produced pheromone of *E. heros* was presented by Borges et al. (1998a). In recent field tests we evaluated the response of stink bugs to natural (airborne extracts) and synthetic racemic mixtures of methyl 2,6,10-trimethyltridecanoate. The presence of stink bugs and their egg parasitoids was recorded in pheromone-baited traps. During these tests, *P. guildinii* was caught in the baited traps in significantly greater numbers than other insects, i.e., *P. guildinii* comprised 63% of the species present in the baited traps, followed by *E. heros* (10%) and *N. viridula* (10%). Other species, such as *Thyanta perditor*, *Acrosternum aseadum*, and *Edessa meditabunda*, comprised less than 5% of the catch (Borges et al., 1998b).

In this paper we report the identification of methyl 2,6,10-trimethyldodecanoate and methyl 2,6,10-trimethyltridecanoate in the airborne volatiles collected from *P. guildinii* males.

METHODS AND MATERIALS

Insects. P. guildinii adults were obtained from a soybean field (1 ha) at Cenargen (National Research Center for Genetic Resources and Biotechnology),

and their offspring were reared on sunflower seeds, soybeans, and green beans at 26 ± 0.5°C and 65 ± 10% relative humidity with a 14L : 10D photoperiod. To prevent olfactory interactions between the sexes, males were separated from females after the imaginal molt and cuticular hardening, but before sexual maturity.

Collection of Volatiles. Samples were collected from groups of 16 virgin adult male ($N = 80$ or female ($N = 48$) *P. guildinii* of known age by coaxing the bugs into a 500-ml glass column and trapping volatiles from the air drawn by vacuum (100 ml/min) over the bugs and through 30 mg of activated charcoal for 24 hr. Trapped volatiles were eluted from the filter with 150 μl of *n*-hexane and stored at −20°C (Borges and Aldrich, 1994).

GC-MS Analysis. GC-MS analyses were carried out on a Shimadzu QP-5000 GC-MS spectrometer linked to a TIC detector in splitless injector mode. The DB-5 capillary column (30 m × 0.53 mm × 0.25 μm was operated at 50°C for 1 min, increased to 150°C at a rate of 5°C/min, held at this temperature for 5 min, increased to 280°C at a rate of 10°C/min, and finally held at this temperature for 10 min.

RESULTS

Two male-specific compounds (R_t 27.47 and 29.62 min) were detected in the airborne extract of *P. guildinii*, corresponding to methyl 2,6,10-trimethyldodecanoate (**1**) (Figure 1A and B) and methyl 2,6,10-trimethyltridecanoate (**2**) (Figure 1A and C). The same compounds were previously observed in the pheromonal blend of the stink bug *E. heros* (Aldrich et al., 1994).

Identity of the two compounds was checked by coinjection with a synthetic sample prepared in our laboratory (Ferreira and Zarbin, 1996) and with one prepared by Mori and Murata (1994). Gas chromatograms of volatiles collected from female *P. guildinii* (not shown) totally lacked compounds **1** and **2**.

DISCUSSION

Our finding of methyl 2,6,10-trimethyldodecanoate and methyl 2,6,10-trimethyltridecanoate in volatiles collected from *P. guildinii* explains by *P. guildinii* was attracted to field traps (Borges et al., 1998b) containing synthetic methyl 2,6,10-trimethyltridecanoate during pheromones studies of *E. heros*. Both compounds are present in the pheromone of *E. heros*, and both are part of the *P. guildinii* communication system. Males of *P. guildinii* release them in a 1 : 3 ratio of methyl 2,6,10-trimethyldodecanoate–methyl 2,6,10-trimethyltridecanoate. We have no information on the stereochemical importance of the stereogenic centers in the molecules to attraction of *P. guildinii*.

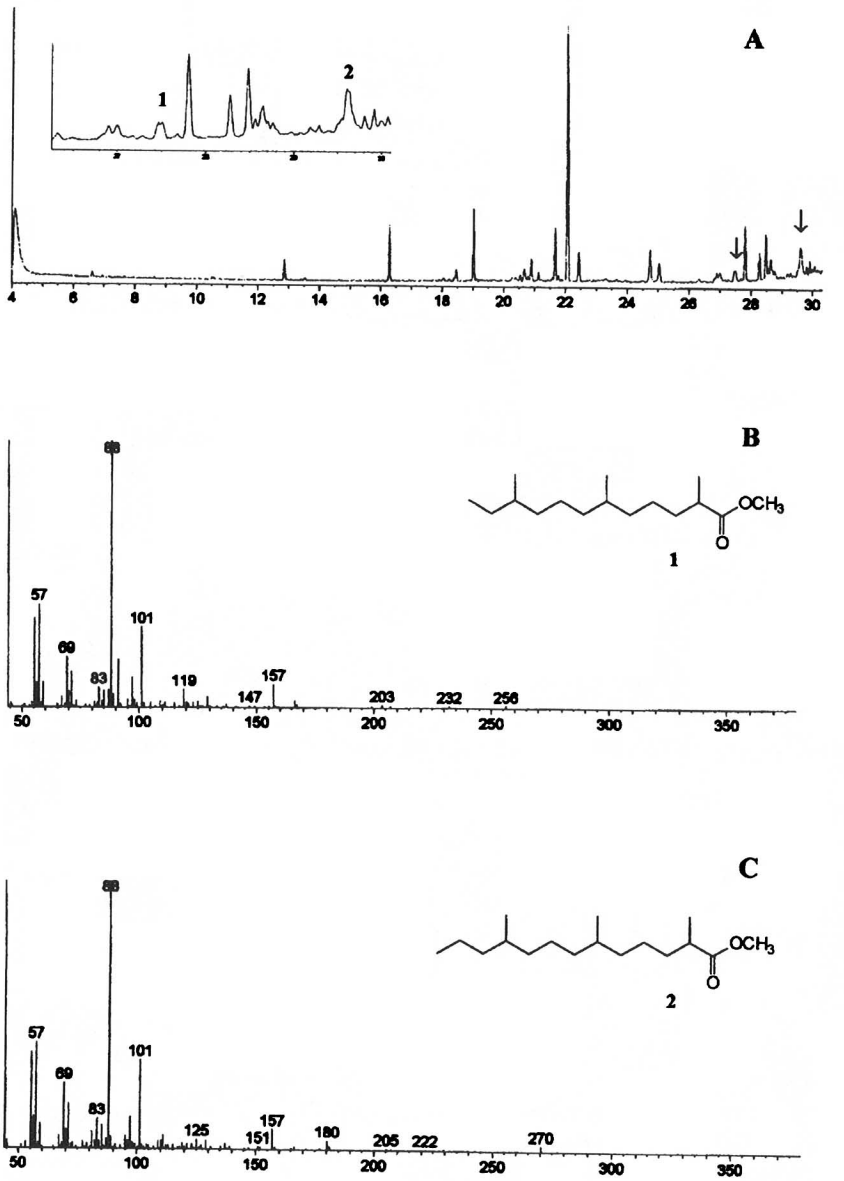

FIG. 1. GC-MS analysis of the airborne pheromone extract of *Piezodorus guildinii*. (A) chromatogram chart (arrows indicate the active compounds in the full chart. **1**: methyl 2,6,10-trimethyldodecanoate, and **2**: methyl 2,6,10-trimethyltridecanoate). (B) Mass spectrum of compound **1**. (C) mass spectrum of compound **2**.

Species-specific blends based on the same compounds among *Euschistus* species were reported by Aldrich et al. (1991). This phenomenon of pheromone sharing was also reported for *N. viridula* and the sister genus *Acrosternum* (Aldrich et al., 1993). Although the different species liberate a similar pheromone blend, unique ratios of these compounds might be involved in species isolation.

Synthetic methyl 2,6,10-trimethyltridecanoate is a very stable molecule (Prof. Kenji Mori, personal communication), and its stability may enable an integrated pest management program (IMP) to adopt another tool to control and monitor *E. heros* and *P. guildinii*. In addition, it may be possible to manipulate a stereoisomeric mixture containing methyl 2,6,10-trimethyl-tridecanoate to make a blend attractive for other species from the soybean stink bug complex.

Additional components from the *P. guildinii* pheromone blend remain to be identified. We believe, however, that these components have little or no influence in the long-distance communication behavior of this species because efficient attraction in the field is possible with the synthetic racemic mixture of methyl 2,6,10-trimethyltridecanoate (Borges et al., 1998b).

Pheromone blends based on the same compounds for two pentatomids species, *N. viridula* and species in the sister genus *Acrosternum*, were reported by Aldrich et al. (1993). In this work, we report the sharing of pheromone compounds between two species of the soybean stink bug complex in Brazil, *P. guildinii* and *E. heros*. Furthermore, we have now identified two male-released compounds from the sex pheromone of the stink bug, *P. guildinii*.

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