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Source: Florida Entomologist, 92(4) : 548-553

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.092.0403

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CHEMICAL ANALYSIS OF FEMALE VOLATILES AND FIELD RESPONSE OF THE COFFEE LEAFMINER MOTH (LEPIDOPTERA: LYONETIIDAE) TO STEREOISOMERS OF ITS MAJOR SEX PHEROMONE COMPONENT

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ABSTRACT

We analyzed the volatiles emitted by Mexican populations of the female coffee leafminer moth *Leucoptera coffeella* that were collected in coffee plantations located in the Soconusco region of Chiapas. Our results confirmed previous results that *L. coffeella* females emitted 5,9-dimethylpentadecane as the major and 5,9-dimethylhexadecane as the minor component. Field response of the coffee leafminer moth to stereoisomers of the major component showed that delta traps baited with (5*S*,9*R*)-dimethylpentadecane caught a significantly greater number of coffee leafminer males when compared to those captured by traps baited with (5*R*,9*R*)-dimethylpentadecane, (5*R*,9*S*)-dimethylpentadecane and the control. The number of coffee leafminer captured by traps baited with (5*S*,9*S*)-dimethylpentadecane was not significantly different from the number captured by traps baited with (5*R*,9*R*)-dimethylpentadecane. The results are discussed in view of the possibility of using stereoisomers as bait for monitoring the leafminer moth.

RESUMEN

Nosotros estudiamos los volátiles emitidos por hembras del minador de la hoja del café, *Leucoptera coffeella*, colectados en plantaciones de café de la región del Soconusco, Chiapas, México. Nuestros resultados confirman los resultados previos que la hembra de *L. coffeella* emite (5,9)-dimetilpentadecano como compuesto mayoritario y (5,9)-dimetilhexadecano como minoritario. Los resultados de la evaluación en campo de los estereoisómeros del compuesto mayoritario mostraron que las trampas delta cebadas con (5*S*,9*R*)-dimetilpentadecano presentaron capturas significativamente mayores comparadas con las capturas de trampas cebadas con (5*R*,9*R*)-dimetilpentadecano, (5*R*,9*S*)-dimetilpentadecano y el control. El número promedio de minadores capturados en trampas cebadas con (5*S*,9*S*)-dimetilpentadecano fue similar al capturado con (5*R*,9*R*)-dimetilpentadecano. Los resultados de este trabajo son discutidos considerando la posibilidad que algunos de estos estereoisómeros puedan ser usados como cebo para el monitoreo del minador de la hoja del café.

Translation provided by the authors.

The coffee leafminer, *Leucoptera coffeella* (Guerin-Meneville), is an important and widely distributed pest among the main coffee producing countries in Latin America (Sanchez-De Leon 1984; Souza & Reis 1992; Barrera 2008). Eggs are laid individually or in small clusters of up to 7 eggs, and total fecundity varies between 30-80 eggs. Upon hatching the larva makes a semi-circular cut at the leaf base and penetrates rapidly into the leaf, where it moves around, mining the palisade parenchyma tissue (Barrera 2008). Several small mines may run together, causing brown spots to appear and in severe attacks major loss of leaf tissue and premature leaf drop occurs, reducing plant vigor and yield (Souza & Reis 1992). In Brazil, this insect is considered a key pest of the coffee plant and causes losses of 50% in production (Souza & Reis 1992). In Mexico, the leafminer is frequently found attacking coffee plants in Chiapas (Segura et al. 2004; De la Mora et al. 2008) and Veracruz (Nestel et al. 1994) but infestation levels are generally low. Nevertheless, the intense use of pesticides has reduced many of the natural enemies of this insect, allowing increased *L. coffeella* populations in some municipalities of Chiapas (Barrera et al. 2003). A sampling made in the Soconusco region of Chiapas revealed that the coffee leafminer is present all year around but principally preceding the rainy season during Mar and Apr (Barrera et al. 2006). Currently, the main method of coffee leafminer control in tropical America is the application of insecticides. However, the use of insecticides increases the likelihood of the coffee beans containing residual pesticides affecting human health and the environment, and consistent use may induce resistance in the coffee leafminer (Fragoso et al. 2002). The use of sexual pheromones of the coffee leafminer may help reduce the use of insecticides, reducing toxic residues in the coffee fruit and preserving its natural enemies in the agro-ecosystem (Michereff et al. 2007). Furthermore, the sexual

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pheromone of the coffee leafminer may be used for monitoring populations of this pest, allowing identification of the main areas of infestation so the control can be specifically directed to these more heavily infested areas. This would increase efficiency and decrease costs and non-target impact (Baca et al. 2008).

Francke et al. (1988) identified 5,9-dimethylpentadecane as major component and 5,9-dimethylhexadecane as minor component in the female produced pheromone and reported that both components are EAD active. However, the absolute configuration of both natural pheromone components still remains to be determined. Lima (2001) found that traps baited with a racemic mixture of 5,9-dimethylpentadecane captured more males than traps baited with pure stereoisomers. The catches of traps baited with the single pure stereoisomers were not significantly different among compounds. Zarbin et al. (2004) reported that (5*S*,9*S*)-dimethylpentadecane elicited higher antennal responses when compared with 3 other possible isomers. Insect pheromones, especially in moths, may vary between populations of the same species in different geographic locations (e.g., Hansson et al. 1990; Battista-Pereira et al. 2000). In this study, we determined the chemical composition of the volatiles released by female coffee leafminer moths present in the Soconusco region of Chiapas, Mexico, and then we evaluated the 4 stereoisomers of the major sex pheromone component as attractants of the coffee leafminer in the field.

Material and Methods

Insects

Insects were reared in the laboratory as described elsewhere (Reis et al. 2000). Larvae were reared on green coffee leaves, where the larvae develop and pupate, and maintained at 25-27°C, 60-70% RH, and a photoperiod of 12:12 (L:D). At emergence, moths were kept separately by sex and fed with 10% honey solution.

Pheromone Collection

For volatile collection ten 1-2-day-old virgin females were placed in a cylindrical glass aeration chamber (20 cm long × 15 cm i.d.). A charcoal-filtered airstream (1 L/min) was maintained through the glass aeration chamber. The female volatiles were collected with Porapack Q (50-80 meshes, Water Associates, Inc., Milford, MA) packed between silanized glass wool plugs in a Pasteur pipette during 24 h. The collected volatiles were eluted from the absorbent with 500 µL of HPLC grade hexane (Sigma-Aldrich, Toluca, Mexico), and concentrated to 200 µL by a slow stream of nitrogen. The extract was stored at 20ºC until chemical analysis.

Chemical Analysis

The gas chromatography-mass spectrometry (GC-MS) analysis was conducted with a Varian Star 3400 CX chromatograph linked to a Varian Saturn 4D mass spectrometer. The samples were analyzed with a factor four capillary column VF-5MS, 30 m × 0.25 mm i.d. × 0.250 µm film thickness, Varian) and two chiral columns: Cyclosilb-B (30 m × 0.25 mm i.d. × 0.25 µm film thickness, Agilent Technologies, Santa Clara, CA), or Cyclodex-B (30 m × 0.25 mm i.d. × 0.25 µm film thickness, Agilent Technologies, Santa Clara, CA) that were programmed at 50°C for 1 min, then 10°C/min until 200°C, and held for 10 min. The carrier gas was helium (1 mL/min). The injector port temperature was held at 250°C. Ionization was by electron impact at 70 eV. Identifications of the volatiles emitted by the coffee leafminer females were based on retention time, mass spectrum compared with the mass spectrum of synthetic standards, or tentatively identified based on the fragmentation pattern suggested by Pomonis et al. (1980). The retention index was calculated based on a standard of hydrocarbons (C7-C30) (Sigma-Aldrich, Toluca, Mexico).

Compounds

The synthesis of (5*R*, 9*R*)-dimethylpentadecane, (5*R*, 9*S*)-dimethylpentadecane, (5*S*, 9*R*)-dimethylpentadecane, (5*S*, 9*S*)-dimethylpentadecane, the 4 stereoisomers (Fig. 1) of the major sex pheromone components of the coffee leafminer, was reported by Kuwahara et al. (2000). The com-

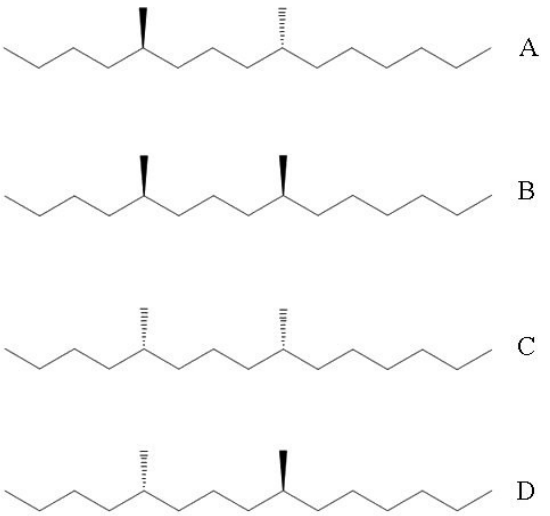


Fig. 1. Structures of stereoisomers of the major sex pheromone component of the coffee leafminer tested in the field. A, (5*R*,9*R*)-dimethylpentadecane; B, (5*R*,9*S*)-dimethylpentadecane; C, (5*S*,9*R*)-dimethylpentadecane; and D, (5*S*,9*S*)-dimethylpentadecane.

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pounds used in this study were supplied by Dr. S. Kuwahara (Laboratory of Applied Bioorganic Chemistry, Division of Life Sciences, Graduate School of Agricultural Science, Tohoku University, Japan). The purity of the compounds was determined by GC-MS as described below.

Study Area

The trial was performed on a coffee farm (14°57'N, 92°11'W, altitude 385 m above sea level) at Manuel Lazos, Municipality of Tuxtla Chico in the Soconusco region of SW Chiapas, Mexico. The experiment was performed in an 8-ha area cultivated with approximately 8-year-old *Coffea arabica* L. cv Bourbon, *Coffea canephora* Pierre ex Froehner and Robusta coffee trees combined with a variety of shade trees including *Cedrela odorata* L., *Citrus sinensis* (L.) Osbeck, *Enterolobium cyclocarpum* (Jacq.) Griseb, *Inga micheliana* Harms, *Musa sapientum* L. and *Pouteria sapota* (Jacq). This area has a humid tropical climate with heavy rain commencing in Mar and ending in Nov, with an annual rainfall of 4160 mm in 2003 and an average annual temperature of 25.5°C.

Field Test

We used white Delta plastic traps (Pherotech, Delta, BC) with a sticky plastic sheet below to capture the insects. Traps were arranged in a randomized plot design with 3 replicates of each treatment. The replicate plots were arranged in parallel lines separated by 30 m. The stereoisomers were dissolved in hexane (HPLC grade) and a solution of 10 µg/µL of each compound was prepared. The lures tested were (5*R*, 9*R*)-dimethylpentadecane, (5*R*, 9*S*)-dimethylpentadecane, (5*S*, 9*R*)-dimethylpentadecane, (5*S*, 9*S*)-dimethylpentadecane, and 500 µg of each stereoisomer was loaded in a rubber septum dispenser (Thomas Scientific, Swedesboro, NJ). Hexane (50 µL) was used as a control. Due to non-availability, the racemic mixture was not included in the experiment. The traps baited with the lures were suspended from the coffee tree branches at a height of approximately 1.5 m between 13 Mar and 25 Apr 2003 and the coffee leafminer male moths captured were recorded every 3 d, giving a total of 11 observation dates. The plastic sticky sheet containing the insects from each trap was labeled and transported to the laboratory where the coffee leafminer was identified under a stereomicroscope. Traps were rotated after each observation date between plots and the lures were replaced with freshly treated septa at 3 weeks. Voucher specimens were placed in the insect collection held at El Colegio de la Frontera Sur, Unidad Tapachula, Mexico.

Statistical Analysis

The data analyses were performed with the computer package Statistica (StatSoft 2003). The number of the coffee leafminer captured per trap was transformed by $(1 + 0.5)^{-1.2}$ by the Box & Cox (1964) transformation for normalizing the data and analyzed by one-way analysis of variance (ANOVA). Significant ANOVAs were followed by a posthoc Tukey test for multiple comparisons of means ($P < 0.05$).

RESULTS

Chemical Analysis

GC-MS analyses of volatiles emitted by the coffee leafminer females showed the presence of 2 saturated branched hydrocarbons identified as 5,9-dimethylpentadecane (RI = 1588) and 5,9-dimethylhexadecane (RI = 1696). The mass spectrum of the 5,9-dimethylpentadecane matched the synthetic compound (Fig. 2A). The purity of stereoisomer synthetics was 99%, determined by GC-MS with traces of hydrocarbons possibly formed during the synthesis (Fig. 2B).

Field Study

The number of coffee leafminer males caught was significantly affected by treatment ($F = 7.93$; $df = 4, 55$; $P < 0.001$) (Fig. 3). Traps baited with (5*S*,9*R*)-stereoisomer captured more males than traps baited with (5*R*,9*R*)-stereoisomer, (5*R*,9*S*)-stereoisomer and control. The catches of traps baited with (5*S*,9*S*)-stereoisomer were intermediate and not significantly different from those captured by traps baited with (5*S*,9*R*)-stereoisomer and (5*R*,9*R*)-stereoisomer. There were no differences in the catches of traps baited with (5*R*,9*R*)-stereoisomer, (5*R*,9*S*)-stereoisomer and control.

DISCUSSION

In this study, we confirmed that the Mexican coffee leafminer population emitted 5,9-dimethylpentadecane and 5,9-dimethylhexadecane such as was reported by Francke et al. (1988). We also obtained a chromatogram with a similar retention time to that reported recently by Lima et al. (2008), who reported 5,9-dimethylpentadecane as the major compound extracted from pheromone glands of virgin females. Our results agree with those of Lima et al. (2008) in that 5,9-dimethylpentadecane is the major component released by *L. coffeella* females. The Mexican population of *L. coffeella* released 5,9-dimethylpentadecane and 5,9-dimethylhexadecane.

The absolute configuration of the sex pheromone components of the coffee leafminer has not been reported. We were not able to separate the

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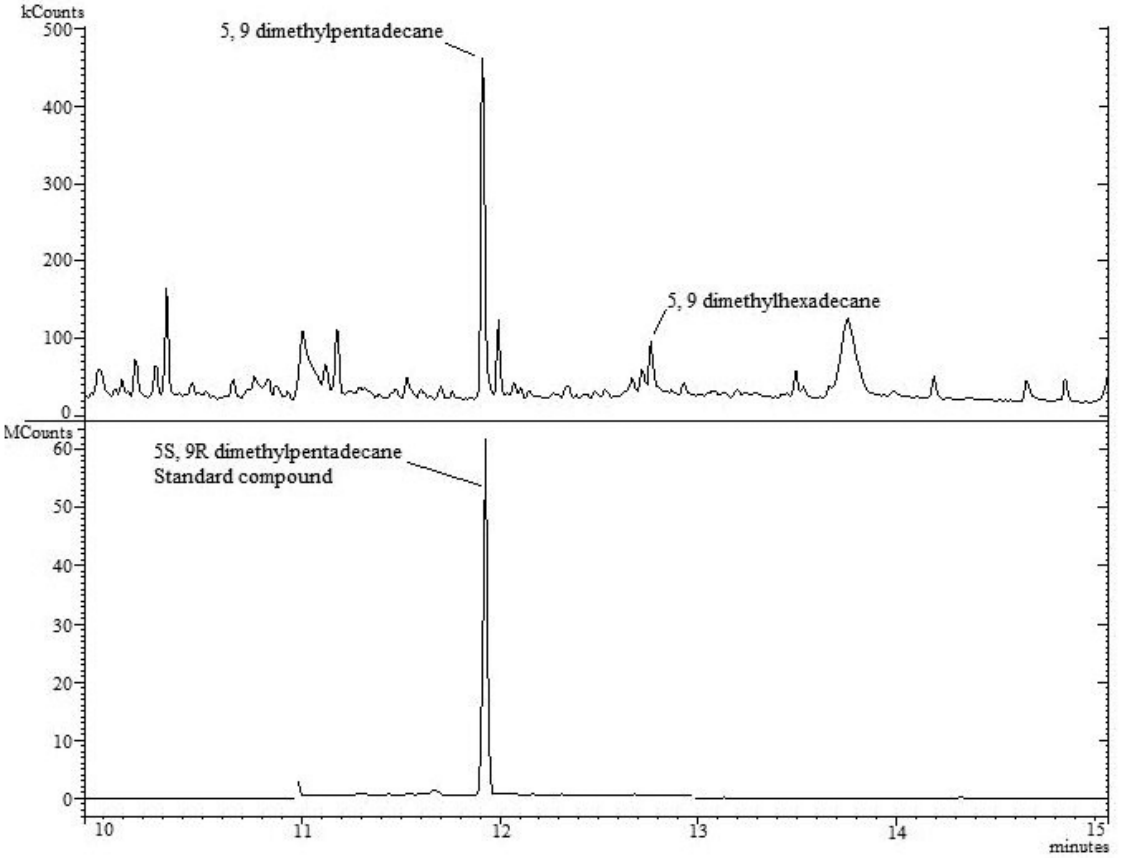


Fig. 2. Trace of the GC-MS (from a factor four capillary column VF-5MS) of pheromone volatiles from virgin females of the coffee leafminer showing both sex pheromone components (A), and 100 ng of synthetic (5*S*,9*R*)-dimethylpentadecane (B).

isomers present in the pheromone volatiles collected from females or synthetic stereoisomers with 2 different chiral columns because the enantiomers of branched aliphatic hydrocarbons do not possess a functional group that permits derivatized into diastereoisomers that could be separated by chromatographic methods or by spectroscopic techniques such as Nuclear Magnetic Resonance (Meierhenrich et al. 2003). However, the enantiomers of a few methyl-substituted alkanes have been separated with enantioselective gas chromatography. For example, Chow et al. (2004) separated stereoisomers of 7,11-dimethylheptadecane under enantiosoelective GC conditions, with a modified cyclodextrin phase. Further studies will be necessary to determine the absolute configuration of the female-produced sex pheromone by using techniques such as enantioselective (ciclodextrin) gas chromatography and gas chromatography coupled electroantennography.

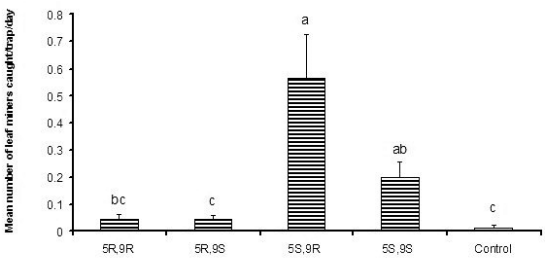


Fig. 3. Mean number of coffee leafminer males caught with delta traps baited with the (5,9)-dimethylpetandecane stereoisomers. Bars with the same letters are not significantly different (Tukey test, $P > 0.05$).

We report for the first time that the coffee leafminer was primarily captured with traps baited with the isomers (5*S*,9*R*)-dimethylpentadecane and (5*S*,9*S*)- dimethylpentadecane. The number of coffee leafminer captured in the present study was very low compared to that reported in Brazil (Lima 2001; Michereff et al. 2007). One possibility is that population levels of

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the coffee leafminer in Mexico are lower than in Brazil. A second possibility is that a mixture of major component isomers might be necessary to improve male captures. Lima (2001) evaluated the effect of the following ratios of the binary blend of 5*R*,9*S*-/ 5*S*,9*S*- dimethylpentadecane: 0:100, 20:80, 50:50, 80:20, and 100:0 on male catches. He found that traps baited with the single 5*S*, 9*S*- isomer captured more than traps baited with any other treatments. The least male captures were obtained by traps baited with the single 5*R*,9*S*-isomer in the ratio 80:20, which suggests that this isomer inhibited the attraction of males to the 5*S*,9*S*-isomer. Furthermore, he found that traps baited with a racemic mixture of dimethylpentadecane captured more *L. coffeella* males than traps baited with single pure stereoisomers. There were no significant differences in the number of males captured by traps baited with the single pure stereoisomers, which is somewhat unexpected because it seem likely that males would at least be attracted more to the stereoisomer emitted by females. In contrast, we found that males were mostly attracted to the 5*S*, 9*S*-isomer, but whether this is the isomer released naturally by *L. coffeella* females remains to be investigated. However, because the racemic mixture was not included in our study, it is difficult to compare our results with those reported by Lima (2001). A third possibility is that we only used the major pheromone component, and the presence of the minor component might be necessary to improve male attraction. To our knowledge nobody has evaluated the biological activity of the minor components released by *L. coffeella* females, but in some moth species the presence of the minor components in the blend are critical for male attraction (Christensen 1997).

In conclusion, the results of this study confirm that 5,9-dimethylpentadecane and 5,9-dimethylhexadecane are released by the coffee leafminer moth present in the Soconusco region of Chiapas, Mexico. Field tests showed that coffee leafminer males responded significantly to 2 stereoisomers of the major component: (5*S*,9*R*)-dimethylpentadecane, and (5*S*,9*S*)-dimethylpentadecane.

Acknowledgments

We thank S. Kuwahara ((Laboratory of Applied Bioorganic Chemistry, Division of Life Sciences, Graduate School of Agricultural Science, Tohoku University, Japan) for supplying the stereoisomers used in this study, Joel Herrera-Muñoz, Antonio Satiesteban, and Armando Virgen for technical assistance, Javier Valle-Mora for statistical advice (ECOSUR), and 2 anonymous reviewers for suggestions to the manuscript. This study was part of the project: "Bioecología y manejo de plagas del café en el Soconusco y Sierra de Chiapas" supported by Fundación Produce Chiapas.

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