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A NEW COMPONENT OF THE MALE PAPAYA FRUIT FLY (DIPTERA: TEPHRITIDAE) SEX PHEROMONE

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Toxotrypana curvicauda Gerstaecker (Diptera: Tephritidae) is the principal tephritid pest of papaya (*Carica papaya* L.; Brassicales: Caricaceae) in the Americas (White & Elson-Harris 1992). Studies have been carried out on the identification of the male sex pheromone (Chuman et al. 1987), behavioral responses to the male sex pheromone (Landolt et al. 1985) and a pheromone bait-trap developed for monitoring and control of papaya fruit flies (Landolt & Heath 1988; Landolt & Heath 1990). 2-methyl-6-vinylpyrazine (2,6mvp) was reported as the main component of the sexual pheromone of the papaya fruit fly and was successfully synthesized (Chuman et al. 1987). Recently, another pyrazine was collected as a minor component from male emissions, but could not be identified (Robledo 2008). The amount of volatile chemicals released increases when other males and host fruit are present (Robledo & Arzuffi 2012), thus facilitating the collection of sufficient amounts unidentified pyrazine from male emissions. The aim of this study was to identify the pyrazine found in lesser amounts in male emissions and to test the biological activity through wind tunnel tests on *T. curvicauda* flies.

Larvae of *T. curvicauda* were obtained from infested *C. papaya* that were collected from Yautepec, Morelos, Mexico (N 18° 05' W 99° 03'; 1,100 masl) and were placed in sifted sterilized soil. Once the adults emerged, they were maintained in a breeding chamber (12:12 h L:D at 25 °C and 50-60% RH). The experiments were conducted from Sep to Feb, under the above laboratory conditions. One h posteclosion, males were placed in an acrylic cage (30 × 30 × 30 cm) and females were placed individually into plastic flasks (9 × 4 cm). They were maintained with a sugar solution (2%), and were isolated from visual and olfactory cues before tests in a separate laboratory. Female adults (6-8-day old) were used for testing.

Fifty males (5-9-day old) were placed in an acrylic cage (30 × 30 × 30 cm) and exposed for 1 h (11:00 - 12:00 h) to 3 unripe papaya fruits (7-10 cm length; Robledo & Arzuffi 2012). Subsequently, the males were placed in a glass container (30 cm ht × 4 cm external diam) (Heath & Manukian 1992). Volatiles were drawn from the container using purified air, previously passed through an

activated charcoal filter, into a glass volatile collection trap (13 cm ht × 0.6 cm external diam) containing 250 mg of Super Q adsorbent (Alltech Assoc, Inc., Deerfield, Illinois) at a flow rate of 1L/min. Two replicate collections were carried out between 12:00 and 15:00 h (peak calling period; Robledo 2008). Subsequently, volatiles were eluted from the adsorbent with 500 µL of hexane (HPLC, JT Baker) and were re-concentrated to 100 µL, using nitrogen flow. The volatile collections from males and the synthetic pyrazines were analysed by a gas chromatography/mass spectrometry detector, according to Robledo and Arzuffi (2012). The compounds were identified by comparing Kovat's index (IR) and mass spectra of the synthetic standards, with reference to the spectral library (NIST/EPA/NIH 2002).

2,6mvp and 2,5-dimethyl-3-vinylpyrazine (2,5dm3vp) were synthesized by means of a one pot reaction (Robledo et al. 2009). They were synthesized using a 2,6-dimethylpyrazine/Hoffman reaction and a 2,3,5-Trimethylpyrazine/Mannich base reaction, respectively.

A transparent plexiglas wind tunnel (120 × 30 × 30 cm) was used for attraction assays (Robacker 1999). All the tests were carried out between 13:00 and 16:00 h. The following stimuli were tested: male volatile collections, synthetic 2,6mvp (2 µL -2.5 µg/µL- and 2µL hexane, Baker, HPLC grade); synthetic 2,5dm3vp (2 µL-0.375 µg/ µL- and 2µL hexane); synthetics mixture (2,6mvp 2 µL-2.5 µg/ µL- and 2,5dm3vp 2µL -0.375 µg/ µL-; proportion 1:0.15); and control (hexane 4µL). In preliminary studies the synthetic mixtures tested in the wind tunnel were: 1:0.10, 1:0.12 and 1:0:0.15; the blend 1:0.15 was the most stable for the application of stimulus and chromatographic analysis. For each stimulus, twelve flies were used, 4 µL of the stimulus was individually placed on filter paper (0.5 × 2.0 cm, Whatman no. 1), then left for 20 s to allow the solvent evaporate at the same height as the air flow entrance. The female fly was released at this height but at the opposite end of the tunnel. Each stimulus consisted of one dose used for 5 min for one fly, and then replaced. To assess the attraction response, both the percentage of flies that moved towards the source and percentage that landed on the source were recorded and ana-

lysed using χ^2 with Yates correction (Systat Software, Port Richmond, California).

In addition to the previously identified 2,6mvp (relative amount $84.9 \pm 6.7\%$; IR 1025), 2,5dm3vp was also identified (relative amount $10.2 \pm 4.1\%$; IR 1102), with a proportion of 1:0.12. Previously, identification of 2,5dm3vp had not been possible, as compared to the other pyrazine, its quantity was very small (Robledo 2008). This problem was overcome by stimulating males by exposure to papaya, to increase the quantities of both pyrazines (Robledo & Arzuffi 2012). The presence of 2,5dm3vp in addition to 2,6mvp in volatile collections from *T. curvicauda* males coincides with that reported with other fruit flies, where collections contained more than one compound (Heath et al. 2000; Nation 2002). The qualitative differences or the complexity in the number of pheromonal components are key factors in attracting fruit fly females (Jang et al. 1994; Heath et al. 2000; Ro-backer et al. 2009).

The percentages of attraction to the source caused by each pyrazine were similar to those caused by the male volatile collections; however, only a blend of these pyrazines resulted in a similar percentage of landings on the source; both presented significantly different results to those obtained with 2,6mvp and 2,5dm3vp ($\chi^2 = 23.386$, gf:1, $p < 0.001$ and $\chi^2 = 12.6$, gf:1, $p < 0.001$, respectively; Fig. 1). This suggests synergism between pyrazines that induces landing. This study contributes to the knowledge of the mating system of *T. curvicauda* and may have a potential impact on practical applications of synthetic pheromone lures for pest management.

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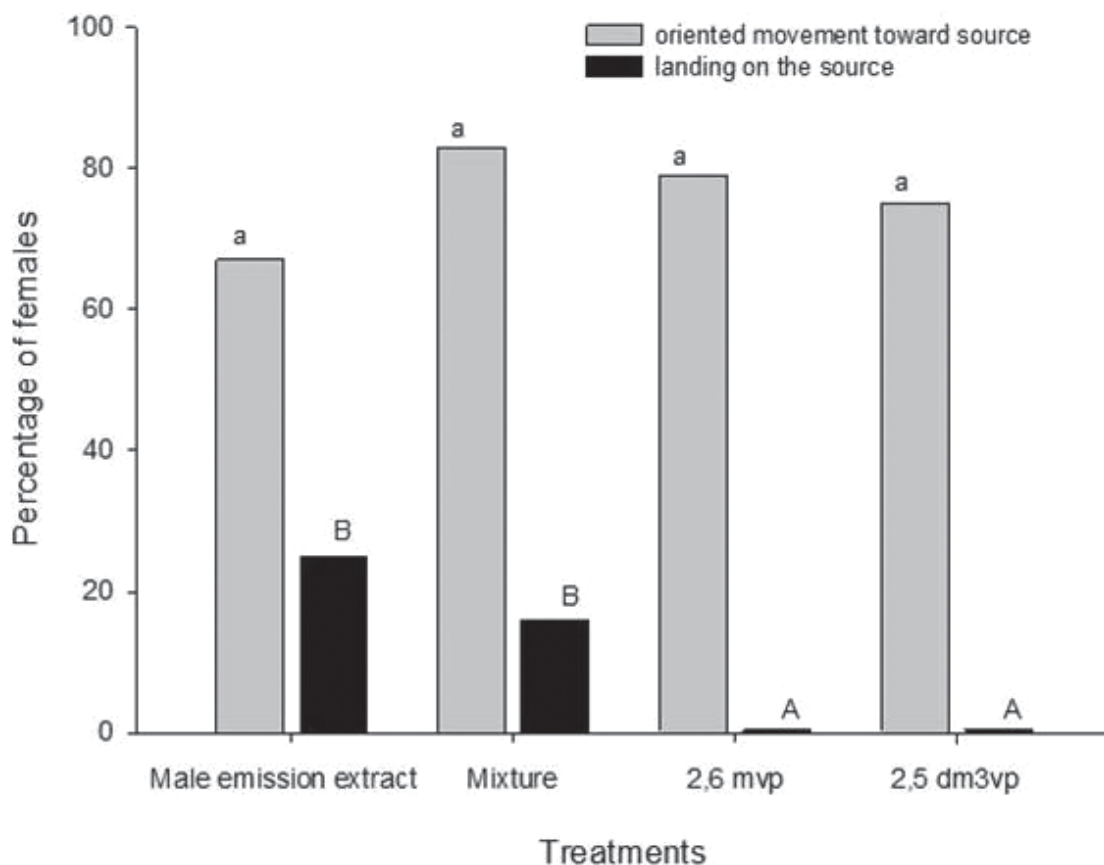


Fig. 1. Percentage of females that oriented toward the source of volatiles and landed on the source stimulated by the male volatile collections, 2-methyl-6-vinylpyrazine (2,6 mvp), 2,5-dimethyl-3-vinylpyrazine (2,5dm3vp) and the 2-pyrazine mixture (2,6 mvp + 2,5dm3vp) in a wind tunnel. Different small letters indicate differences in movement towards the source and different capital letters indicate differences in landing on the source ($P < 0.05$).

SUMMARY

In addition to the major component, 2-methyl-6-vinylpyrazine, 2,5-dimethyl-3-vinylpyrazine was collected as a minor component from calling male papaya fruit flies *Toxotrypana curvicauda* Gerstaecker (Diptera: Tephritidae). Volatile chemicals emitted by calling males were identified by gas chromatography/mass spectrometry detector analyses. Attraction response was verified by wind tunnel bioassays for the male volatile collections and for both synthetic pyrazines. The percentages of attraction to the source caused by each pyrazine were similar to those caused by the male volatile collection; however, only a blend of the pyrazines resulted in a similar percentage of landings on the source.

Key Words: ethenylpyrazine, papaya fruit fly, GC-MS, EAG

RESUMEN

Además de la 2-metil-6-vinilpirazina, se identificó la 3-etenil-2,5-dimetilpirazina en extractos de las emisiones de machos de moscas de la fruta de papaya *Toxotrypana curvicauda* Gerstaecker (Diptera: Tephritidae) utilizando análisis por cromatografía de gases acoplada a espectrometría de masas. También, se verificó la actividad biológica mediante bioensayos en túnel de viento con las pirazinas sintetizadas, comparadas con el extracto de las emisiones de machos, donde los porcentajes de atracción a la fuente; fueron similares. Sin embargo, sólo la mezcla de las dos pirazinas resultó en aterrizaje en la fuente, como el provocado por el extracto de las emisiones de machos.

Palabras Clave: etenilpirazina, mosca del fruto de la papaya, GC-MS, EAG

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