

Fruit compounds affect male sexual success in the South American fruit fly, *Anastrepha fraterculus* (Diptera: Tephritidae)

M. T. Vera^{1,2}, M. J. Ruiz^{1,2}, A. Oviedo¹, S. Abraham^{1,2}, M. Mendoza¹, D. F. Segura^{2,3}, N. A. Kouloussis^{4,5} & E. Willink¹

1 Sección Zoología Agrícola, Estación Experimental Agroindustrial Obispo Colombres, Tucumán, Argentina

2 CONICET, Tucumán, Argentina

3 Laboratorio de Genética de Insectos de Importancia Económica, IGAEF, INTA Castelar, Buenos Aires, Argentina

4 Laboratory of Applied Zoology and Parasitology, School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece

5 Department of Entomology, University of California, Davis, CA, USA

Keywords

exposure to fruit volatiles, guava, male enhancement, sterile insect technique, α -copaene

Correspondence

M. T. Vera (corresponding author), EEAOC, Av. William Cross 3150, Las Talitas (4101), Tucumán, Argentina. E-mail: tvera@eeaoc.org.ar

Received: December 31, 2009; accepted: February 12, 2010.

doi: 10.1111/j.1439-0418.2010.01516.x

Abstract

Although exposure to plants has been shown to influence sexual behaviour in a number of phytophagous insect species, a relatively small number of fruit flies have been investigated in that respect. Here, we evaluated the effect of exposure to the pulp of guava and mango and to essential oils emanating from glands in the flavedo area of lemons on the mating success of *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) males. We also evaluated different durations of exposure and the need for physical contact with the fruit. Results showed that exposure to guava increased the mating success of both wild and laboratory males relative to non-exposed males. In addition, exposed wild flies copulated earlier than non-exposed males. Physical contact with the fruit or ingestion of compounds was not a prerequisite for this phenomenon to occur, since just the exposure to volatiles resulted in a significant enhancement in mating success. Exposure to mango did not affect male sexual performance. In contrast, exposure to lemon for 1 day decreased mating success of males relative to unexposed males, whereas exposure for longer periods seemed to eliminate this effect resulting in comparable mating success rates between treated and untreated males. These results provide a better understanding of the sexual behaviour of this species and may also find application for its control.

Introduction

Exposure to plant chemicals has been shown to influence sexual behaviour in a number of phytophagous insect species. In some tephritid fruit flies, males gain a mating advantage after exposure to certain plants (Nishida et al. 2000a; Tan 2000; Papadopoulos et al. 2008). Males are attracted to compounds emanated by these plants and either feed on them or become arrested in their vicinity by the plant volatiles. For example, males of the Oriental fruit fly, *Bactrocera dorsalis* (Hendel), and other *Bactrocera* species, are strongly attracted to methyl

eugenol (Metcalf 1990), they ingest it and use it to synthesize a sex pheromone component (Tan and Nishida 1996). Males that have consumed methyl eugenol release a sex pheromone that attracts more females than the pheromone of methyl eugenol-deprived males, thereby gaining an advantage in mating competition (Shelly and Dewire 1994). Similarly, an increase in mating success has been found in *B. dorsalis* males that feed on flowers of tropical plants containing methyl eugenol (Nishida et al. 1997). Another example is the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). Mediterranean fruit fly males gain a mating advantage after

exposure to certain fruit compounds but without the need to ingest them. This is the case of the sesquiterpene α -copaene, a hydrocarbon that also acts as an attractant to *C. capitata* males (Flath et al. 1994a,b) and has proved to enhance male mating performance (Shelly 2001). This compound is present in a wide range of natural sources including the bark and fruit of guava (*Psidium guava* L.); in the fruit of mango (*Mangifera indica* [L.]) and several citrus species, as well as in orange (*Citrus sinensis* Osbeck) oil, ginger (*Zingiber officinale* Roscoe) root oil (GRO) and manuka (*Leptospermum scoparium* Forst and Forst) oil (Nishida et al. 2000b; Papadopoulos et al. 2008; Shelly 2008; Shelly et al. 2008 and references therein). Although widely distributed, its concentration varies among plant species and organs.

Many species of the family Tephritidae are economically important pests and impose quarantine restrictions that interfere with international trade. The increasing interest to reduce the use of insecticides to control these flies promotes the implementation of environmental friendly methods worldwide, such as the Sterile Insect Technique (SIT; Knippling 1955). To a large extent, the success of the SIT depends on the ability of sterile males to attract and inseminate wild females. To increase sterile male mating success, major aspects related to male sexual competitiveness have been studied (Hendrichs et al. 2002). For *C. capitata*, the finding that exposure to GRO enhances sexual performance of sterile males (Shelly and McInnis 2001) triggered a series of studies that resulted in its incorporation into SIT programs (Shelly et al. 2007a,b; Shelly 2008).

Within America, the South American fruit fly, *Anastrepha fraterculus* (Wiedemann), is a major fruit pest with a distribution that ranges from southern USA to central Argentina (Salles 1995; Malavasi et al. 2000). It attacks more than 100 host plants (Norrbon 2004), many of which have a high commercial value. Currently, the only control method available to suppress *A. fraterculus* is the use of insecticides. However, environmentally safe methods, such as the SIT, are being proposed as alternatives (Ortíz 1999). Therefore, a deep understanding of the sexual behaviour of the target species is essential for SIT success. In this respect, Malavasi et al. (1983) and Segura et al. (2007) found that *A. fraterculus* has a lek mating system. Males aggregate in mating arenas or leks and release a sexual pheromone that attracts females and upon encounter males initiate sexual courting (de Lima et al. 1994; Salles 2000). Leks are located in particular parts of the tree and pheromone calling is a good

predictor of male copulatory success (Segura et al. 2007). Courtship seems less complex compared with that of *C. capitata* given that successful displays are relatively shorter and no fixed sequence of behaviours was identified (Gómez-Cendra 2007). Pheromone composition was found to differ between two sexually isolated populations (Cáceres et al. 2009), and females from one population showed a tendency to avoid leks formed by males from a different population (Vera et al. 2006). The short and apparently simple courtship, the differences in pheromone composition and the lack of attraction towards leks formed by males from a different population point out the important role of chemical communication on female decisions.

The present study examines whether exposure to guava fruit, the primary host of *A. fraterculus* in Argentina, affects male mating success. Laboratory and wild males were exposed to the fruit for different lengths of time. In addition, we evaluated the effect of mango and lemon as well as the need for physical contact with the fruit. We found that fruit volatiles had an impact on male sexual success increasing our understanding of the reproductive biology of this species and at the same time, gaining new insights for its management.

Material and Methods

Laboratory conditions and insects used

The experiments were conducted in the laboratory at $25 \pm 2^\circ\text{C}$, $70 \pm 10\%$ r.h. Illumination was provided by fluorescent tubes and *Anastrepha fraterculus* adult flies were obtained from a colony established at the Agriculture Zoology laboratories of the Estación Experimental Agroindustrial Obispo Colombes, Tucumán, Argentina. This colony was initiated in 1997 with pupae obtained from infested guavas, collected in the vicinity of Tafi Viejo, Tucumán province (northwest Argentina) (Jaldo 2001) and it has been maintained following standard procedures (Jaldo et al. 2001; Vera et al. 2007). Given that laboratory rearing can affect both male sexual displays and female choice in other tephritid (Briceño and Eberhard 1998, 2000), in some tests, we also used wild flies, which were recovered from infested guavas collected at Horco Molle (Tucumán, Argentina). Fruits were placed in plastic trays with sand to allow pupation. Following emergence, the two sexes were sorted by sex and kept in groups of 25 flies in 750 cc plastic containers containing food and water. Females were fed the standard diet used

for rearing (Jaldo et al. 2001), which contains sugar, hydrolysed yeast (Yeast Hydrolyzated Enzymatic; MP Biomedicals®, Aurora, OH, USA), hydrolysed corn (Gluten Meal; ARCOR®, Tucumán, Argentina) and vitamin E. Males were fed with sugar and brewers yeast (CALSA®, Tucumán, Argentina) (3 : 1 ratio). Laboratory flies were used in an experiment 14–16 days after emergence, whereas wild flies were used 21 days after emergence. This holding period ensured that for both laboratory and wild flies, males and females were sexually mature when tested (Jaldo 2001; Petit-Marty et al. 2004; Jaldo et al. 2007).

Fruit used and conditions of exposure

The exposure effects of guava, lemon, *Citrus limon* (L.) Burm, and mango were evaluated. Guavas were collected from trees occurring in a natural forest in Horco Molle, whereas lemon and mango were purchased in the local market. The general procedure involved exposure of a fruit species to a group of males for a given period of time. Prior to exposure, fruit were washed with tap water. In the case of guava and mango, the fruit was cut in halves, allowing access to the pulp. In the case of lemon, four discs of peel (ca. 4.5 cm in diameter and 0.5 mm deep) were cut and removed with a sharp razor following Papadopoulos et al. (2001). This procedure exposed the essential oil glands present in the flavedo but prevented exposing the pulp. Fruit was first ventilated for 15 min and then placed in plastic cages (12 l) housing 40–60 males, along with adult food and water. Flies had free access to the fruit except for the last experiment (see below). Regardless of the exposure time assigned, fruit exposure ended ca. 18 h before the test, time at which males were labelled and a total of 10 males were placed in the testing cage. Control males, which were not exposed to the fruit, were also placed in the testing cage. The effect of the three fruit species was evaluated at four different exposure times: 1, 5, 10 or 15 days. In guava, 20 replicates were performed with laboratory flies per exposure time and 8 replicates with wild flies. In mango and lemon, six replicates were performed only with laboratory flies.

Evaluation of male mating success

The effect of fruit type and exposure time on male mating success was evaluated in plexiglass testing cages (35 × 25 × 20 cm, with screen covered open-

ings on the top and a cloth-sleeve opening on one side) that contained an artificial twig with leaves providing a surface for resting and mating activities. To distinguish between exposed and non-exposed flies, males were labelled with a dot of water-based paint on the thorax 24 h the day before the test, immediately after fruit exposure finished. Randomly assigned colours identified different type of males. Ten treated males (i.e. exposed to a particular fruit and duration), 10 unexposed males (i.e. control) and 10 females (of the same origin of the male) were released into such a testing cage and the number of couples obtained by each type of male was recorded. Males were released in the testing cages immediately after being marked (i.e. the day before the test) while females were introduced in each cage 5 min before the lights of the room turn on (7:30–8:00 AM) on the day of the evaluation. An observer registered the occurrence of pairs in copula, which were removed from the cages as they formed. For each couple, the colour of the male and the time at which copulation started and finished were recorded. This allowed calculating the mating duration and the time elapsed since the release of the females and the initiation of the copula (from herein referred to as latency). Given that, Argentinian populations of *A. fraterculus* exhibit a narrow period of mating activity early in the morning (Petit-Marty et al. 2004; Vera et al. 2006), each test was run for 2 h.

Evaluation of exposure only to the fruit volatiles

To test the effect of guava and lemon volatiles alone, one group of males was exposed to the fruit as before whilst for a second group, the physical contact with the fruit was prevented and consequently males were exposed only to the fruit volatiles. This was possible by placing the fruit in a small plastic container that had been covered with a cloth mesh. Males landing on the mesh were kept at a distance of 1–3 cm from the fruit. For guava, the exposure time was 7 days, whereas for lemon, we exposed the males either for 1 or for 7 days. This exposure times were chosen given the results obtained in the previous test. To assure that males would feed on the fruit, in one treatment we exposed a group of males to guava for 7 days in a cage that did not contain any other food source. The effect of fruit volatiles was evaluated using a slightly different method. In this case, one treated male and one control male were released in 750 cc plastic containers containing one virgin female. As in the previous experiment, males were released the afternoon before the evaluation, right after being marked,

whereas females the morning of the evaluation just before the lights turn on in the laboratory. In this case, only laboratory flies were evaluated.

Data analysis

In the experiments using groups of competing flies, the number of matings obtained per replicate during the 2 h observation period in each cage was used for the statistical analysis. Cages (i.e. replicates) with two or less matings were discarded (20% or less of all possible matings). Mean numbers within each treatment (fruit or exposure time) were compared using paired *t*-test. Latency and mating duration times were compared within each fruit species by means of a two-way ANOVA where male type (exposed or control) and exposure time were the independent variables. In the experiment where only one female and two males were caged in each container, data were analysed by means of a *G*-test with Yates' correction and no records of latency or mating duration were performed. All analyses were performed with InfoStat (2004).

Results

Effect of guava

Wild males exposed to guava for 15 days showed a significant mating advantage over unexposed males ($t_5 = 4.33$; $P = 0.008$; fig. 1a). Although a tendency for more copulations appeared in the other exposure times and for laboratory flies, the differences were not significant (fig. 1b). When data from all the exposure times were pooled, wild exposed males achieved a mean (\pm SE) of 3.37 ± 0.26 matings, whereas control males obtained only 1.83 ± 0.19 matings ($t_{30} = 5.01$; $P < 0.001$). Pooled data for laboratory males showed the same trend: exposed males achieved a mean of 3.96 ± 0.20 matings, whereas control males obtained a mean $3.07 (\pm 0.23$ SE) ($t_{65} = 2.62$; $P < 0.01$). Sexual enhancement was more noticeable in wild males, but the difference between the two types of flies was not statistically significant (Homogeneity $\chi^2 = 3.47$, $P = 0.063$).

Exposed wild males started mating earlier than untreated wild males, but no effect of exposure times and no interaction were detected (ANOVA: $F_{1,146} = 5.09$, $P = 0.026$ for male type; $F_{4,146} = 1.34$, $P = 0.258$ for exposure time; and $F_{4,146} = 1.05$, $P = 0.385$ for the interaction; table 1). For mating duration, there were no significant effects ($F_{1,141} = 2.25$, $P = 0.066$ for exposure time; and $F_{1,141} = 0.001$,

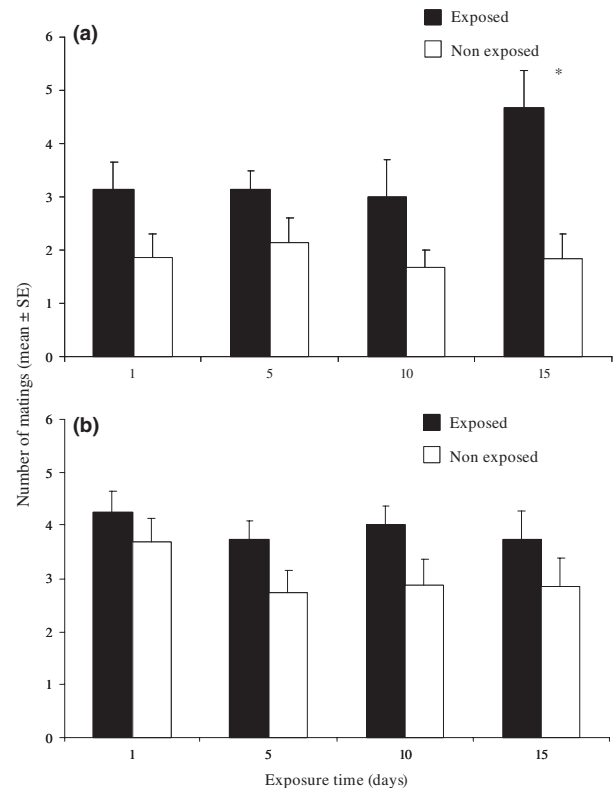


Fig. 1 Number of matings achieved (mean \pm SE) by (a) wild or (b) laboratory *Anastrepha fraterculus* males after exposure to guava fruits for different lengths of time. Mating success was evaluated in experimental cages in which 10 exposed and 10 control males competed for 10 virgin females ($n = 6, 6, 5$ and 5 cages or replicates for 1, 5, 10 and 15 days of exposure, respectively, for wild flies; and $n = 19, 18, 15$ and 11 cages for 1, 5, 10 and 15 days of exposure, respectively, for laboratory flies). *Significant differences (*t*-test, $P < 0.05$).

$P = 0.973$ for male type), but the interaction was significant ($F_{4,141} = 3.93$, $P = 0.005$; table 1).

With respect to laboratory flies, results were non-significant both for latency [$F_{3,206} = 0.96$, $P = 0.412$ for exposure time; $F_{1,206} = 0.79$, $P = 0.374$ for male type (i.e. exposed or non-exposed); and $F_{3,206} = 2.03$, $P = 0.111$ for the interaction] and for mating duration ($F_{3,199} = 2.02$, $P = 0.112$ for exposure time; $F_{1,199} = 1.17$, $P = 0.281$ for male type; and $F_{3,199} = 0.40$, $P = 0.750$ for the interaction; table 1).

Effect of mango and lemon

Exposure of laboratory males to lemon for 1 day resulted in a reduced mating success ($t_5 = -3.16$, $P = 0.025$; fig. 2a). However, this effect depended on the duration of exposure, as it was not observed in males exposed for longer periods ($t_5 = -0.67$, $P = 0.530$ for 5 days exposure; $t_5 = 0.00$, $P = 1.000$

Table 1 Latency (time elapsed before copulation started) and mating duration in minutes (mean \pm SE) for *Anastrepha fraterculus* males exposed to guava, lemon or mango fruits compared with unexposed control males

Fruit	Latency (n)		Mating duration (n)	
	Exposed	Unexposed	Exposed	Unexposed
Guava (wild)	35 \pm 2 (99) a	45 \pm 4 (57) b	46 \pm 3 (96) a	48 \pm 4 (55) a
Guava (laboratory)	50 \pm 3 (126) a	53 \pm 4 (88) a	48 \pm 2 (122) a	45 \pm 2 (85) a
Lemon (laboratory)	39 \pm 4 (29) a	34 \pm 3 (41) a	47 \pm 3 (50) a	47 \pm 3 (60) a
Mango (laboratory)	33 \pm 3 (31) a	33 \pm 4 (32) a	41 \pm 4 (52) a	48 \pm 4 (61) a

Within each fruit, data from different exposure times were pooled. For each variable within each fruit, means followed by a different letter were statistically different ($P < 0.05$).

for 10 days exposure and $t_2 = 0.38$, $P = 0.742$ for 15 days exposure).

Laboratory males exposed to mango did not gain a mating advantage over control males (fig. 2b). This was irrespective of exposure time ($t_5 = -0.24$, $P = 0.822$ for 1 day exposure; $t_5 = 0.17$, $P = 0.872$ for 5 days exposure; $t_5 = 0.25$, $P = 0.809$ for 10 days exposure and $t_1 = -1.00$, $P = 0.500$ for 15 days exposure).

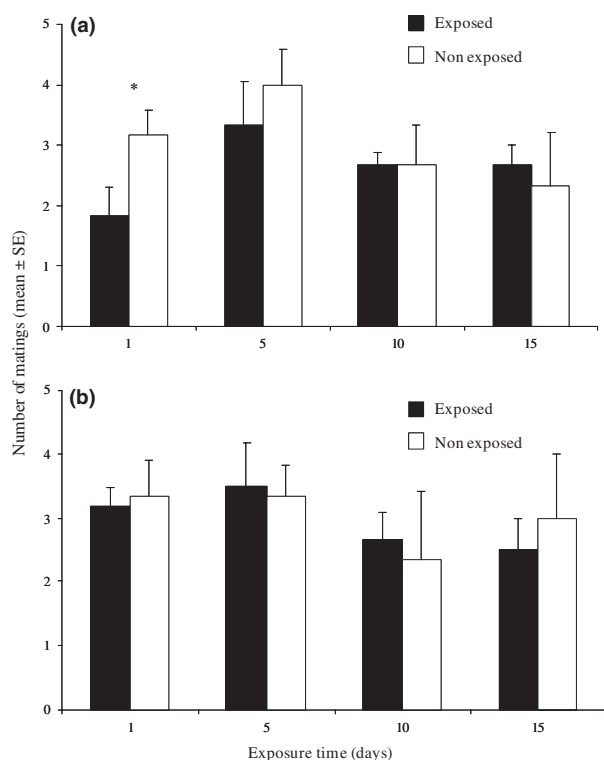


Fig. 2 Number of matings achieved (mean \pm SE) by *Anastrepha fraterculus* laboratory males after exposure to (a) lemon or (b) mango fruits for different lengths of time. Mating success was evaluated in experimental cages in which 10 exposed and 10 control males competed for 10 virgin females ($n = 5, 5, 5$ and 2 cages for 1, 5, 10 and 15 days of exposure to lemon, respectively; and $n = 6, 6, 6$, and 2 cages for 1, 5, 10 and 15 days of exposure to mango, respectively). *Significant differences (t -test, $P < 0.05$).

For males exposed to lemon, there was an effect of the exposure time on latency ($F_{3,62} = 4.63$, $P = 0.006$), no effect of male type ($F_{1,62} = 0.41$, $P = 0.526$) and no interaction between factors ($F_{3,62} = 1.07$, $P = 0.368$; table 1). Males exposed to lemon for 5 days started copulating earlier (mean = 22 \pm 2 min, $n = 25$) than males exposed for 10 days (mean = 48 \pm 6 min, $n = 18$), and control males ($t_{23} = 2.58$, $P = 0.017$). Mating duration was not affected by any of the analysed factors ($F_{3,102} = 2.46$, $P = 0.067$ for exposure time; $F_{1,102} = 0.17$, $P = 0.682$ for male type; and $F_{3,102} = 1.27$, $P = 0.288$ for the interaction).

For males exposed to mango, there was an effect of exposure time ($F_{3,55} = 4.12$, $P = 0.011$), no effect of the type of male ($F_{1,55} = 0.04$, $P = 0.842$) and no interaction ($F_{3,55} = 0.33$, $P = 0.803$). Males exposed for 1 day started copulating later (mean = 48 \pm 6 min, $n = 18$) than males exposed for 5 (mean = 28 \pm 3 min, $n = 18$) or 10 days (mean = 24 \pm 4 min, $n = 16$; Tukey test, $P < 0.05$). Mating duration did not differ among exposure times or male type ($F_{3,105} = 0.26$, $P = 0.8529$ for exposure time; $F_{1,105} = 1.18$, $P = 0.2794$ for male type; and $F_{3,105} = 0.78$, $P = 0.5077$ for the interaction; table 1).

Role of fruit volatiles

Percentages of matings achieved by males exposed to guava and lemon volatiles alone or that had direct contact to the fruit, as well as the significance of the G -test, are presented in table 2. Exposure to guava volatiles only resulted in a significant increase in the mating success of males. The same occurred for males exposed to lemon volatiles only irrespective of the duration of exposure.

Discussion

The present study showed that fruit exposure can affect *A. fraterculus* male sexual success. Exposure to

Table 2 Number of matings achieved by fruit-exposed and control *Anastrepha fraterculus* males under different exposure conditions for guava and lemon fruits

Fruit	Type	Exposure time	Exposed	Unexposed	Total ¹	Percentage ²	G-test ³	P
Guava	Volatiles and food	7 days	35	13	80	72.9	9.51	0.0020
	Fruit contact and food	7 days	27	17	76	61.4	1.85	0.1733
	Fruit contact, no food	7 days	10	9	59	52.6	0.00	1.0000
Lemon	Volatiles and food	7 days	27	13	60	67.5	4.30	0.0381
	Volatiles and food	1 day	74	25	123	74.8	24.28	<0.0001
	Fruit contact and food	7 days	15	7	30	68.2	2.27	0.1322
	Fruit contact and food	1 day	34	18	91	65.4	4.39	0.0362

To test the effect of guava and lemon volatiles alone, some males had contact to the fruit, whereas for others the physical contact with the fruit was prevented and consequently males were exposed only to the fruit volatiles.

¹Total number of females evaluated including those that did not mate.

²Matings achieved by treated males.

³With Yates' correction.

guava resulted in a clear mating advantage, whereas the effects of exposure to lemon depended on the experimental conditions. The enhancing effect of guava did not require physical contact or ingestion of fruit compounds, given that exposure to volatiles was sufficient for males to acquire a mating advantage.

Exposure to guavas resulting in an increased male mating advantage has also been found in *C. capitata* (Shelly and Villalobos 2004). Here, we found that the number of matings showed a tendency to be higher for treated males and when the data for different lengths of exposure times were pooled, the differences between exposed and unexposed males became statistically significant. This impact of guava on *A. fraterculus* male copulatory success was evident both for wild and laboratory flies. In addition, this mating advantage was revealed, in wild flies, by the time copulation started. Exposed males attracted, courted and mated with females earlier than non-exposed males. However, no influence of fruit exposure was detected for mating duration. These results suggest that fruit exposure somehow affects female choice but once copulation started it seems to have no further influence on female decisions to enhance their reproductive success. Moreover, given the resemblances to what was found for *C. capitata*, it can be suggested that chemical cues are involved.

Unlike guava, exposure to mango had no apparent effect on male mating success. This agrees with the lack of effect of mango exposure on male sexual enhancement in *C. capitata* (Shelly et al. 2008). Although mango is a natural source of α -copaene, which in *C. capitata* is responsible of increased male mating success (Shelly 2001), this compound is generally present at very low concentrations (see refer-

ences in Shelly et al. 2008). It may be the case then that the quantities are not sufficient to enhance male mating success, or as proposed by Shelly et al. (2008), the presence of other compounds diminished or blocked any α -copaene effect.

The results obtained in the case of males exposed to lemon were contradictory. Under certain experimental conditions, exposure to lemon for 24 h resulted in a decreased copulatory success, whilst under a different exposure condition it resulted in a clear advantage. We observed that exposure to lemon induced, in some cases, a high mortality of flies, particularly in the experiment where males were exposed to lemon only for 24 h (data not shown). Hence, in this experiment, the group of males exposed only for 24 h could have included moribund or less vigorous males and this can be the explanation for the lower mating performance. Higher exposure times may have included only those males that survived lemon exposure, and that were in general more vigorous. The fact that males exposed to lemon volatiles only showed a higher mating advantage seems to point into the same direction. Some compounds of citrus oils, at given concentrations, can be toxic to fruit fly adults and larvae (Greany et al. 1983; Salvatore et al. 2004; Papachristos et al. 2009). If noxious compounds are not volatile and only through contact or ingestion can affect the flies, then males exposed to volatiles should exhibit a higher mating success than those males that have directly accessed the fruit. Still, this cannot explain why in the first experiments there was a detrimental effect of males exposed for 24 h while in the last experiment there was an enhancing effect. Experiments

were run at different times of the year and therefore we cannot yet rule out a changing composition of the lemon oil glands through the seasons. The fact that the test we used to evaluate mating performance differed between the first and the second experiments could also account for the different response after lemon exposure. The experimental set up has been shown to influence the results obtained in similar tests for *C. capitata* (Papadopoulos et al. 2001, 2006).

Detrimental effects of certain compounds that also elicit mating advantage have been reported previously. A direct application of GRO on the abdomen of *C. capitata* males resulted in a decreased mating advantage, suggesting a negative effect of GRO at high doses (Papadopoulos et al. 2006). Similarly, citrus oil acts as an attractant to *C. capitata* at low concentration, whereas higher concentrations deter direct contact with the oil (Papadopoulos et al. 2001). Moreover, the effect of citrus oil exposure showed a dose–response relationship in *C. capitata* with lower doses being ineffective in increasing mating success of the males, intermediate doses increasing mating success and higher doses killing a percentage of males (Kouloussis et al., this volume).

In conclusion, the present study reports the enhancement of male sexual performance in *A. fraterculus* as a response to fruit volatiles exposure. The fact that exposure to only volatiles is sufficient to elicit the effect could indicate that *a*-copaene, present in guava and lemon, is the responsible compound. However, given that plant volatiles are a complex mixture of compounds, belonging to the same or to other chemical groups, other compounds could also be involved. The fact that GRO, a rich source of *a*-copaene, is currently being used in *C. capitata* operational SIT programmes to enhance sterile male performance is encouraging and envisions that, if this compound is identified as responsible for the *A. fraterculus* male enhancement effect observed in this study, the implementation of its use at a large scale also in this pest species will be rather straightforward.

Acknowledgements

This work was supported by FAO/IAEA grant 14111 to M.T.V. We thank Compañía Argentina de Levaduras S.A. (CALSA) for providing brewer's yeast and ARCOR S.A. for providing corn protein for the adult diets. We thank Nikos Papadopoulos (University of Thessaly, Greece) for his revision and comments in an early draft of the manuscript.

References

- Briceño RD, Eberhard WG, 1998. Medfly courtship duration: a sexually selected reaction norm changed by crowding. *Ethol. Ecol. Evol.* 10, 369–382.
- Briceño RD, Eberhard WG, 2000. Possible Fisherian changes in female mate-choice criteria in a mass-reared strain of *Ceratitis capitata* (Diptera: Tephritidae). *Ann. Entomol. Soc. Am.* 93, 343–345.
- Cáceres C, Segura DF, Vera MT, Wornoyaporn V, Cladera J, Teal P, Sapountzis P, Bourtzis K, Zacharopoulou A, Robinson AS, 2009. Incipient speciation revealed in *Anastrepha fraterculus* (Diptera: Tephritidae) by studies on mating compatibility, sex pheromones, hybridization, and cytology. *Biol. J. Linn. Soc.* 97, 152–165.
- Flath RA, Cunningham RT, Mon TR, John JO, 1994a. Additional male Mediterranean fruit fly (*Ceratitis capitata* Wied.) attractants from angelica seed oil (*Angelica archangelica* L.). *J. Chem. Ecol.* 20, 1969–1984.
- Flath RA, Cunningham RT, Mon TR, John JO, 1994b. Male lures for Mediterranean fruit fly (*Ceratitis capitata* Wied.): structural analogues of α -copaene. *J. Chem. Ecol.* 20, 2595–2609.
- Gómez-Cendra P, 2007. Estudios genéticos y comportamentales sobre la mosca sudamericana de la fruta (*Anastrepha fraterculus*). PhD Thesis, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.
- Greany PD, Styer SC, Davies PL, Shaw PE, Chambers DL, 1983. Biochemical resistance of citrus to fruit flies. Demonstration and elucidation of resistance to the Caribbean fruit fly *Anastrepha suspensa*. *Entomol. Exp. Appl.* 34, 40–50.
- Hendrichs J, Robinson AS, Cayol JP, Enkerlin W, 2002. Medfly areawide sterile insect technique programmes for prevention, suppression or eradication: the importance of mating behavior studies. *Fla. Entomol.* 85, 1–13.
- InfoStat, 2004. InfoStat, versión 2004. Manual del Usuario, 1st edn. Grupo InfoStat, FCA, Universidad Nacional de Córdoba. Editorial Brujas. Argentina.
- Jaldo HE, 2001. Estudios biológicos y poblacionales de *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae). PhD Thesis, Facultad de Agronomía y Zootecnia, Universidad Nacional de Tucumán, Argentina.
- Jaldo HE, Gramajo MC, Willink E, 2001. Mass rearing of *Anastrepha fraterculus* (Diptera: Tephritidae): a preliminary strategy. *Fla. Entomol.* 84, 716–718.
- Jaldo HE, Willink E, Liedo P, 2007. Demographic analysis of mass-reared *Anastrepha fraterculus* (Diptera: Tephritidae) in Tucumán, Argentina. *Rev. Ind. Agr. Tuc.* 84, 15–20.
- Knipling GF, 1955. Possibilities of insect control of eradication through the use of sexually sterile males. *J. Econ. Entomol.* 48, 459–462.

- Kouloussis NA, Katsoyannos BI, Papadopoulos NT, Ioannou CS, Iliadis IV, 2010. Enhanced mating competitiveness of *Ceratitidis capitata* males following exposure to citrus compounds. *J. App. Entomol.* (submitted).
- de Lima IS, Howse PE, Salles LAB, 1994. Reproductive behavior of the South American fruit fly *Anastrepha fraterculus* (Diptera: Tephritidae): laboratory and field studies. *Physiol. Entomol.* 19, 271–277.
- Malavasi A, Morgante JS, Prokopy RJ, 1983. Distribution and activities of *Anastrepha fraterculus* (Diptera: Tephritidae) flies on host and non host trees. *Ann. Entomol. Soc. Am.* 76, 286–292.
- Malavasi A, Zucchi RA, Sugayama R, 2000. Biogeografia. In: *Moscas-das-frutas de importância econômica no Brasil. Conhecimento básico e aplicado.* Ed. by Malavasi A, Zucchi RA, Holos Editora, Ribeirão Preto, Brasil, 93–98.
- Metcalf RL, 1990. Chemical ecology of Dacine fruit flies (Diptera: Tephritidae). *Ann. Entomol. Soc. Am.* 83, 1017–1030.
- Nishida R, Shelly TE, Kaneshiro KY, 1997. Acquisition of female attractive fragrance by males of the oriental fruit fly from a Hawaiian lei flower, *Fagraea berteriana*. *J. Chem. Ecol.* 23, 2275–2285.
- Nishida R, Shelly TE, Kaneshiro KY, Tan KH, 2000a. Roles of semiochemicals in mating systems: a comparison between oriental fruit fly and medfly. In: *Area-wide control of fruit flies and other insect pests.* Ed. by Tan KH, Penerbit University Sains Malaysia, Penang, Malaysia, 631–637.
- Nishida R, Shelly TE, Whittier TS, Kaneshiro KY, 2000b. α -Copaene, a potential rendezvous cue for the Mediterranean fruit fly, *Ceratitidis capitata*? *J. Chem. Ecol.* 26, 87–100.
- Norrbom AL, 2004. Host plant database for *Anastrepha* and *Toxotripiana* (Diptera: Tephritidae: Toxotripianini). [WWW document]. URL <http://www.sel.barc.usda.gov:8080/diptera/Tephritidae/TephIntro.html>.
- Ortíz G, 1999. Potential use of the sterile insect technique against the South American fruit fly. In: *The South American Fruit Fly, Anastrepha fraterculus* (Wied.): advances in artificial rearing, taxonomic status and biological studies. IAEA TEC-DOC 1064. Ed. by International Atomic Energy Agency, The International Atomic Energy Agency, Vienna, Austria, 121–130.
- Papachristos DP, Kimbaris AC, Papadopoulos NT, Polissiou MG, 2009. Toxicity of citrus essential oils against *Ceratitidis capitata* (Diptera: Tephritidae) larvae. *Ann. Appl. Biol.* 155, 381–389.
- Papadopoulos NT, Katsoyannos BL, Kouloussis NA, Hendrichs J, 2001. Effect of orange peel substances on mating competitiveness of male *Ceratitidis capitata*. *Entomol. Exp. Appl.* 99, 253–261.
- Papadopoulos NT, Shelly TE, Niyazi N, Jang E, 2006. Olfactory and behavioral mechanisms underlying enhanced mating competitiveness following exposure to ginger root oil and orange oil in males of the Mediterranean fruit fly, *Ceratitidis capitata* (Diptera: Tephritidae). *J. Insect Behav.* 19, 403–418.
- Papadopoulos NT, Kouloussis NA, Katsoyannos BI, 2008. Effects of plant chemical on the behavior of the Mediterranean fruit fly. In: *Fruit flies of economic importance: from basic to applied knowledge proceedings of the 7th international symposium on fruit flies of economic importance, 10–15 September 2006, Salvador, Brazil.* Ed. by Sugayama RL, Zucchi RA, Ovruski SM, Sivinski J, Press Color Graficos Especializados, Brotas, Bahia, Brazil, 97–106.
- Petit-Marty N, Vera MT, Calcagno G, Cladera JL, Segura DF, 2004. Sexual behavior and mating compatibility among four populations of *Anastrepha fraterculus* (Diptera: Tephritidae) from Argentina. *Ann. Entomol. Soc. Am.* 97, 1320–1327.
- Salles LAB, 1995. *Bioecologia e Control das Moscas das Frutas Sul-Americanas.* EMBRAPA-CPACT, Pelotas, Brazil.
- Salles LAB 2000. *Biologia e ciclo de vida de Anastrepha fraterculus.* In: *Moscas-das-frutas de Importância Econômica no Brasil. Conhecimento Básico e Aplicado.* Ed. by Malavasi A, Zucchi RA, Holos Editora, Ribeirão Preto, Brasil, 81–86.
- Salvatore AS, Borkosky S, Willink E, Bardón A, 2004. Toxic effect of lemon peel constituents in *Ceratitidis capitata*. *J. Chem. Ecol.* 30, 323–333.
- Segura DF, Petit-Marty N, Sciurano RB, Vera MT, Calcagno G, Allinghi A, Gomez-Cendra P, Cladera JL, Vilardi JC, 2007. Lekking behavior of *Anastrepha fraterculus* (Diptera: Tephritidae). *Fla. Entomol.* 90, 154–162.
- Shelly TE, 2001. Exposure to α -copaene and α -copaene-containing oils enhances mating success of male Mediterranean fruit fly (Diptera: Tephritidae). *Ann. Entomol. Soc. Am.* 94, 497–502.
- Shelly TE, 2008. Aromatherapy and medfly SIT. In: *Fruit flies of economic importance: from basic to applied knowledge proceedings of the 7th international symposium on fruit flies of economic importance, 10–15 September 2006, Salvador, Brazil.* Ed. by Sugayama RL, Zucchi RA, Ovruski SM, Sivinski J, Press Color Graficos Especializados, Brotas, Bahia, Brazil, 59–69.
- Shelly TE, Dewire AM, 1994. Chemically mediated success in male Oriental fruit flies (Diptera: Tephritidae). *Ann. Entomol. Soc. Am.* 87, 375–382.
- Shelly TE, McInnis DO, 2001. Exposure to ginger root oil enhances mating success of irradiated, mass-reared males of Mediterranean fruit fly (Diptera: Tephritidae). *J. Econ. Entomol.* 94, 1413–1418.
- Shelly TE, Villalobos EM, 2004. Host-plant influence on the mating success of male Mediterranean fruit fly: variable effects within and between individual plants. *Anim. Behav.* 68, 417–426.

- Shelly TE, Edu J, Smith E, Hoffman K, War M, Santos R, Favela A, Garagliano R, Ibewiro B, McInnis DO, 2007a. Aromatherapy on a large scale: exposing entire adult holding rooms to ginger root oil increases the mating competitiveness of sterile males of the Mediterranean fruit fly in field cage trials. *Entomol. Exp. Appl.* 123, 193–201.
- Shelly TE, McInnis DO, Rodd C, Edu J, Pahio E, 2007b. Sterile insect technique and Mediterranean fruit fly (Diptera: Tephritidae): assessing the utility of aromatherapy in a Hawaiian coffee field. *J. Econ. Entomol.* 100, 273–282.
- Shelly TE, Cowan AN, Edu J, Pahio E, 2008. Mating success of male Mediterranean fruit flies following exposure to two sources of α -copaene, manuka oil and mango. *Fla. Entomol.* 91, 9–15.
- Tan KH, 2000. Behaviour and chemical ecology of *Bactrocera* flies. In: *Area-wide control of fruit flies and other insect pests*. Ed. by Tan KH, Penerbit University Sains Malaysia, Penang, Malaysia, 647–656.
- Tan KH, Nishida R, 1996. Sex pheromone and mating competition after methyl eugenol consumption in the *Bactrocera dorsalis* complex. In: *Fruit fly pests – a world assessment of their biology and management*. Ed. by McPherson BA, Steck GJ, St. Lucie Press, Delray, FL, USA, 147–153.
- Vera MT, Cáceres C, Wornoyaporn V, Islam A, Robinson AS, de la Vega MH, Hendrichs J, Cayol JP, 2006. Mating incompatibility among populations of the South American fruit fly *Anastrepha fraterculus* (Wied.) (Diptera: Tephritidae). *Ann. Entomol. Soc. Am.* 99, 387–397.
- Vera MT, Abraham S, Oviedo A, Willink E, 2007. Demographic and quality control parameters of *Anastrepha fraterculus* (Diptera: Tephritidae) artificial rearing. *Fla. Entomol.* 90, 53–57.