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Lemon and *Schinus polygama* essential oils enhance male mating success of *Anastrepha fraterculus*

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Abstract

The sterile insect technique (SIT) is an environmentally friendly pest control method that relies on the introduction of sterility into the pest population by the release of sterile males. Many Tephritidae fruit fly pests are currently being controlled with SIT. Sterile insect technique efficacy can be increased if the sexual success of mass-reared sterile males is enhanced. Phytochemicals influence the sexual behaviour of many species of phytophagous insects. Here, we evaluated the possibility of using essential oils to enhance male sexual success of the highly polyphagous fruit fly pest *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae), also known as the South American fruit fly. In laboratory tests, we determined the effect of exposing males to volatiles from seven essential oils [*Baccharis spartioides* (Hook. & Arn) Cabrera (Asteraceae), *Schinus polygama* (Cav.) Cabrera, *Schinus areira* (L.) (Anacardiaceae), *Zingiber officinale* (Roscoe) (Zingiberaceae), *Citrus limon* (L.) Burm. F., *Citrus paradisi* Macfad., and *Citrus sinensis* (L.) Osbeck (Rutaceae)] and two monoterpenes (limonene and citral) that are present at high proportions in some of the oils we evaluated. One exposed and one non-exposed (control) male were placed in a mating arena with one virgin female. We recorded the type of male chosen as mating partner, copula start time, and copula duration. Laboratory males exposed to the volatiles of *C. limon* and *S. polygama* essential oils achieved more matings than non-exposed males. The rest of the oils had no effect on male mating success. In addition, limonene-exposed males obtained significantly more matings than non-exposed males, and citral induced a detrimental effect. Exposure to the volatiles of the various essential oils and monoterpenes did not impact on copula start time and copula duration. We discuss the role of essential-oil volatiles on *A. fraterculus* males' sexual behaviour and its implications for SIT.

Abbreviated abstract

Sterile insect technique (SIT) efficacy can be increased if mass-reared sterile males' sexual success is enhanced. We evaluated the possibility of using phytochemicals to enhance male sexual success of *Anastrepha fraterculus* (Diptera: Tephritidae) under laboratory conditions. Essential oils of *Citrus limon* (Rutaceae) and *Schinus polygama* (Anacardiaceae) and limonene enhanced male mating success. We discuss the role of exposure to essential-oil volatiles on *A. fraterculus* male sexual behavior and its implications for SIT.

Graphic for Table of Contents

graphical abstract EEA.pdf

Introduction

The Tephritidae family (Diptera) comprises approximately 5 000 species, including destructive pests of fruit and vegetable production (White & Elson-Harris, 1992; Norrbom, 2004). Most of the economically important species are highly polyphagous (Norrbom & Kim, 1988; Norrbom, 2004) with a wide distribution and of quarantine concern. The need to reduce population levels below the threshold of economic damage is high and many countries implement area-wide integrated pest management (AW-IPM) strategies. The growing demand to reduce the use of insecticides promotes the implementation of environmentally friendly control methods, such as the sterile insect technique (SIT) in such AW-IPM programs (Enkerlin, 2005). In the SIT, mass-reared sterile males are released in the field, where they are expected to mate with wild females and to transfer sterile sperm. Mated females lay unfertile eggs, resulting in the concomitant reduction of the target population (Knippling, 1959; Dyck et al., 2005; Dunn & Follett, 2017). The acceptance of sterile males by wild females in the field is therefore crucial for SIT success, and hence in-depth knowledge of the mating behaviour of target species becomes essential, particularly those factors that could enhance sterile male mating success (Hendrichs et al., 2002; Pereira et al., 2013a).

The South American fruit fly, *Anastrepha fraterculus* (Wiedemann), is a cryptic species complex (Hendrichs et al., 2015) considered as a highly polyphagous tephritid, and a serious pest in several countries of South America. This species complex is composed of at least eight morphotypes (Hernández-Ortiz et al., 2004, 2012, 2015) and it is present from the USA (Texas) to Argentina (Hernández-Ortiz & Aluja, 1993; Salles, 1995; Steck, 1999; Malavasi et al., 2000). It has been reported to infest more than 100 host plants species, including major fruit crops (Norrbom & Kim, 1988; Norrbom, 2004; Hernández-Ortiz et al., 2020), and it poses quarantine restrictions in most non-American countries (Guillén & Sánchez, 2007). Consequently, great research efforts have been allocated to the development and implementation of SIT against *A. fraterculus* (Ortiz, 1999; Cladera et al., 2014). Key issues involved mating compatibility among morphotypes (Juárez et al., 2015; Vaníčková et al., 2015), description of the sexual behaviour (Roriz et al., 2018), determination of sterility doses (Allinghi et al., 2007; Mastrangelo et al., 2010), and rearing protocols (Vera et al., 2014; Goane et al., 2018).

Like many other Tephritidae pest species, *A. fraterculus* has a lek-based mating system (Malavasi et al., 1983; Segura et al., 2007). Males aggregate in groups on host and non-host plant species and perform sexual displays that attract females in a clearly defined area (Aluja, 1994; Shelly, 2018). During the sexual display, males produce visual, vibratory, and chemical signals,

which include the extrusion of the salivary glands, protrusion of the anal tissue, and wing fanning (Sivinski et al., 2001). Females presumably integrate these signals when selecting a mate and males are subjected to strong sexual selection pressure (Shelly, 2018).

The sexual behaviour of many phytophagous insects is influenced by plants in a variety of ways, including serving as sexual encounter sites and synergizing or increasing male sexual signals to optimize their attractiveness to females (Landolt & Phillips, 1997). Because of their practical and theoretical relevance, the effects of plants on insect sexual behaviour has been well studied in several Tephritidae species (Shelly, 2008; Segura et al., 2018). For example, males of certain *Bactrocera* species, such as *Bactrocera dorsalis* (Hendel), are attracted to methyl eugenol (ME), a phenylpropanoid produced by orchid flowers. Upon ingestion, ME is metabolized and then incorporated into the pheromone, allowing males to gain a mating advantage over males that do not ingest ME (Nishida et al., 1988; Hee & Tan, 2004). Similarly, males of *Ceratitis capitata* (Wiedemann) increase their mating success after exposure to the volatiles of the essential oils of ginger root, *Zingiber officinale* (Roscoe) (Zingiberaceae) (Shelly, 2001; Shelly & Mcinnis, 2001), citrus fruits (Shelly et al., 2004; Papadopoulos et al., 2006; Kouloussis et al., 2013), *Schinus polygama* (Cav.) Cabrera (Anacardiaceae), and *Baccharis spartioides* (Hook. & Arn.) Cabrera (Asteraceae) (Jofré-Barud et al., 2014). Kouloussis et al. (2013) found that the exposure of males to a mixture of specific components of citrus oil (i.e., limonene, linalool, β -myrcene, α -pinene, and geraniol) likewise boosted male mating competitiveness, but when they tested these compounds singly, the effect was moderate yet non-significant. In contrast, Juan-Blasco et al. (2013) found that exposure to the volatiles of linalool singly was sufficient to improve *C. capitata* male mating success. For *A. fraterculus*, an increase in male mating success was found after exposure to the volatiles of fresh guava (*Psidium guava* L.) and lemon [*Citrus limon* (L.) Burm. F. (Rutaceae)] fruits (Vera et al., 2013; Bachmann et al., 2015). Furthermore, exposure to a mixture of seven compounds (limonene, β -myrcene, (*E*)- β -ocimene, α -humulene, *E*-2-hexenal, ethyl butanoate, and ethyl hexanoate) which are released by guava fruit also triggered a significant boost of male mating competitiveness (Bachmann et al., 2015). For *Anastrepha ludens* (Loew), exposure to the volatiles of grapefruit essential oil (*Citrus paradisi* Macfad.) increased male mating success (Morató et al., 2015). Exposure of males to ginger-root oil volatiles seems to have a wide range of effects in *Anastrepha* spp. For *Anastrepha serpentina* (Wiedemann) there was an enhancing effect when males were 9 or 12 days old, but there was no effect when they were younger; for *Anastrepha obliqua* (Macquart) there was no effect of exposure on male mating success and for *A.*

ludens there was a detrimental effect when males were 6 days old, and no effect when males were older (Flores et al., 2011). For *A. fraterculus*, the lack of consistency in experiments (Mendoza, 2010) imposes further investigations.

Given the relevance of male sexual performance for the success of SIT, and in continuity with our previous investigations of the effect of *Citrus* spp. fruit volatiles on the sexual performance of *A. fraterculus* (Vera et al., 2013) and those of the effect of essential oils from non-host species on *C. capitata* (Jofre Barud et al., 2014; Jofre Barud, 2018), here we tested in the laboratory the effect of seven essential oils – namely *C. limon*, *C. paradisi*, *Citrus sinensis* (L.) Osbeck, *S. polygama*, *Schinus areira* (L.) (Anacardiaceae), *B. spartioides*, and *Z. officinale* – and two monoterpenes – limonene and citral – on *A. fraterculus* male mating competitiveness in order to identify essential oils or compounds that could be explored at a larger scale for their implementation as a pre-release method to enhance SIT efficacy.

Materials and methods

Insects

Flies were obtained from a colony of the Brazilian 1 morphotype within the *A. fraterculus* cryptic species complex, kept at IGEAF (INTA Castelar, Buenos Aires, Argentina). This colony was initiated in 1997 with pupae obtained from infested guavas collected in Tafi Viejo (Tucumán, Argentina). For rearing details of this colony see Jaldo et al. (2001).

Pupae were placed in 12-l plastic containers (22 × 30 × 19 cm) with water and sugar and maintained under laboratory conditions (24-26 °C and 70-80% r.h.) until adults emerged. Every 24-48 h, flies that had emerged were sorted by sex. Females were placed in cylindrical, 1-l plastic containers (11 cm high, 12 cm diameter) in groups of 15 individuals, whereas males were placed in 12-l plastic containers in groups of 300 individuals. This procedure is routinely done to avoid discarding large amounts of females in case a male is mistakenly transferred into the females' cage while sorting the sexes. Adults were provided with water and food. Females were fed the standard diet used for rearing *A. fraterculus* in Argentina, which consists of yeast hydrolysate (Yeast Hydrolysate Enzymatic; MP Biomedicals, Aurora, OH, USA), sugar, and hydrolysed maize (Gluten Meal; Arcor, Tucumán, Argentina) (1:3:1 ratio), and a vitamin complex (Jaldo et al., 2001). This diet has been proven highly nutritious and a great boost to reproduction in females (Jaldo et al., 2001). Males were fed the same diet that we used in our previous studies focused on evaluating the role of fruit volatiles as male enhancers (Vera et al., 2013; Bachmann et al., 2015,

2019; Bachman, 2016), which consists of brewer's yeast (Calsa; El Manantial, Tucumán, Argentina) and sugar (1:3 ratio). Flies were kept in separate rooms during the period of sexual maturation (10-12 days) to avoid any effect of pheromone exposure before the mating test.

Essential oils and monoterpenes

The essential oils evaluated were all obtained from fresh material. The plant material of *B. spartioides* and *S. polygama* was collected in the department of Jáchal (30°13'00"S, 68°35'00"W); the plant material of *S. areira* was collected in the department of Ullum (31°25'00"S, 68°44'00"W), both in San Juan province, Argentina. The aerial parts of the plants (150 g) were subjected to steam distillation for 2 h using a Clevenger type apparatus. The roots of *Z. officinale* (2 kg) were purchased at the local market and subjected to distillation for 6 h. Fruits from *C. limon*, *C. paradisi*, and *C. sinensis* were collected in the experimental field at Estación Experimental Obispo Colombres (26°47'15.45"S, 65°11'23.72"W), Tucumán province, Argentina. The day after harvest, fruit were washed with tap water, dried at room temperature, and the flavedo was removed from the peel. Peel compounds were extracted with ethyl ether by immersion. Ether extracts were filtered, and the solvent was evaporated using a rotary evaporator at room temperature, as detailed in Ruiz et al. (2015). Table 1 shows the proportion of the chemical groups and major compounds for each essential oil. The detailed chemical characterization of the essential oils can be found in Jofré-Barud (2018) for *B. spartioides*, *S. polygama*, *S. areira*, and *Z. officinale*, and in Ruiz et al. (2015) for *C. limon*, *C. paradisi*, and *C. sinensis*. The monoterpenes (R)-limonene 98% and citral (mixture of neral and geranial) 95% were purchased from Sigma-Aldrich (Buenos Aires, Argentina). These two compounds were chosen as they are the two dominant compounds (more than 50%; Table 1) found in *Citrus* spp. essential oils (limonene) and in *Z. officinale* essential oil (citral). Essential oils and monoterpenes were stored at -20 °C before use.

Exposure to the volatiles of essential oils and monoterpenes

The day before the mating test, males were transferred in groups of 60 individuals into 3-l glass containers covered with a nylon fabric where they were exposed to 20 µl of a given essential oil or monoterpene for 1.5 h. The essential oil or monoterpene was delivered inside a 400-µl glass insert vial that was placed in the centre of the glass container immediately after the flies were released. During exposure, cages were devoid of food and water. Upon completion of the exposure time,

males were transferred in groups of 15 individuals to 1-l plastic containers with food and water, where they were kept until the mating test. Non-exposed males were treated in the same way but were not exposed to essential oils or monoterpenes.

Mating tests

To assess the effect of exposure to different essential oils or monoterpenes on male mating success, we followed the mating test protocol described by Bachmann et al. (2015), which consisted of assessing the mate choice of a single female when presented with one exposed male and one non-exposed male in a mating arena (1-l plastic container). Males were marked by feeding on diets supplemented with dyes of different colours (Fleibor; Tablada, Buenos Aires, Argentina). Previous experiments have shown that this marking technique has no adverse effect on the flies. Two colours were used, one for exposed males and the other for non-exposed males, and each mating test the colour assigned to a male type was alternated. Mating tests started when lights were turned on in the experimental room (09:00 hours), in concordance with the period of sexual activity of the Brazilian 1 *A. fraterculus* from Argentina (Petit-Marty et al., 2004; Vera et al., 2006). An observer monitored the flies continuously during 2 h for the occurrence of copulation. Whenever a mating couple was detected, the uncoupled male was removed, and male dye colour, copulation start time, and copulation end time were recorded. The experiment was performed on five different days (mating tests), and each day, 50 mating arenas (50 replicates) were observed for each essential oil (except *Z. officinale*) and monoterpene with a total of 250 mating arenas evaluated in each case. For *Z. officinale*, we ran the experiment on four different days (200 mating arenas).

Data analysis

The effect of exposure to volatiles on male mating success was evaluated by comparing the number of copulations achieved by treated and non-treated males for each essential oil or monoterpene by means of a G test of goodness of fit to an equal proportion hypothesis, with the Yates correction for continuity (recommended whenever the degree of freedom equals 1; Zar, 1996). Possible bias on the probability of mating attributable to the colour of the male was explored by means of a generalized linear model. The number of matings achieved by the males was the response variable and colour was the fixed factor. The error distribution was fitted to a binomial family with a logit link function. The time it took the female to choose a male (latency to

mate) and copula duration were compared by a Wilcoxon paired test for each essential oil or compound. For the three variables, the data from the mating arenas of the different mating tests were pooled. Data were analysed with InfoStat (Di Rienzo et al., 2018).

Results

Mating success

The effect of exposure to volatiles on mating success depended on the essential oil or compound evaluated (Table 2, Figure 1). Females mated significantly more often with males exposed to the volatiles of the essential oils of *S. polygama* and lemon, whereas the rest of the oils did not affect male mating success (Figure 1A). Likewise, males exposed to limonene obtained significantly more matings than non-exposed males (Figure 1B). In contrast, males exposed to citral achieved significantly fewer matings than non-exposed males (Figure 1B). Dye colour showed no effect on female choice, irrespective of whether the males were exposed or not (exposed: $F_{1,42} = 0.87$, $P = 0.39$; non-exposed: $F_{1,42} = 1.16$, $P = 0.25$). Therefore, the probability of mating for a given male was independent of its colour.

Latency to mate and copula duration

Exposure to the volatiles of the various essential oils and monoterpenes did not affect latency to mate (Table 3), nor did it affect copula duration (Table 4). On average copulations started 19.5 min after females' release (median = 15 min; Q1 = 5 min; Q3 = 25 min). Copulations lasted on average 59.7 min (median = 55.5 min; Q1 = 44 min; Q3 = 70 min).

Discussion

The present study aimed to determine whether exposure to volatiles of certain essential oils or monoterpenes confers a mating advantage to *A. fraterculus* males. Our results showed that out of the seven essential oils chosen based on previous results, the volatiles of *C. limon* and *S. polygama* essential oils conferred to males a mating advantage over non-exposed males. Of the single compounds evaluated, limonene, but not citral, increased mating success.

Volatiles exposure and mating success

Previous studies have reported that exposure to the volatiles of lemons resulted in a mating

advantage in *A. fraterculus* males (Vera et al., 2013). Similarly, for *C. capitata* a mating advantage was reported for males exposed to lemon and *S. polygama* essential oils (Kouloussis et al., 2013; Jofré-Barud et al., 2014). These two essential oils are different in their chemical composition, but interestingly elicited the same response. The essential oil from lemon largely consists of monoterpene hydrocarbons (91.9%), including limonene as the predominant compound (Ruiz et al., 2015), whereas the essential oil of *S. polygama* is composed mainly of monoterpene hydrocarbons and hydrocarbon sesquiterpenes with no single compound being predominant (Jofré-Barud et al., 2014; Jofré-Barud, 2018). Male enhancement in *A. fraterculus* was also achieved after exposure to guava volatiles (Vera et al., 2013; Bachmann et al., 2015, 2019), in which there is no predominant chemical group (Pino et al., 2001) and in an artificial mixture of seven compounds present in the guava volatiles, including limonene, in even proportions (Bachmann et al., 2015). Therefore, further studies are required in order to identify candidate compound(s) responsible of male enhancement. Regarding limonene alone, no enhancing effect was found in *C. capitata* (Juan-Blasco et al., 2013; Kouloussis et al., 2013), and, to our knowledge, there are no reported studies on other *Anastrepha* species. Therefore, this study represents the first report of an enhancing effect for this compound.

On the other hand, exposure to the volatiles of the essential oils of grapefruit, orange, ginger root, *B. spartioides*, and *S. areira* did not increase male mating success. Other authors tested the effect of these oils on other species of fruit flies, and results were dependent on the species evaluated (Segura et al., 2018). For example, grapefruit essential oil enhanced mating success in *A. ludens* and *C. capitata* (Shelly, 2009; Morató et al., 2015). Here, although we did not find a significant effect of grapefruit essential oil, there was a tendency to a higher male mating success. Orange essential oil enhanced *C. capitata* male mating success in a dose-dependent manner, and high doses resulted in complete mortality of the exposed males (Kouloussis et al., 2013). In previous experiments, *A. fraterculus* males also showed a dose-dependent response after exposure to the volatiles of wounded peel of Valencia oranges (unpubl.). In addition, our results confirm the lack of effect of ginger root oil on *A. fraterculus* male mating competitiveness (Mendoza, 2010). The effect of exposure to ginger-root oil volatiles in other *Anastrepha* species varies, from an enhancing effect to no effect or even a detrimental effect (i.e., females mated more frequently with non-exposed males) (Flores et al., 2011). The ginger root oil we used had large amounts of citral, which agrees with our finding that citral significantly decreased the number of matings achieved by exposed males. This is also consistent with the detrimental effect of ginger

root oil found for *A. ludens* (Flores et al., 2011) and indicates a difference from what is found for *C. capitata* and *Ceratitis quilici* de Meyer et al., where ginger root oil produces a consistent enhancing effect (Segura et al., 2018).

Two hypotheses were proposed to explain how plant-derived volatiles enhance fruit fly male mating success (Segura et al., 2018). The first, referred as 'perfume' effect hypothesis, proposes that males retain the plant-derived volatiles in their cuticle. The retained compound(s) are attractive to the female, and increase male mating probabilities. Under this hypothesis, Shelly et al. (2007) explained why *C. capitata* females were attracted to ginger root oil-exposed dead males and not to non-exposed dead males. The second hypothesis states that males became more active after exposure to plant-derived volatiles because they increase their sexual activity (Shelly, 2001; Papadopoulos et al., 2006; Bachmann et al., 2015). For *A. fraterculus*, evidence obtained so far supports the idea that the enhancing effect is better explained by an increase of sexual signalling, rather than by a 'perfume' effect (Bachmann, 2016; further discussed in Segura et al., 2018). This second hypothesis also explains detrimental effects and increased mortality: if the compounds responsible for this increment in sexual signalling are modulators of the nervous system, exposure above a certain dose may be toxic (Ibrahim et al., 2001). Both limonene and citral have been proven to be toxic for *A. fraterculus* embryo and larvae, with citral being more toxic than limonene (Ruiz et al., 2014). This might explain why the same concentration and exposure times of these two compounds generated opposite results; limonene stimulated males, whereas citral had a detrimental effect. This effect on the nervous system could also explain the differences among oils from lemon (with an enhancing effect), grapefruit (marginal effect), and orange (no effect), as the concentration of limonene was 73, 83, and 96%, respectively. A correlation in the response after exposure to volatiles and the toxicity level of the major compound present in the tested essential oils, might also explain why ginger root oil (with high content of citral) enhances *C. capitata* males and has no effect on *A. fraterculus* males, as citral is less toxic for *C. capitata* immatures than for *A. fraterculus* (Ruiz et al., 2014). However, the positive effect found after limonene exposure does not support this hypothesis, as it was delivered as a pure compound. Thus, it is likely that the enhancing effect of limonene in orange and grapefruit essential oils was masked by some detrimental effect attributed to another compound in these essential oils. More studies are necessary to evaluate the importance of doses and companion compounds in the mixture.

Exposure to volatiles and latency to mate

Exposure to volatiles had no significant effect on latency to mate. This variable has been associated with male reproductive success in *C. capitata* (Ben-Yosef et al., 2008). In the case of *A. fraterculus* Brazilian 1 morphotype, for which the time of sexual activity during the day is narrow and restricted to the early morning (Roriz et al., 2018), the number of sexually receptive females in the field is, in general, low. Therefore, the males chosen first would be those with higher chances to gain several mates in the same day or engage in longer copulations. In our study, the lack of effect of volatiles exposure on latency to mate is in agreement with the results of Vera et al. (2013) who found that exposure to guava volatiles had no effect on latency to mate for *A. fraterculus* laboratory males when evaluated under laboratory conditions, and with those of Bachmann (2016) who found no difference in the time to mate between laboratory males exposed to the volatiles of guava fruit and non-exposed males when mated with wild females, or when wild males, exposed and non-exposed, when mated with wild females. This is in contrast with Vera et al. (2013) who found that wild males exposed to the volatiles of guava fruit mated sooner than non-exposed males. Differences between wild and laboratory flies could be attributed to the fact that laboratory strains are subjected to unintentional selection pressure that may result in flies mating sooner and females mating often, making the effect of exposure to volatiles not evident when evaluated in laboratory flies; yet, it cannot explain the differences between wild flies in the results from Bachmann (2016) and Vera et al. (2013), which differed in the experimental set-up. Other studies that evaluated *A. fraterculus* latency to mate in laboratory and wild flies showed that latency to mate was similar for both fly origins (Vera et al., 2006; Allinghi et al., 2007; Liendo et al., 2013), suggesting there is no evidence of any change in terms of this trait related to the origin of the flies. Therefore, it is possible that the differences found are attributable to the experimental set-up. Unfortunately, by the time we ran the experiment, the infestation levels in the field and the availability of fruit were not sufficient to collect material from the wild in order to compare the effect of essential-oil volatiles and their major compounds in the laboratory and wild flies simultaneously.

Exposure to volatiles and copula duration

Exposure to volatiles also had no significant effect on copula duration. This is in agreement with Vera et al. (2013) for *A. fraterculus* and Flores et al. (2011) for other *Anastrepha* species but contrasts with Bachman et al. (2019), who found that copulations involving males exposed to

guava volatiles lasted longer than those involving non-exposed males. Although there is some controversy about the duration of copula as an indicator of male reproductive success in tephritid fruit flies, the evidence collected so far for *A. fraterculus* indicates that longer copulas correlate with lower tendency to remate and longer refractory period in the female (Abraham et al., 2011) and in higher fecundity (Bachmann et al., 2019). This high fecundity without remating would ensure high fitness to the male and, in the context of the SIT, may imply higher induced sterility in the wild population (Bachmann et al., 2019).

In all, two essential oils and one compound could be identified that enhance male mating success in *A. fraterculus* under laboratory conditions. Further validation under more natural conditions and in situations in which laboratory males compete with wild males for wild females is now required. Given the need to enhance sterile males' mating competitiveness, this finding has important implications for future management of this serious fruit pest. The implementation of pre-release treatments that enhance sterile male mating success allows the optimization of the sterile:fertile ratio in the field, reducing operational costs.

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Figure caption

Figure 1 Percentage of mating achieved by *Anastrepha fraterculus* males exposed to the volatiles of various (A) essential oils or (B) monoterpenes (black bars) vs. non-exposed (white bars) under laboratory conditions. G test of goodness of fit to an equal proportion hypothesis, with Yates correction for continuity: * $0.01 < P < 0.05$, ** $P < 0.01$.

Table 1 Major chemical group and compounds found in the seven essential oils evaluated for their effect on the sexual performance of *Anastrepha fraterculus* males

Chemical group	<i>Citrus limon</i> ¹	<i>Citrus sinensis</i> ¹	<i>Citrus paradisi</i> ¹	<i>Schinus polygama</i> ²	<i>Schinus areira</i> ²	<i>Baccharis spartioides</i> ²	<i>Zingiber officinale</i> ²
Monoterpene hydrocarbons (total)	91.9	97.9	85.2	66.2	65.6	69.1	16.1
	Limonene (72.5) ³	Limonene (95.6)	Limonene (82.7)	α -Thujene (21.0)	α -Phelandrene (18.8)	β -Phelandrene (27.3)	Camphene (6.4)
	γ -Terpinene (9.4)			δ -2-Carene (12.7)	Limonene (9.6)	Sabinene (16.6)	
	β -Pinene (6.1)			Bicyclogermacrene (8.1)	Camphene (9.2)	β -Pinene (12.1)	
				α -Phelandrene (5.5)	β -Phelandrene (7.3)		
					α -Pinene (5.6)		
Monoterpene oxygenated (total)	4.8	0.9	2.1	2.1	2.3	10.8	72.7
							Z-citral (21.5)
							E-citral (30.4)
							1-8 Cineol (10.1)
Sesquiterpene hydrocarbons (total)	1.1	0.1	1.6	23.7	19.1	1.8	2.8
				Byciclogermacrene (8.1)			
Sesquiterpene oxygenated (total)	-	-	-	3.7	7.2	5.8	0.5
Aliphatic hydrocarbons (total)	-	-	0.6	1.2	0.2	1.1	-
Aromatic hydrocarbons (total)	-	-	-	0.1	2.4	-	-
Coumarins (total)	0.3	-	7.4	-	-	-	-
Total	98.6	99.7	99.8	97.9	98.3	95.5	99.2

¹The detailed chemical characterization can be found in Ruiz et al. (2015).

²The detailed chemical characterization can be found in Jofré-Barud (2018).

³Values represent % concentration of components of the total chemical profile. Only components with 5% or higher are listed.

Table 2 Number of matings achieved by *Anastrepha fraterculus* males exposed or non-exposed to seven essential oils or two compounds

EO source / compound	Exposed	Non-exposed	χ^2 (d.f. = 1) ¹	P
<i>Citrus limon</i> (lemon)	63	29	11.93	<0.001
<i>Citrus sinensis</i> (orange)	70	63	0.27	0.21
<i>Citrus paradisi</i> (grapefruit)	77	55	3.36	0.067
<i>Schinus polygama</i>	79	46	8.28	0.004
<i>Schinus areira</i>	72	56	1.76	0.18
<i>Baccharis spartioides</i>	64	46	2.64	0.10
<i>Zingiber officinale</i> (ginger)	44	38	0.31	0.58
Limonene	69	37	9.20	0.002
Citral	54	84	6.14	0.013

¹ Based on G test of goodness of fit, with Yates correction.

Table 3 Latency to mating (min) between exposed and non-exposed *Anastrepha fraterculus* laboratory males under laboratory conditions for the essential oils (EO) or monoterpenes evaluated in the laboratory mating test

EO source / compound	Exposed		Non-exposed		W ²	P
	Median	(Q1-Q3, n) ¹	Median	(Q1-Q3, n)		
<i>Citrus limon</i> (lemon)	12	(7-17, 82)	15	(7-20, 42)	2829	0.28
<i>Citrus sinensis</i> (orange)	7	(5-15, 70)	5	(5-15, 63)	3963	0.24
<i>Citrus paradisi</i> (grapefruit)	15	(3-25, 77)	15	(5-35, 55)	3803	0.50
<i>Schinus polygama</i>	15	(5-25, 79)	10	(5-20, 46)	2542	0.065
<i>Schinus areira</i>	20	(10-30, 72)	25	(15-35, 56)	3977	0.079
<i>Baccharis spartioides</i>	15	(5-20, 64)	15	(5-20, 46)	2577	0.88
<i>Zingiber officinale</i> (ginger)	10	(10-20, 64)	10	(10-20, 38)	1576	0.99
Limonene	18	(10-37, 69)	15	(10-40, 37)	2057	0.60
Citral	15	(10-30, 55)	15	(5-37, 83)	3967	0.53

¹Q1, first quartile; Q3, third quartile; n, number of couples.

²Wilcoxon paired test.

Table 4 Copula duration (min) between exposed and non-exposed laboratory *Anastrepha fraterculus* males under laboratory conditions for the essential oils (EO) or monoterpenes evaluated in the laboratory mating test

EO source / compounds	Exposed		Non-exposed		W ²	P
	Median	(Q1-Q3, n) ¹	Median	(Q1-Q3, n)		
<i>Citrus limon</i> (lemon)	50.5	(43-67, 82)	50	(31-60, 42)	2399	0.23
<i>Citrus sinensis</i> (orange)	54.5	(35-64, 70)	57	(40-69, 63)	4360	0.53
<i>Citrus paradisi</i> (grapefruit)	50	(40-65, 77)	55	(40-72, 55)	3808.5	0.49
<i>Schinus polygama</i>	70	(60-85, 79)	65	(55-80, 46)	2652.5	0.21
<i>Schinus areira</i>	50	(35-65, 72)	55	(45-70, 56)	3950.5	0.10
<i>Baccharis spartioides</i>	65	(50-75, 64)	70	(60-80, 46)	2655.5	0.53
<i>Zingiber officinale</i> (ginger)	67.5	(55-80, 44)	65	(60-80, 38)	1613.5	0.73
Limonene	50	(40-64, 69)	46	(35-63, 37)	1875	0.49
Citral	53	(37-63, 55)	50	(40-63, 83)	3803	0.93

¹Q1 = first quartile; Q3 = third quartile; n = number of couples

²Wilcoxon paired test

