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THE EFFECT OF GINGER OIL ON THE SEXUAL PERFORMANCE OF *ANASTREPHA* MALES (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Aromatherapy treatment using ginger oil has been shown to be effective in increasing the sexual competitiveness of males of the Mediterranean fruit fly, *Ceratits capitata* (Wiedemann) and can partially remedy the detrimental effects associated with the mass-rearing and irradiation processes that are used in Sterile Insect Technique (SIT) programs. In this study, we evaluated the effect of ginger oil treatment on the sexual performance of males of 3 *Anastrepha* fruit fly species of economic importance (*A. ludens* (Loew), *A. obliqua* (Macquart), and *A. serpentina* (Wiedemann)) when they were at 6, 9, and 12 d old. The experiments were carried out under semi-natural conditions, and the number and duration of copulations achieved by each type of male were determined. Our results indicate that the male response to ginger oil exposure was different for each of the evaluated species. For *A. ludens,* we observed a detrimental effect on the number of copulations obtained by 6 d-old males but not by 9 or 12 d-old males. For *A. obliqua*, treatment with ginger oil did not significantly affect the evaluated parameters at any age. For treated *A. serpentina* males, 9 and 12 d-old males showed greater sexual performance when compared to untreated males. Copula duration was not affected by ginger oil treatment in any of the species. We determined that aromatherapy using ginger oil does not have the same beneficial effect in *A. ludens* and *A. obliqua* as it does in *C. capitata*, but in *A. serpentina*, the use of this product offers a great potential and might render important benefits in SIT applications.

Key Words: tropical fruit flies, sexual behavior, aromatherapy, mating competitiveness

RESUMEN

El tratamiento de aromaterapia con aceite de jengibre ha demostrado ser una opción eficaz para incrementar la competitividad sexual de los machos de la mosca del Mediterráneo *Ceratits capitata* (Wied), el cual puede remediar parcialmente los efectos negativos asociados a los procesos de cría masiva e irradiación de los insectos que se utilizan en programas con la Técnica del Insecto Estéril (TIE). En este estudio evaluamos el efecto del tratamiento con aceite de jengibre en el desempeño sexual de los machos de tres especies de moscas de la fruta del género *Anastrepha* importantes económicamente (*A. ludens* (Loew), *A. obliqua* McQuart y *A. serpentina* (Wied.)), en las edades de 6, 9 y 12 días. Los experimentos se llevaron a cabo bajo condiciones seminaturales donde se determinó el número y la duración de las cópulas alcanzadas por cada tipo de machos. Nuestros resultados indican que la respuesta de los machos a la exposición de aceite de jengibre fue diferente en cada una de las especies evaluadas. En *A. ludens* se observó un efecto detrimental en el número de cópulas alcanzado por los machos de 6 días de edad, pero no así en las edades de 9 y 12 días donde los machos tratados no mostraron diferencias con el testigo. En *A. obliqua*, el tratamiento con aceite de jengibre no presentó efecto significativo en ninguno de los parámetros evaluados. En *A. serpentina*, los machos tratados en las edades de 9 y 12 días de edad mostraron un mejor desempeño sexual en comparación con los no tratados. El tratamiento con aceite de jengibre no afectó la duración de la cópula en ninguna de las especies evaluadas. Se determinó que la aromaterapia con aceite de jengibre no tiene el mismo efecto benéfico en *A. ludens* y *A. obliqua* que en *C. capitata*; pero en *A. serpentina* el uso de este producto ofrece un gran potencial y puede tener importantes beneficios en la aplicación de la TIE.

Translation provided by authors.

The Sterile Insect Technique (SIT) requires artificially reared male insects after being released into the field to be successful in competing for female mates with wild males (Robinson et al. 2002). This technique has proven to be effective against fruit flies (Enkerlin 2005), the screwworm, *Cochliomyia hominivorax* (Coquerel) (Vargas-Terán et al. 2005), a few lepidopteran pests (Bloem et al. 2005; Blomefield et al. 2010), and tsetse flies (Feldman et al. 2005). However, the maintenance of breeding colonies under mass-rearing conditions tended to reduce the mating performance of sterile males (Pereira et al. 2007), which was reflected by low acceptance of these males by wild females in the field (Lance et al. 2000).

Different strategies have been proposed to enhance the sexual performance of sterile males.

For example, the use of ginger oil has been successful in increasing the sexual competitiveness of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), males (Shelly et al. 2002; 2003). This simple and inexpensive strategy (Shelly et al. 2002; Shelly et al. 2006a, b) results in changes in male behavior or in male pheromone production or release, which enhances the sexual competitiveness of sterile males (Briceño et al. 2007; Barry et al. 2003; Shelly & McInnis 2001a) and does not cause any negative effects on the survival or re-mating time of females (Shelly et al. 2002; 2004a; Morelli et al. 2010).

Ginger oil can feasibly be used in packing and emergence facilities prior to the release of sterile *C. capitata*, regardless of whether PARC boxes (Shelly et al. 2004b) or screens that are arranged in emergence towers (Shelly et al. 2006a) are used as the packing units. In addition, field studies have indicated that the release of *C. capitata* males that had been previously treated with ginger oil led to a significant increase of sterile eggs in the treated plot compared to the control (Shelly et al. 2007a).

Technologies developed for a particular species of fruit fly sometimes have the potential to be used for other species. For instance, the effects of a juvenile hormone analogue (methoprene), which accelerated the sexual maturation of *Anastrepha suspensa* (Loew), (Teal et al. 2000), have been successfully demonstrated in other species of *Anastrepha* and in *Bactrocera cucurbitae* (Coquillett) (Teal et al. 2007; Haq et al. 2010).

In this study, we aimed to determine whether ginger oil has a beneficial effect on the sexual performance of males of 3 economically important species of the genus *Anastrepha*: (i) the Mexican fruit fly, *A. ludens* (Loew), which is considered to be the major pest of citrus fruits in Mexico and several countries of Central America; (ii) the West Indian fruit fly, *A. obliqua* (Macquart), which attacks mango fruits in commercial orchards that are mainly situated at lower altitudes (Hernández-Ortiz & Aluja 1993); and (iii) *A. serpentina* (Wiedemann), a tropical fruit fly that is the major pest of Zapotaceae fruits from the lower Rio Grande valley of Texas to Brazil (Aluja et al. 1996; Hernández-Ortiz & Aluja 1993).

Materials and Methods

Biological Material

Fertile pupae of *A. ludens* and *A. obliqua* were obtained from the Moscafrut mass-rearing facility, and the pupae of *A. serpentina* were obtained from the Methods Development Unit. Flies were reared according to standard protocols (Domínguez et al. 2010; Artiaga-López et al. 2004; Pinson et al. 2006), and pupae from each species were kept in glass cages (30 × 30 × 30 cm) for emergence and sexual maturation. Two days after emergence, males and females were placed in separate cages with water and food (3:1 sugar: hydrolyzed protein) and were maintained in laboratory conditions of 26 ± 1 °C and 70 ± 5% RH before testing in field conditions.

Study Area

Tests were performed under semi-natural conditions in the gardens of the Moscafrut facility (SAGARPA-IICA), which is located in Metapa de Dominguez, Chiapas, Mexico. Five charcoal-colored field cages 3 m diam × 2 m high made of fiberglass insect screening (Saint Gobain Technical Fabrics, Valley Forge, Pennsylvania, USA) were installed under shade trees. Both mango and orange trees were placed inside each cage to create a natural setting, and the environmental conditions during the evaluations ranged from 28 to 34 °C and 60-80% RH.

Treatments

The effects of ginger oil treatments were evaluated on males of 3 different ages (6, 9, and 12 d-old) of each species. Using the same glass cages as described above, we divided the fruit flies into groups of 50 males by age and species and replicated each treatment ten times. Each replicate consisted of 50 treated males, 50 non-treated males (control), and 50 mature females, which were all released into a field cage. In total, we used 1,500 treated males (500 per age category), 1,500 control males and 1,500 females (a total of 4,500 flies) for each species. The ages of the treated males were established according to their reported sexual maturity and release age (7-d-old) of sterile flies (Hernández et al. 2010). Mass-reared *A. ludens* reached sexual maturity at 8 d (Dominguez et al. 2010), whereas *A. obliqua* and *A. serpentina* achieved sexual maturity at 6 d (Meza-Hernández et al. 2004; Martínez et al. 1995). The ages of the females of all species ranged from 11-12 d.

Ginger Oil Exposure

Treated males were exposed to ginger oil for 24 h before testing. A 9 cm^2 piece of filter paper that had been immersed in 2 mL of ginger oil (Citrus and Allied Essences Ltd., Lake Success, New York), following the recommended dose of 36 mL per trailer of 132 m^3 (0.27 mL/m^3 (Shelly et al. 2008b), was placed in a Petri dish lid inside a room of 1.60 × 1.50 × 3.25 m (0.26 mL/m^3). Untreated males and females were kept in a different room but under the same environmental conditions of 26 ± 1 °C and 70 ± 5% R. H., with a photoperiod of 14:10 (L: D). To differentiate between treatments during the tests, the untreated group

of flies was given food mixed with McCormick® green food coloring (McCormick & Company, Inc., Sparks, Maryland, USA) (Arevalo et al. 2009), which formed a colored mark in the digestive tract of those flies that could be seen through the abdomen. This marking method was alternated between treatments and control flies to avoid any possible bias.

Field Cage Tests

Anastrepha obliqua males (50 treated and 50 control flies) were released at 0500 h, and females (50) were released 15 min later. Flies were observed continuously and each couple was caught and placed in a glass vial (5 mL) where they were held until the end of mating. The times of onset and termination of mating were recorded. Observations were ended at 1000 h, which was the end of the major mating period. *A. ludens* and *A. serpentina* mate at twilight, therefore the release of the males of these species (50 treated and 50 controls) was performed at 1600 h, and the females were released 15 min later. Observations were made as above, and ended after sunset. For each species and age, we determined the number of matings initiated per 10 min time period, total number of matings per observation period and the duration of each mating.

Data Analysis

We used separate paired *t*-tests to compare the average number of copulations per cage and the average duration of mating for each species at each age. The Bartlett's test for unequal variances showed homogeneity ($P < 0.05$), so data were not transformed (Zar 1999). Distribution of number of matings initiated per 10 min period were compared for treated and untreated males by the log-rank test using JMP 5.0.1.2 software (SAS Institute Inc. 2003).

Results

The effect of ginger oil on mating was different for each of the evaluated species. For *A. ludens*, 6 d old control males had significantly more copulations than treated males ($t = 9.65$, df = 9, $P < 0.001$), while there were no significant differences for 9 and 12 d old flies ($t < 1.90$, df = 9, $P > 0.083$, Table 1). For *A. obliqua,* the results showed no significant differences for 6 d old ($t = 1.62$, df = 9, $P = 0.139$), 9 d old ($t = 1.83$, df = 9, $P = 0.101$) or 12 d old ($t = 0.32$, df = 9, $P = 0.755$) flies. For *A. serpentina*, 6 d-old treated and control males achieved a similar number of copulations ($t = 1.56$, df = 9, $P = 0.152$); however, at 9 d and older, the ginger-treated males copulated significantly more than the control males ($t > 6.5$, df = 9, $P < 0.001$) (Table 1).

The duration of copulations ranged from 27 to 43 min for *A. obliqua*, from 40 to 55 min for *A. ludens,* from 23 to 30 min for *A. serpentina.* No differences between treatments were observed for the durations of copulations in any fruit fly species (Table 2).

The distribution of copulations (Fig. 1) during the period of sexual activity for *A. obliqua* was not different between couples formed by females and control males or treated males for the 3 ages tested ($\chi^2 < 0.71$, df = 1, $P > 0.401$). For *A. ludens,* a significant difference was observed in the 6 d old group (the activity began 40 min later in treated males compared to control males ($\chi^2 = 9.49$, df = 1, $P = 0.002$) but not in the 9 d old group ($\chi^2 = 1.13$, df = 1, $P = 0.287$) or 12 d old group ($\chi^2 = 1.38$, df = 1, $P = 0.239$). For *A. serpentina*, there was no difference in the distribution of copulas through time for any of the tested ages ($\chi^2 < 2.00$, df = 1, $P > 0.157$) (Fig. 1).

Discussion

In this study, we found that ginger oil exposure had different effects on male mating in the 3 species studied. Our results showed that 6 d old treated males of *A. ludens* achieved a smaller number of copulations than untreated males. Ginger oil was predicted to have either a beneficial effect as it does on *C. capitata* (Shelly et al. 2001a; 2002; 2003; 2006a) or no effect (Shelly et al. 2008a), but a detrimental effect was not predicted to occur. To our knowledge, this is the first

Table 1. Number of copulations (average ± SE) achieved by ginger oil-treated and untreated males of *Anastrepha obliqua, A. ludens* and *A. serpentina* that were 6, 9 and 12 days old.-

	6 d		9 d		12 d	
	Treated	Control	Treated	Control	Treated	Control
A. obliqua	15.1 ± 1.7	13.0 ± 0.9 ns	18.6 ± 1.2	14.6 ± 1.5 ns	11.5 ± 2.3	10.4 ± 1.7 ns
A. ludens	9.0 ± 0.8	22.9 ± 1.4*	18.0 ± 1.4	15.2 ± 1.1 ns	14.6 ± 1.6	19.7 ± 1.2 ns
A. serpentina	6.0 ± 1.3	7.9 ± 1.2 ns	10.2 ± 1.1	3.3 ± 0.5 *	10.4 ± 1.4	1.7 ± 0.6 *

ns: no significant difference ($P > 0.05$).
*significant difference ($P < 0.05$) between treated and control flies at each age of each species.

TABLE 2. DURATION OF COPULATION IN MINUTES (AVERAGE ± SE) OF GINGER OIL TREATED AND UNTREATED *A. OBLIQUA, A. LUDENS* AND *A. SERPENTINA* MALES THAT WERE 6, 9 AND 12 DAYS OLD.

	6 d		9 d		12 d	
	Treated	Control	Treated	Control	Treated	Control
A. obliqua	39.7 ± 2.5	42.9 ± 3.3	34.7 ± 4.6	31.50 ± 3.1	29.3 ± 3.1	29.4 ± 2.9
A. ludens	40.3 ± 1.7	44.1 ± 3.8	57.9 ± 4.7	53.72 ± 4.9	52.8 ± 4.2	54.1 ± 4.4
A. serpentina	27.6 ± 3.7	29.9 ± 1.2	29.2 ± 1.2	27.80 ± 2.0	23.4 ± 1.9	25.0 ± 2.8

No significant differences were found between treated and control flies at any age of any species ($P > 0.05$).

report of a negative effect of ginger oil on the sexual performance of male fruit flies, and the reasons for this occurrence are still unclear. Some studies have tried to explain the chemical, physiological or behavioral factors responsible for the enhanced mating for *C. capitata* males exposed to ginger oil (Papadopoulos et al. 2006; Shelly et al. 2007b). The complete explanation remains elusive, but some evidence seems to indicate that ginger oil interacts with cuticular odors to produce a scent that was attractive to females. We hypothesized that the opposite may be happening in 6 d-old *A. ludens* males, i.e., the development of non-beneficial odors with the consequence being a diminished number of copulations. Related to this, Vera et al. (2010) reported that exposure of *A. fraterculus* males to essential oils of lemon, *Citrus limon* (L.), for 1 d decreased mating success

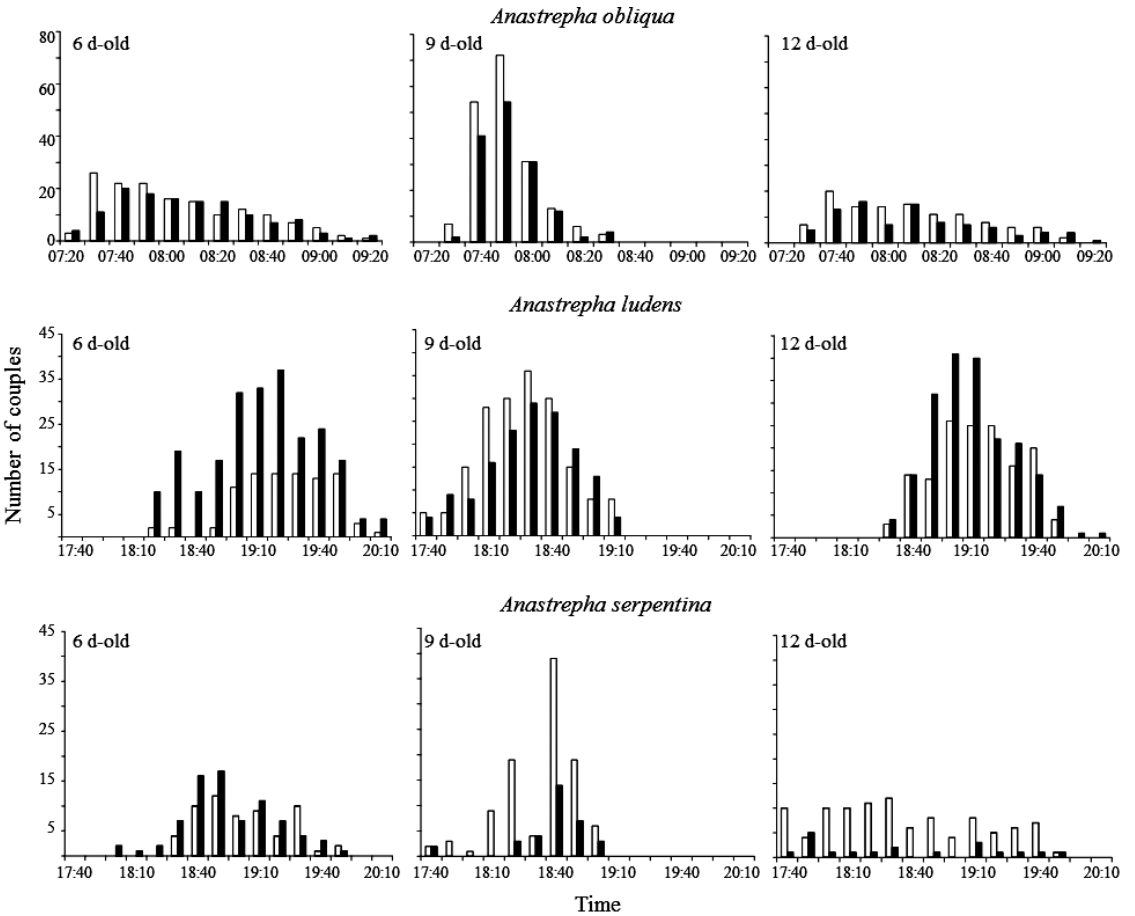

Fig. 1. Distribution of matings during the period of sexual activity of control males (■) and males treated with ginger oil (□) of *Anastrepha obliqua*, *A. ludens* and *A. serpentina* under semi-natural conditions.

relative to unexposed males, although this effect was not apparent following longer exposure periods. The reason for these findings was also not clear, but the authors discussed the possibility that some compounds of citrus oil could be toxic to adult fruit flies and could predominantly affect those moribund or less vigorous males. In addition, we observed that 6 d old *A. ludens* males that had been exposed to ginger oil were affected negatively, and the onset of copulation of these flies was delayed. This finding was contrary to what had been reported for *C. capitata* (Shelly et al. 2003), whose period of latency was not affected by exposure to ginger oil. Following previous statements in respect to the interaction between ginger oil and cuticular odors, we hypothesize that the formation of copulations could be restarted once the non-beneficial odors in *A. ludens* 6 d-old males has dissipated.

A. serpentina males showed partial benefits of ginger oil exposure. Ginger oil exposure had a positive effect on the number of copulations obtained by 9 and 12 d old males. Shelly and McInnis (2001a) showed that 1 to 3 d old *C. capitata* males responded similarly when exposed to ginger oil, achieving more copulations than unexposed males. Sexual maturity of a male *A. serpentina* is reached at 6 d (Martinez et al. 1995), which was the precise age at which we observed no effect of ginger oil exposure on sexual competitiveness. This could simply indicate that the males were not physiologically ready for mating, considering that the period for sexual maturing was spent in an environment of 26 °C. However, under laboratory conditions (26-28 °C), *A. serpentina* males start calling at 5 d and reach a peak of sexual activity at 8-9 d (Castrejon-Gomez et al. 2007). In *A. obliqua,* ginger exposure did not affect, either positively or negatively, the mating capacity of 6, 9, or 12 d old males, and again, we do not have a clear reason for this observation. However, following the hypothesis that different effects are derived from the interaction between ginger oil and cuticular odors (depending of the fruit fly species involved), we suggest that in the case of *A. obliqua* males, the ginger oil did not cause any change in cuticular scent that affected behavior of males or females.

It is known that in several insect pests, host plant chemicals may influence developmental rates, the progress of maturation, as well as the pheromone composition of the insects, all leading to reproductive success (Landolt & Phillips 1997; Papadopoulos et al. 2008). To explain the significance of these phytochemicals on insect responses, 2 hypotheses (the ancestral host hypothesis and sexual selection by female choice hypothesis) have been invoked, and these hypotheses are not necessarily logical alternatives to one another (Raghu 2004). The work from Vera et al. (2010) showed that exposure of *A. fraterculus* males to guava (their primary host) volatiles resulted in a clear mating advantage to exposed males without requiring physical contact or ingestion of fruit. Because our tested males exhibited different, species-dependent responses to ginger oil exposure and because they were not confronted with volatiles from their primary hosts, it was not easy to determine the most feasible hypothesis to explain our results. This scenario led us to suppose that a variation of an intrinsic physiological characteristic (possibly a chemical receptor) could be responsible for the differential effect of ginger oil that was observed in the 3 *Anastrepha* species studied.

In contrast, the duration of copulation was not affected by exposure to ginger in any of the fruit fly species studied. This coincided with the findings by Shelly et al. (2003), which demonstrated that treatment with ginger did not significantly affect the duration of copulation in male *C. capitata*. Shelly & Kennelly (2002) also found that the type of diet that was given to *C. capitata* males did not affect the duration of copula in this species. However, environmental factors such as land elevation (Shelly et al. 2003) and food quality (Perez-Staples & Aluja 2004) were reported to affect copula duration in *C. capitata* and *A. striata* males, respectively.

Data showing the positive effects of ginger oil on *C. capitata* sexual performance are abundant and cover different scenarios related to the packing and field release procedures of sterile insects (e.g., McInnis et al. 2010; Paranhos et al. 2010), as well as key parameters for evaluation of mating competitiveness (Shelly 2001a, b; Shelly & McInnis 2001a, b; Shelly et al. 2002, 2003, 2005, 2006a). However, our data from testing *Anastrepha* spp. led us to different scenarios and conclusions for each evaluated species, as follows: 1) Ginger oil had no effect on the sexual performance *A. obliqua* males. 2) Ginger oil reduced the number of matings achieved by 6 d old *A. ludens* males, and did not improve the sexual performance of 9 and 12 d old males. The mechanisms underlying both findings are unknown and should be addressed in future studies. It is known that *A. ludens* and *A. obliqua* belong to the same *fraterculus* intrageneric group, are polyphagous and share some hosts, such as mangoes (Norrbom & Kim 1988; Hernandez 1992), which could explain the similar effects observed and suggests that for both species it is necessary to evaluate new alternatives related to essences of their host fruits. Indeed this was done for *A. fraterculus* males (Vera et al. 2010), where exposure to volatiles of guava increased its sexual competitiveness. 3) For *A. serpentina*, the use of ginger oil might be a helpful when applying the SIT technique to this species, as all of the benefits of ginger oil previously reported for sterile males of Mediterranean fruit flies appear to be true for *A. serpentina* males as well.

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