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Enhancing mating performance after juvenile hormone treatment in *Anastrepha fraterculus*: a differential response in males and females acts as a physiological sexing system

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Abstract

Methoprene (a mimic of juvenile hormone) treatment can reduce the time required for sexual maturation in *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) males under laboratory conditions, supporting its use as a treatment for sterile males within the context of the sterile insect technique (SIT). We evaluated sexual behaviour, mating competitiveness of methoprene-treated males, and female readiness to mate after methoprene-treatment in field cages. The study involved two strains of *A. fraterculus* from Argentina and Peru, which show several polymorphisms in relation to their sexual behaviour. We also analyzed whether methoprene treatment affected male and/or female behaviour in the same way in these two strains. Methoprene-treated males were equally competitive with untreated mature males, and became sexually competitive 6 days after emergence (3–4 days earlier than untreated males). In contrast, methoprene did not induce sexual maturation in females or, at least, it did not induce a higher rate of mating in 7-day-old females. These results were observed both for the Argentina and the Peru strains. Altogether, our results indicate that methoprene treatment produces sexually competitive males in field cages. In the absence of a genetic sexing system, and when sterile males and females of *A. fraterculus* are released simultaneously, the fact that females do not respond as do males to the methoprene treatment acts as a physiological sexing effect. Therefore, in the presence of mainly sexually immature sterile females, released sexually mature sterile males would have to disperse in search of wild fertile females, thereby greatly reducing matings among the released sterile insects and thus enhancing sterile insect technique efficiency.

Introduction

The South American fruit fly, *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae), is a major fruit pest in several South American countries. It has a wide distribution ranging from southern USA to central Argentina (Salles, 1995; IAEA, 1999; Steck, 1999). It is a polyphagous species that attacks more than 80 host species (Norrbom, 2004), many of them, such as peach, guava, plum, mango, and apple, are of high commercial value.

Increased pressure to reduce the use of insecticides (currently the only method available to control this pest) has prompted the development of environmentally friendly methods, such as the use of natural enemies and the sterile insect technique (SIT) (Ortíz, 1999; Ovruski et al., 1999). In support of future SIT programmes, baseline data have been collected on mass rearing and quality control (Jaldo et al., 2001; Vera et al., 2007), radiation biology (Allinghi

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et al., 2007a,b), and male survival (Gómez Cendra et al., 2007). Key aspects of the sexual behaviour have also been addressed. Malavasi et al. (1983) and Segura et al. (2007) found that *A. fraterculus*, as other *Anastrepha* species, employs a lek mating system, where females evaluate males within a lek on the basis of morphological characters (Sciurano et al., 2007) and probably also behavioural displays. Mating compatibility among different populations, both within Argentina (Petit-Marty et al., 2004) and elsewhere in South America (Vera et al., 2006), has also been evaluated.

One difficulty in using the SIT to control this species is the long precopulatory period of adult males. As for many other *Anastrepha* species, sexual maturation is a slow process in *A. fraterculus*, and sterile flies must be maintained at the fly handling facilities for several days before their release, increasing operational costs considerably (food, space, and staff) (Enkerlin, 2007). Holding adults may also lead to physical damage to the flies (Teal & Gómez-Simuta, 2002), sometimes forcing the release of sexually immature flies, which are not able to compete with wild males.

Sexual maturation in males is hormonally coordinated in insects (Happ, 1992). Juvenile hormone (JH), a sesquiterpenoid, plays a major role during reproduction in many, and perhaps all, insects (Wyatt & Davey, 1996; Gilbert et al., 2000; Wilson et al., 2003). Although the role of the JH is better understood in females than in males, a clear role for this hormone during male sexual maturation has been reported in a variety of insects. Maturation of male accessory glands has been the main function reported for JH (Happ, 1992; Wilson et al., 2003 and references therein). In tephritid flies, Teal et al. (2000) showed that topical application of JH III, or the synthetic mimics methoprene or fenoxycarb, to newly emerged males accelerates sexual maturation, sex pheromone release, and mating of *Anastrepha suspensa* (Loew). Moreover, this treatment significantly improved male participation in leks and sexual success (Pereira, 2005). Sexual signalling in males is tightly coordinated with reproductive maturity (Nation, 1972, 1974, 1990), so JH analogues were probably stimulating development of the male reproductive system as in other insects.

Improvement in mating performance after methoprene treatment seems to be a widespread phenomenon within the Tephritidae, as similar results have been reported for *Anastrepha ludens* (Loew) and *Anastrepha obliqua* (Macquart) (Teal et al., 2007), *Bactrocera tryoni* (Froggat) (Smallridge et al., 2006), and *Bactrocera cucurbitae* (Coquillett) (I Haq, C Cáceres, AS Robinson, J Hendrichs & C Stauffer, unpubl.). In *A. fraterculus* males, Segura et al. (2006) found a reduction in the mean time of maturation from 7 to 4 days during laboratory studies on pheromone release and mating activity. However, no studies have described the sexual behaviour of methoprene-treated males in a more natural setting. The use of methoprene has been proposed as a tool that could make SIT more efficient, as treated males will reach sexual maturity earlier and so can be released without long holding times (Teal et al., 2007). In order to validate the potential use of this approach, studies are needed that assess the mating competitiveness of methoprene-treated males. For success of SIT, sterile males not only have to survive and reach sexual maturity but also to compete in the field with wild males for wild females.

There have been no evaluations of the effect of methoprene on *A. fraterculus* females. As there is no genetic sexing strain (GSS, genetically modified strains that allow sex differentiation in immature stages and the release of sterile male flies only) for this species, both males and females would be treated with methoprene prior to field release. It has been postulated that vitellogenesis, the process of yolk protein synthesis and oocyte uptake, is regulated both by JH and ecdysteroids (Gruntenko et al., 2005). Given that ovarian maturation is temporally coordinated with female receptivity (Cusson & McNeil, 1989; Gadenne, 1993; Cusson et al., 1994; Picimbon et al., 1995), methoprene treatment could potentially accelerate the readiness of sterile females to mate. However, the influence of JH on the onset of female receptivity varies among species (Ringo, 2002), and Richard et al. (1998, 2001) and Gruntenko et al. (2005) proposed that an ecdysteroid plays a major role in the control of oogenesis, while JH acts during the initial stage of vitellogenesis.

If JH alone accelerates sexual maturation in *A. fraterculus* females, then there is a chance that these sterile females will mate with the sterile males, reducing the chances that the sterile males attract and mate with wild fertile females. However, if methoprene does not accelerate maturation in females but does so in males, then this physiological treatment could produce an effect similar to that of a GSS that is, sterile males would be ready to mate while sterile females (although treated) would be immature and therefore will not interfere with those males (as the females would not respond to pheromone released by the males). In addition, the relatively high mortality of sterile flies in the field (Hendrichs et al., 1993; Gómez Cendra et al., 2007) would eliminate a majority of the sterile females before they reach full maturity. Under this assumption, the overall result would be an increase in the number of sexually mature sterile males available to mate with wild females.

In agreement with previous reports on the high levels of phenotypic variability found among different populations of *A. fraterculus* (reviewed in Steck, 1999), Vera et al. (2006) reported a high degree of pre-zygotic isolation among

several strains from South America, especially between flies from Argentina and Peru. Tests carried out to re-examine the degree of isolation (Cáceres et al. in press) showed that the sexual isolation was related to differences in the time of sexual activity, male sexual pheromone composition, and male location during leking. The considerable differences in sexual behaviour between flies from these two strains could also involve differences in the sexual maturation process. Therefore, assessment of JH treatment should be carried out in parallel to analyse its effectiveness in these two strains.

The objectives of the present study on *A. fraterculus* strains from Argentina and Peru, were (1) to evaluate the sexual behaviour and competitiveness of methoprene-treated males in field cages by comparing the sexual performance of young treated males to that of already mature untreated males, and then determining the minimum age at which treated males become sexually competitive, and (2) to examine the effect of methoprene treatment on the process of female sexual maturation.

Materials and methods

Insects

Two strains of *A. fraterculus* from Argentina and Peru were obtained in 2004 and have been reared at the FAO/IAEA Laboratories at Seibersdorf, Austria, on artificial diet. Both strains were maintained under the same rearing conditions (25 ± 1 °C, 60 ± 10% r.h., and L14:D10 photoperiod).

Methoprene treatment

Pupae were placed in emergence cages and within the first 3 h of emergence, 1 µl of a 5 µg µl^{-1} solution of methoprene (11-methoxy-3,7,11-trimethyl-2E,4E-dodecadienoate) dissolved in acetone was applied to the thorax (following procedures in Teal et al., 2000). After treatment, flies were placed in laboratory cages (ca. 20 l) with adult food (3:1 sugar:hydrolyzed yeast) and vials containing water. Treated males and females were kept in separate cages. Untreated flies of the same batches were also sorted by sex and placed in similar cages. Untreated males and females were tested at sexual maturity, at 10 and 14 days of age (Jaldo, 2001).

Field cages procedure

All experiments were performed in field cages at the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria, following standard procedures (FAO/IAEA/USDA, 2003). This involved the release of two types of males (i.e., treated and untreated, or males from two different strains) plus one (unisexual test) or two (bisexual test) types of females inside a cage (4 m^2 base and 1.8 m in height). In each cage, one *Citrus sinensis* Osbeck (Rutaceae) (L.) tree (1.7 m high with a canopy of about 1.5 m in diameter) provided an arena for resting and mating activities of the flies. Tests were performed in a greenhouse with controlled minimum temperature (>18 °C) and relative humidity (60–80%).

For identification, flies were marked with a dot of water-based paint on the thorax 48 h before the test. We used a different, randomly assigned colour for each kind of male and female. This procedure does not affect sexual performance of *A. fraterculus* (Petit-Marty et al., 2004).

The occurrence of mating pairs was observed during the experiment. For each mating pair, the type of male and female, the location on the tree, the time at which it was detected, and the duration of mating were recorded. Mating duration was determined by transferring the mating pair gently into a vial and checking every 10 min until the flies separated.

Experiments

Competitiveness of 7-day-old treated males. Twenty-five methoprene-treated 7-day-old males, 25 untreated 10-day-old males, and 25 untreated 14-day-old females were released in a field cage. A control test was run in parallel, in which the number and type of flies released were the same as above, but the 7-day-old males were untreated. This experiment was conducted separately with flies from the Argentina and the Peru strains. Each cage test was considered as a replicate, and eight replicates were conducted for each strain.

Minimum age at which males become competitive. Based on the sexual maturation curve of *A. fraterculus* in the laboratory (Segura et al., 2006), treated males might be able to compete with mature untreated males even when younger than 7 days old. In the field cage, this was assessed by releasing 5-, 6-, or 7-day-old treated males and untreated 10-day-old males. Twenty-five males of each type and 25 mature females were released in each cage. Eight replicates were performed for each age and strain.

Effect of methoprene treatment on females. In order to assess if methoprene treatment also accelerates female sexual maturation, 25 treated females and males (7 days old) were released inside a field cage along with 25 mature untreated males (10 days old) and 25 mature untreated females (14 days old). For the control, 7-day-old treated females were replaced with 7-day-old untreated females. Six replicates were performed for each age and strain.

Data analysis

Two indices that describe the competitiveness of both females and males, female and male relative performance

indexes (FRPI and MRPI, respectively) were calculated (Cayol et al., 1999). The significance of the FRPI and MRPI was evaluated using a χ^2-test of goodness of fit, assuming equal performance for both types of males/females. Mean FRPIs or MRPIs were compared between treatments and strains by means of a two-way analysis of variance (ANOVA).

The effect of methoprene on the general sexual behaviour of treated males and females was evaluated by the time at which mating started, mating duration, and mating location. Mating start time (referred to as latency) was calculated as the time elapsed from the release of the females to the beginning of a given mating. Mating duration was determined as the time the mating pair disengaged minus the time they started to mate. For experiments 1 and 2, the location of the mating pairs was also analyzed, as this variable reflects the location chosen by the males to release sexual pheromone (Segura et al., 2007). The location was described in terms of the tree canopy (upper, middle, and low), and the quadrant (northern, western, southern, and eastern). Differences in latency and mating duration were evaluated by means of a one-way ANOVA [or a non-parametric alternative – Kruskal–Wallis or Mann–Whitney test – if the heteroscedasticity was mild or preceded by data transformation if the heteroscedasticity was severe (Zar, 1996)]. To analyze differences in the location, the distribution of mating pairs was compared by a χ^2-test of heterogeneity. All analyses were performed with STATISTICA for Windows (StatSoft, 2000).

Results

Competitiveness of 7-day-old treated males

When 7-day-old untreated males from the Argentina strain were caged with 10-day-old males, they obtained only 30% of the matings. Following treatment, they obtained 50% of the matings (Figure 1A). The mean MRPIs (Table 1) showed that when 7-day-old males were treated with methoprene they were as competitive as 10-day-old untreated males. A χ^2 goodness of fit test showed in all cases that there were no differences with mature males when the young males were treated, but significant differences appeared when the young males were not treated (Table 1). When 7-day-old males from the Peru strain were treated with methoprene, they obtained a similar percentage of matings as 10-day-old males, but this percentage was reduced when 7-day-old males were not treated (Figure 1B). The mean MRPI for the treated males of both strains was close to zero (Table 1), and in all the replicates χ^2-tests revealed no significant differences. When males were not treated, statistical differences were detected (although individual χ^2-tests showed no differences in three out of eight replicates). A two-way ANOVA detected significant differences between the MRPIs of treated and untreated 7-day-old males, but no effect of the strain was found (ANOVA; strain: $F_{1,28} = 0.129$, $P = 0.723$; treatment: $F_{1,28} = 52.947$, $P<0.001$; interaction: $F_{1,28} = 3.67$, $P = 0.066$).

When latency was compared between mature and young (treated and untreated) males, significant differences were

Table 1 Mean male reproductive performance index (MRPI) (experiments 1 and 2), and female reproductive performance index (FRPI) (experiment 3) with their standard error (SEM) for the Argentina strain (ARG) and the Peru strain of *Anastrepha fraterculus* and treatment. The χ^2-value and its associated P-value are presented (departures from zero were evaluated through a χ^2-test of goodness-of-fit)

Experiment	Strain	Age/treatment	MRPI/FRPI ± SEM[1]	χ^2	P-value
1	ARG	7 days treated	–0.025 ± 0.079a	0.026	0.872
	ARG	7 days untreated	–0.411 ± 0.044b	28.961	<0.001
	PERU	7 days treated	0.139 ± 0.037a	3.286	0.073
	PERU	7 days untreated	–0.524 ± 0.106b	28.405	<0.001
2	ARG	5 days treated	–0.898 ± 0.068a	81.520	<0.001
	ARG	6 days treated	–0.128 ± 0.062b	2.219	0.136
	ARG	7 days treated	0.057 ± 0.071b	0.416	0.591
	PERU	5 days treated	–0.859 ± 0.047a	82.286	<0.001
	PERU	6 days treated	–0.031 ± 0.112b	0.343	0.558
	PERU	7 days treated	–0.083 ± 0.109b	0.757	0.384
3	ARG	7 days treated	–0.623 ± 0.081a	54.535	<0.001
	ARG	7 days untreated	–0.448 ± 0.047a	33.800	<0.001
	PERU	7 days treated	–0.607 ± 0.077a	62.583	<0.001
	PERU	7 days untreated	–0.510 ± 0.058a	38.502	<0.001

Within each experiment, means followed by the same letter did not differ (P>0.05).

[1]This column presents mean MRPIs for experiments 1 and 2, and mean FRPIs for experiment 3.

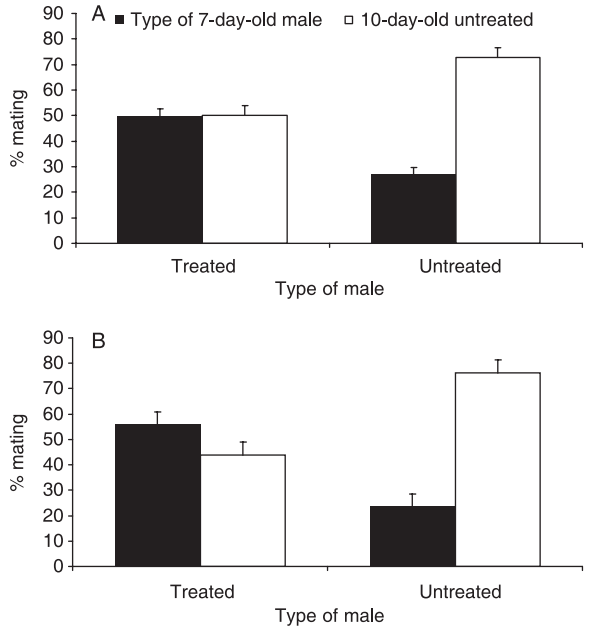

Figure 1 Mean (+ SE) percentage of matings achieved by 7-day-old methoprene-treated or untreated, and 10-day-old untreated (and already mature) males, for the (A) Argentina or (B) Peru strains of *Anastrepha fraterculus*. Eight replicates were performed for each strain.

found for both Argentina and Peru treated males and 10-day-old males, although for the Argentina strain, the latency was lower for 10-day-old males whereas for the Peru strain, the latency was lower for 7-day-old treated males (Table 2). On the other hand, mean mating duration (Table 2) did not show differences between 7-day-old treated and 10-day-old untreated males for both strains. Only in the control experiment with untreated males from Argentina was there a difference in the mating duration between males.

The comparison of the location where males engaged in a mating (Table 2) showed no differences between 7-day-old (treated or untreated) and 10-day-old males from Argentina. For Peru, significant differences in the distribution of the males among heights as well as quadrants were found, but only with 7-day-old treated males.

Minimum age at which males become competitive

When the treated males from the Argentina strain were 5 days old, they were only involved in 5.1 ± 3.5% of all the matings, while the mature untreated males achieved all other matings (Figure 2A). However, 6- and 7-day-old treated males were as competitive as mature males, obtaining 43.6 ± 3.2 and 55.6 ± 3.1% of the matings, respectively (Figure 2A). The mean MRPI in these experiments and the χ^2-test showed that these values were always significantly different from zero when 5-day-old treated males were tested, but did not differ from zero when treated males were 6 or 7 days old (Table 1).

As with males from Argentina, 5-day-old treated Peru males were unable to compete with mature males (they participated only in 7.8 ± 2.7% of the matings). However, 6- and 7-day-old treated males were as competitive as mature males, obtaining 49.1 ± 5.3 and 47.1 ± 6.1% of the matings, respectively (Figure 2B). χ^2-tests showed that the MRPIs were statistically different from zero in every replicate when the males were 5 days old, but only in one replicate out of eight when the males were 6 or 7 days old (Table 1).

Table 2 Mean latency (minutes to start of mating) and mating duration (min) for each type of *Anastrepha fraterculus* male, strain, and treatment in experiment 1. Differences in latency times were evaluated through a one-way analysis of variance or a Mann–Whitney test, and the corresponding F or Z-values and the P-values are presented. Results from the χ^2-test of heterogeneity comparing the matings found at different height and quadrants of the tree are also presented; n represents sample size

			Latency			Duration			Height		Quadrant	
Strain	Treatment	Male	Mean ± SEM (n)	F	P-value	Mean ± SEM (n)	F	P-value	χ^2	P-value	χ^2	P-value
ARG	Yes	7 days old	52 ± 8 (77)	1.962[1]	0.049	78 ± 7 (77)	0.207	0.650	2.890	0.236	0.342	0.952
		Mature[2]	31 ± 5 (77)			83 ± 7 (77)						
	No	7 days old	26 ± 8 (45)	4.265	0.041	66 ± 4 (45)	4.801	0.030	1.178	0.555	1.919	0.589
		Mature[2]	48 ± 5 (109)			54 ± 2 (109)						
PERU	Yes	7 days old	69 ± 4 (94)	7.890	0.006	33 ± 1 (94)	1.054	0.306	6.062	0.048	12.671	0.005
		Mature[2]	89 ± 5 (69)			35 ± 1 (69)						
	No	7 days old	67 ± 9 (35)	1.085	0.299	34 ± 2 (35)	0.241	0.625	1.361	0.508	2.590	0.459
		Mature[2]	82 ± 11 (96)			35 ± 1 (96)						

[1]Z value (Mann–Whitney test) is presented for this case only.
[2]Mature control males were 10 days old and untreated.

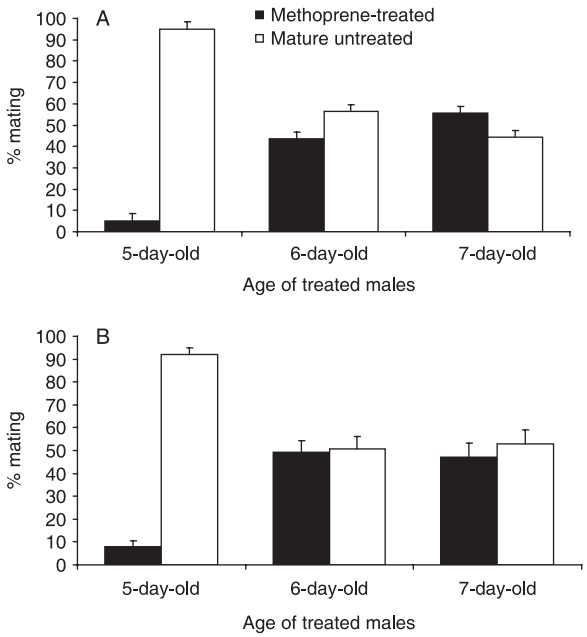

Figure 2 Mean (+ SE) percentage of mating obtained by 5-, 6-, or 7-day-old treated males, and 10-day-old mature and untreated males for (A) Argentina or (B) Peru strain of *Anastrepha fraterculus*. Eight replicates were performed for each age and strain.

A two-way ANOVA test showed that the MRPIs did not differ between the two strains, but showed significant differences among males from different ages (ANOVA; strain: $F_{1,42} = 0.001$, $P = 0.983$; treatment: $F_{2,42} = 68.052$, $P<0.001$; interaction: $F_{2,42} = 1.113$, $P = 0.338$). Multiple comparisons using a Tukey' test showed that the MRPIs obtained for 5-day-old males from both strains were significantly lower than those obtained for the 6- or 7-day-old males, which did not differ between themselves (Table 1).

When the latency was compared between treated and untreated males, no differences were found at any age for either strain (Table 3). A similar result was found for the mating duration, although 7-day-old Argentina males mated for significantly shorter time periods than 10-day-old males (Table 3).

No differences were detected across heights or quadrants when the location of matings was compared between males of different ages from Argentina (Table 3). The same results were obtained for Peru males, except for the 7-day-old treated males whose location differed significantly from mating pairs that involved mature males (Table 3). In this last case, mating 7-day-old males tended to be grouped in the NE quadrant, while mature males showed no tendency to mate in a particular quadrant.

Effect of methoprene treatment on females

The mean percentage of mating obtained by each type of female for the Argentina strain (7-day-old treated and untreated, and 14-day-old untreated) is shown in Figure 3A. For this strain, 14-day-old females were much more active than 7-day-old females, and they participated in most of the matings: 83.9 ± 5.1% when compared to the untreated 7-day-old females and 75.7 ± 2.6% when compared to treated 7-day-old females (Figure 3A). The FRPIs

Table 3 Mean latency (minutes to start of mating) and mating duration (min) for each type of *Anastrepha fraterculus* male, strain, and treatment in experiment 2. Differences in latency times were evaluated through a one-way analysis of variance or a Mann–Whitney test, and the corresponding F or Z-values and the P-values are presented. Results from the χ^2-test of heterogeneity comparing the matings found at different height and quadrants of the tree are also presented; n represents sample size

		Latency			Duration			Height		Quadrant	
Strain	Male	Mean ± SEM (n)	F	P-value	Mean ± SEM (n)	F	P-value	χ^2	P-value	χ^2	P-value
ARG	5 days old	24 ± 10 (5)	0.608	0.437	49 ± 9 (5)	1.300	0.257	1.986	0.372	5.437	0.143
	Mature[2]	35 ± 3 (103)			62 ± 2 (103)						
	6 days old	22 ± 3 (59)	0.001	0.978	73 ± 3 (59)	1.927	0.167	2.732	0.259	1.603	0.659
	Mature[2]	22 ± 3 (87)			79 ± 3 (87)						
	7 days old	18 ± 2 (82)	0.201	0.655	73 ± 3 (82)	2.410[1]	0.016	2.114	0.348	4.546	0.208
	Mature[2]	20 ± 2 (72)			87 ± 4 (72)						
PERU	5 days old	145 ± 53 (5)	0.707	0.402	36 ± 3 (5)	0.661	0.418	0.417	0.814	3.177	0.365
	Mature[2]	149 ± 10 (106)			37 ± 1 (106)						
	6 days old	89 ± 9 (68)	0.092	0.762	36 ± 2 (68)	0.002	0.969	5.098	0.078	1.212	0.750
	Mature[2]	93 ± 7 (75)			37 ± 1 (75)						
	7 days old	167 ± 17 (61)	0.001	0.973	34 ± 1 (61)	0.081	0.777	5.187	0.075	12.125	0.007
	Mature[2]	168 ± 14 (71)			34 ± 1 (71)						

[1]Z value (Mann–Whitney test) is presented for this case only.

[2]Mature untreated control males were 10 days old.

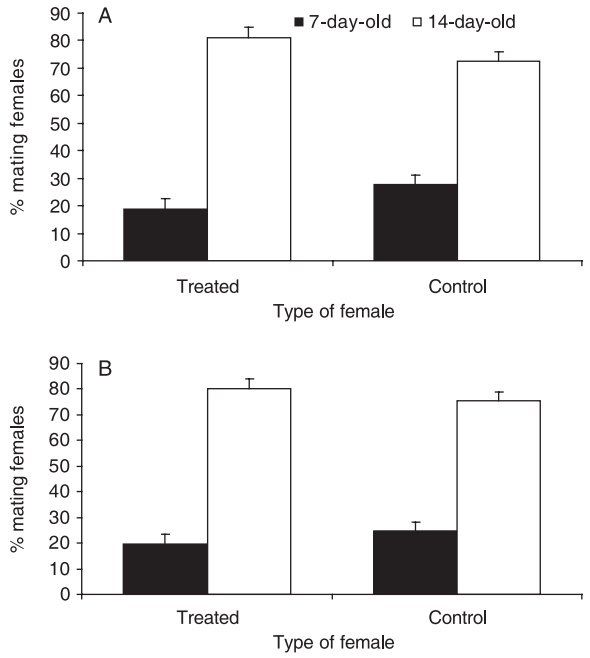

Figure 3 Mean (+ SE) percentage of mating involving different types of females (7 or 14 days old, treated or untreated) for the (A) Argentina or (B) Peru strains of *Anastrepha fraterculus*. Six replicates were performed for each age and strain.

(Table 1) were highly biased towards negative values, being significantly different from zero in all replicates (except for one replicate in the experiment with treated females as reflected by the χ^2-test). For the Peru strain, mature females were involved in more than 75% of the matings (80.4 ± 3.8% with 7-day-old untreated females and 75.7 ± 3.4% with treated females; Figure 3B). When the FRPIs (Table 1) were analyzed, a χ^2-test showed that there were significant differences in the number of matings in which 7- and 14-day-old females had participated, in all replicates. When the FRPIs were compared between strains and treatments, the two-way ANOVA showed that there was no effect of any of these two factors on the FRPIs (ANOVA strain: $F_{1,20} = 0.111$, $P = 0.743$; treatment: $F_{2,20} = 4.131$, $P = 0.056$; interaction: $F_{2,20} = 0.321$, $P = 0.557$).

There was a significant difference in the mean latency (Table 4) between the treated 7-day-old and 14-day-old females for the Argentina strain only. In this case, the younger treated females mated later in the day than the 14-day-old females. On the other hand, mating duration was not significantly different between females of the two strains (Table 4).

Discussion

Methoprene (a JH analogue) treatment produced sexually competitive 7-day-old males in both the Argentina and Peru strains of *A. fraterculus* in field cages, which is in agreement with previous laboratory studies (Segura et al., 2006). Therefore, methoprene treatment may help to overcome the problem of the long process of sexual maturation in relation to SIT for this species. Furthermore, methoprene treatment allowed 6-day-old males from both strains to compete effectively for mates with mature untreated males. Although Segura et al. (2006) reported that in the laboratory, treated males are not fully mature before day 7 after emergence, in the field cage 6-day-old treated males were able to compete with older males. Interestingly, although 5-day-old treated males show a high level of maturity in the laboratory [ca. 85% of the males mate at that age (Segura et al., 2006)], it is clear that

Table 4 Mean latency (minutes to start of mating) and mating duration (min) for each type of *Anastrepha fraterculus* female, strain, and treatment in experiment 3. Differences in latency times were evaluated through a one-way analysis of variance, and the corresponding F and P-values are presented; n represents sample size

			Latency			Duration		
Strain	Treatment	Female	Mean ± SEM (n)	F	P-value	Mean ± SEM (n)	F	P-value
ARG	Yes	7 days old	37 ± 8 (24)	4.029	0.047	53 ± 3 (24)	3.727	0.056
		Mature[1]	23 ± 2 (106)			62 ± 1 (106)		
	No	7 days old	42 ± 6 (55)	1.171	0.281	65 ± 3 (55)	0.466	0.266
		Mature[1]	33 ± 4 (113)			68 ± 2 (113)		
PERU	Yes	7 days old	105 ± 20 (23)	0.215	0.987	36 ± 3 (23)	0.011	0.917
		Mature[1]	107 ± 10 (84)			37 ± 1 (84)		
	No	7 days old	88 ± 16 (35)	0.012	0.913	42 ± 2 (35)	0.860	0.356
		Mature[1]	90 ± 9 (89)			39 ± 1 (89)		

[1]Mature control females were 14 days old and untreated.

they were not mature enough to compete with mature males in the field cage. These differences stress the importance of carrying out these studies in field cages. The practical implication of the results is that a release protocol should be developed so that treated sterile insects can be held for 6 days before release.

For latency, mating duration, and mating location in the tree (a good indicator of male location when they are releasing sexual pheromones), only latency showed differences between males. In experiment 1, 7-day-old treated males from Argentina needed more time to start mating than untreated mature males. However, for the Peru strain this tendency was reversed. When latency was analysed as part of experiment 2, no differences were detected. This may be due to the fact that in experiment 1, males were released nearly at sunrise, but due to logistic limitations the other experiments started ca. 1 h after sunrise. This could have affected the normal temporal distribution of matings, blurring the differences in latency found in experiment 1 for the Argentina strain. The differences in latency found in experiment 3 for the Argentina strain in the case of treated females can be explained by the fact that just a few treated females were involved in matings and this reduced the mean latency. Mating duration and location of the males in the tree did not show major trends, and only showed significant differences in a few cases. Altogether, these results showed that there is almost no detrimental effect of the methoprene treatment on the sexual behavioural patterns of the males.

Female sexual receptivity is tightly coordinated with ovarian development, which in turn is hormonally regulated (Ringo, 2002). However, the relative importance of the hormones involved in this process (JH and ecdysteroids) seems to differ markedly among species. In *A. fraterculus* females, methoprene treatment did not accelerate sexual maturation, or at least, did not induce a higher rate of mating in 7-day-old females. These results support the treatment of pupae or teneral sterile flies with methoprene for SIT. As there is no GSS for *A. fraterculus*, SIT relies on bisexual releases of sexually mature flies. Under such conditions, a majority of released sterile females start to mate at the same time as the released sterile males. This normally results in a reduced impact, as sterile males will predominantly mate with the mature virgin sterile females, using up their sperm loads and limiting their exposure to wild females. Following methoprene treatment, sterile males would be released along with sterile females and if methoprene treatment produced the same effect on both sexes, the reduced impact would remain. However, these results show that methoprene treatment does not accelerate sexual maturation in females and that the treatment actually induces physiological separation of the sexes regarding their readiness to mate. This could be envisaged as a 'sexing effect' of the methoprene, given that the final result is the presence in the field of sterile males ready to mate together with a majority of unreceptive sterile females. Therefore, sterile males will not be distracted and will disperse from their release site in search for mates, in a way similar to the release of only males as it would happen with a genetic sexing strain. Nevertheless, ca. 20% of 7-day-old females were ready to mate, and future studies should be directed at finding a pre-release treatment that reduces this percentage. Given that 6-day-old treated males are as competitive as mature males, those studies should include field cage tests that compare the readiness to mate of 6-day-old treated and untreated females with that of mature females, because this 1 day less could help to reduce the percent of receptive young females even further (i.e., <20%).

Argentina and Peru strains showed similar responses to methoprene treatment. Males accelerated their process of sexual maturation, and females were unaffected. This is in agreement with the data obtained from other *Anastrepha* and *Bactrocera* species (Teal et al., 2000; Pereira, 2005; Smallridge et al., 2006; I Haq, C Cáceres, AS Robinson, J Hendrichs & C Stauffer, unpubl.), for which a similar increase in competitiveness has been reported in methoprene-treated males.

Methoprene treatment of young *A. fraterculus* males will enable them to compete with wild males following release, which can result in the reduction of operational costs for SIT. In addition, the novel finding that methoprene treatment does not accelerate sexual maturation in females is promising as it may promote the dispersal of males from the release site, although this needs to be confirmed. Additional studies should include field cage tests with irradiated (and thus sterile) methoprene-treated males and wild males to evaluate their competitiveness and capacity to induce sterility in wild females, and also studies on female re-mating following matings with treated and untreated males. Treated males may be effective in performing their courtship and be accepted by the females, but this may not be accompanied by an adequate effect on female receptivity (either by the transfer of male accessory gland products or sufficient amounts of sperm) and result in females that are still receptive and will therefore search for new mates. Finally, any possible detrimental effects of methoprene treatment on survival and dispersal should be assessed.

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