Artículo científico

Chemosterilization of *Anastrepha fraterculus* (*Diptera: Tephritidae*) with an insect growth regulator

Quimioesterilización de *Anastrepha fraterculus* (*Diptera: Tephritidae*) con un regulador del crecimiento de insectos

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Abstract

Anastrepha fraterculus is a fruit pest of economic importance in South America and northwestern Argentina. Currently, it is controlled with toxic baits but there is a demand for environmentally acceptable methodologies. The use of inhibitors of the chitin synthesis is proposed as a tool to sterilize adults in the field. The aim of this study was to evaluate the efficacy of different doses of lufenuron to generate chemosterilization in *A. fraterculus*. Three experiments were performed. In the first two, the product was incorporated into the diet of the adults from emergence to sexual maturity. Treated individuals from one sex were crossed with untreated individuals from the other sex. Eggs were collected and allowed embryonic development. In the third trial, untreated males and females were crossed and allowed to copulate. Subsequently, females were exposed to the product and the eggs were collected. Egg hatch was affected in the three experiments in a dose dependent way. In Experiment 3, the effect of the product was evidenced 48 hours after exposure. Fecundity was also affected when the females were the treated sex. The results show that exposure of adults to lufenuron reduces egg hatch in *A. fraterculus* encouraging further investigations to adapt this methodology for this species.

Key words: South American fruit fly, lufenuron, benzoylureas.

Resumen

Anastrepha fraterculus es una plaga de frutales de importancia económica en Sudamérica y el noroeste argentino. Actualmente es controlada con cebos tóxicos pero existe una demanda de metodologías ambientalmente aceptables. El uso de inhibidores de la síntesis de quitina, se plantea como una herramienta para esterilizar adultos en el campo. El objetivo de este trabajo fue evaluar la eficacia de diferentes dosis de Lufenuron para generar quimiosterilización en *A. fraterculus*. Se realizaron tres experimentos. En los dos primeros, el producto se incorporó en la dieta de adultos desde la emergencia hasta la madurez sexual. Se cruzaron individuos tratados de un sexo con individuos no tratados del otro sexo. Se colectaron los huevos y se permitió el desarrollo embrionario. En el tercero, se cruzaron machos y hembras no tratados y se les permitió copular. Posteriormente, se expuso a las hembras al producto y se colectaron los huevos. El porcentaje de eclosión se vio afectado en los tres experimentos en una forma dosis dependiente. En el tercer experimento, el efecto del producto se manifestó 48 horas después de la exposición. La fecundidad solo se vio afectada cuando el sexo tratado fueron las hembras. Los resultados muestran que la exposición de adultos a lufenuron permite reducir la eclosión de huevos en *A. fraterculus* sentando bases para investigaciones tendientes a adaptar esta metodología para esta especie.

Palabras clave: Mosca sudamericana de la fruta, lufenuron, benzoilurea.

Introduction

The South American fruit fly, *Anastrepha fraterculus* (Wiedemann) is a fruit pest of economic importance to many countries in America. It is originated in South America and is present from the south of the United States of America (Texas) to Argentina (Salles, 1995; Steck, 1999; Malavasi, 2000). In South America, this species is found in two broad, apparently unconnected

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bands: one along the western edge, including both highland and lowland areas of the Andean range, and the other along the east coast (Malavasi, 2000) and recent records report its presence in the Amazonas (Zucchi *et al.*, 2011). This highly destructive pest has been reported to infest more than 100 host plants including major fruit crops (Norrbom and Kim, 1988; Norrbom, 2003). Both direct damage to the fruit as well as quarantine restrictions for fruit export to many countries results in great losses for producers (Steck, 1999).

In Argentina, *A. fraterculus* occurs in the northeast and northwest of the country overlapping its distribution with that of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), (Ovruski *et al.*, 2003; Segura *et al.*, 2006). It infests mainly guavas (*Psidium guava*), mango (*Mangifera indica*), peaches (*Prunus persica*), and to a lesser extent some citrus species such as grapefruit (*Citrus paradisi*) (Ovruski *et al.*, 2003; Segura *et al.*, 2006; Oroño *et al.*, 2008). The losses that these two species impose to the local production are estimated to be 15 to 20 % of the fruit production (Guillén and Sánchez, 2007).

Currently, the only method available to suppress *A. fraterculus* populations is the application of an insecticide, which depending on the region is the organophosphorate malathion, cypermethrin or the bacteria-derived spinosad. Any of them impose concerns to the environment and human health. Therefore, environmentally safe methods, such as the sterile insect technique (SIT) (Knipling, 1955), are being proposed as alternatives (Ortiz, 1999). The SIT relies in the mass rearing of the insect, its sterilization, often by irradiation, and its release in the field. Once in the field, the sterile males copulate with wild females rendering non-viable offspring. This technique, is widely used to control several insect pests (Enkerlin, 2005) and in Argentina it is used to control *C. capitata* (de Longo *et al.*, 2000; Guillén and Sánchez 2007), The success of the suppression and eradication programmes against *C. capitata* in the region of Patagonia and the province of Mendoza, areas where *A. fraterculus* is not present, has encouraged the use of the SIT in those areas where the two species coexist (Guillén and Sánchez, 2007). This has strengthened the need to develop the SIT against *A. fraterculus* leading to huge research efforts that focused on different aspects of *A. fraterculus* biology, artificial rearing protocols and determination of irradiation doses for sterilization. However, this technique is still not readily available. In this respect, the use of other options, particularly for small areas should be explored.

Chemoesterilization is among the other control alternatives available (Tunaz and Uygun, 2004). It has been proven that several insect growth regulators (IGRs) are capable of inducing sterility in several insects such as cockroaches (Mosson *et al.*, 1995), moths (Butter *et al.*, 2003) and beetles (Avila *et al.*, 1999). Within the Diptera, this also happens in *Drosophila melanogaster* Meigen (Wilson and Cryan, 1997) and several fruit flies from the Tephritidae family. In *C. capitata* Casaña-Giner *et al.* (1999) reported that several phenyl-benzoylureas with fluorinated alkoxy substituents in the phenyl group proved to reduce egg hatch. Among them, lufenuron was the one showing the highest activity. This compound has also been shown to have an effect in the sterility of some species of *Anastrepha* (Moya *et al.*, 2010) and in *Bactrocera dorsalis* (Hendel) and *Bactrocera latifrons* (Hendel) (Chang *et al.*, 2012). In most of the cases the sterilization effect applied to both sexes; that is, when males and females were fed with lufenuron and then coupled with flies from the opposite sex not fed with lufenuron, viability of the G1 was significantly reduced. In addition, for *C. capitata* it has been shown that lufenuron can be applied in the field in traps which contain a lure to attract the flies and a phagostimulant bait (Navarro-Llopis *et al.*, 2004; 2007; 2010; Alemany *et al.*, 2008); results showed a significant decrease in the adult population and in fruit damage.

The present work aimed at evaluating the sterilizing effect of lufenuron on *A. fraterculus* adults. The impact was evaluated by scoring egg hatch and we also determined the effect of lufenuron on fecundity.

Material and methods

We performed three different experiments. In Experiment 1, we applied lufenuron to the diet of the males during the process of sexual maturation; in Experiment 2, we applied lufenuron to the females during the same stage and in Experiment 3, we applied lufernuron to the diet of already inseminated and fertile females. All experiments were performed at the laboratories of Cátedra de Terapéutica Vegetal, Facultad de Agronomía y Zootecnia, Universidad Nacional de Tucumán, Tucumán, Argentina.

Insects

Anastrepha fraterculus adult flies were obtained from a colony established at the laboratories of Zoología Agrícola from the Estación

Experimental Agroindustrial Obispo Colombres, 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 since then, it has been maintained following standard procedures (Salles *et al.*, 1999; Jaldo *et al.*, 2001; 2007; Vera *et al.*, 2007). Pupae (30 gr) were placed in plastic containers and maintained in a room at 25 ± 2 °C and 60 ± 20 RH with a 12:12 L:D cycle until adult emergence. Once adults started emerging they were sorted by sex and transferred to the appropriate cage according to the experiment they were assigned (see below).

Chemicals

Experiments were performed with lufenuron, a chithin synthesis inhibitor from the Benzoil urea group as it is formulated by Syngenta (Match® EC 5%).

Bioessays

To evaluate the effect of lufenuron on the fertility of *A. fraterculus*, three experiments were conducted. In the first, we evaluated the effect of supplying lufenuron along the period of sexual maturation on male fertility; in the second, we evaluated the effect of supplying lufenuron along the sexual maturation period on female fertility and in the third, we evaluated the effect of providing lufenuron on already sexually mature and inseminated females fertility. In all cases, the insecticide was incorporated into the adult diet which was supplied in 50 cc plastic containers. The diet consisted of a mixture hydrolyzed yeast (14.5%, w/w), corn protein (27.3%, w/w), sugar (for human consumption) (57.9%, w/w), one vitamin complex (Dayamineral ®) (0.2%, w/w) and vitamin E (0.2%, w/w). Seven concentrations were evaluated: 0; 100; 500; 1,000; 2,000; 5,000 and 7,000 ppm of active ingredient. After mixing the diet with the lufenuron, the container was ventilated to allow solvent evaporation. For the first two trials, the diet with lufenuron was provided from adult emergence until they were 10 days old. After this time, 25 exposed males were crossed with 25 unexposed females of the same age (trial one) in a plastic cage (12 l) which contained one side with a cloth with a thin layer of silicone as oviposition substrate. Experiment 2 consisted of the reciprocal cross (untreated males mated to treated females). After the cage was set up, we left the flies to copulate freely for 48 h, time at which two eggs collections at 24 h interval were performed. In each egg collection all the eggs up to 100 were placed in a Petri dish over a moistened cloth and a black filter paper. If more than 100 eggs were laid, the remaining was discarded. Eggs were incubated at 25 ± 2°C for 5 days. After this incubation period, egg hatch was scored. In Experiment 3, cages were set up with 25 already sexually mature and mated females. One day before providing the females with the diet with lufenuron (day 0), eggs were collected as described previously. Then, the food was replaced by food with lufenuron at the desired concentration and eggs were collected for 4 days on a daily basis (days 1, 2, 3 and 4 respectively). As in Experiments 1 and 2, eggs were incubated for five days, at which time egg hatch was recorded. Experiments 1 and 2 were replicated 3 times whilst experiment 3 was replicated four times. Water was supplied in containers of 20 ml capacity trough a piece of cotton cloth.

Data analysis

To evaluate the effect of lufernuron on fertility, in Experiments 1 and 2 the data from the two egg collections were averaged to obtain a mean value for each cage (replicate). In those cases were less than 10 eggs were laid, the data was discarded. The effect of lufenuron over egg hatch in Experiments 1 and 2 was analyzed by adjusting the data to a log-logistic model following Seefeldt *et al.* (1995) to estimate the concentrations that inhibited 50% and 99% of the eclosion (EIC_{50} and EIC_{99} respectively). In addition, we performed an ANOVA followed by multiple comparisons Tukey test with the concentration as the fixed factor and egg hatch as the response variable. In Experiment 3 the data was analyzed by means of a generalized linear mixed model in which the fixed factors where the concentration and the time at which egg collections were performed. We included in the model the possible temporal correlation (repeated measures) among collections within each cage as a random factor. The model was fitted to a binomial distribution with a logit link function.

To evaluate the effect of lufenuron on fecundity, in Experiment 1 and 2, we sum the number of eggs laid during the two eggs collections. The analysis performed was the same as for egg hatch: one way ANOVA for each sex, were the concentration was the fixed factor and the number of eggs laid the response variable. In Experiment 3 the data was analyzed by means of a mixed model in which the fixed factors where the concentration and the time at which egg collec-

tions were performed.

We included in the model the possible temporal correlation (repeated measures) among collections within each cage.

Dose-response analysis was performed with R and the remaining tests with INFOSTAT (Di Rienzo *et al.*, 2012) which operates with R.

This effect was significant from 500 ppm (Table 1) and the respective eclosion inhibiting concentrations were 412.2 ppm for EIC_{50} and 7110 ppm for EIC_{99}.

Table 1. Effect of lufenuron on the fertility of *Anastrepha fraterculus* males and females treated during the time of sexual maturation (from emergence up to 10 days-old).

Concentration (ppm)	Males (Experiment 1)	Females (Experiment 2)
0 (control)	82.4 ± 4.7a	64.9 ± 10.9a
100	54.5 ± 17.6ab	0.8 ± 0.5b
500	34.1 ± 14.9bc	0.2 ± 0.2b
2,000	11.6 ± 3.6bc	0.0 ± 0.0b
5,000	2.9 ± 1.0b	0.0 ± 0.0b
7,000	2.0 ± 2.0b	-

Within each sex (column), values with the same letter are equal (Tukey test; $p > 0.05$).

Experiment 2 revealed that lufenuron also affected *A. fraterculus* female fertility ($F = 28.54$; $df = 5,11$; $p < 0.0001$) and this effect was more pronounced than for males (Table 1, Figure 1).

Results

Effect of lufenuron on fertility

In Experiment 1, the fertility of *A. fraterculus* males was significantly affected by lufenuron ($F = 13.51$; $df = 5,11$; $p = 0.0002$). This was evidenced by the fact that treated males were able to induce sterility when mated with untreated virgin females (Figure 1).

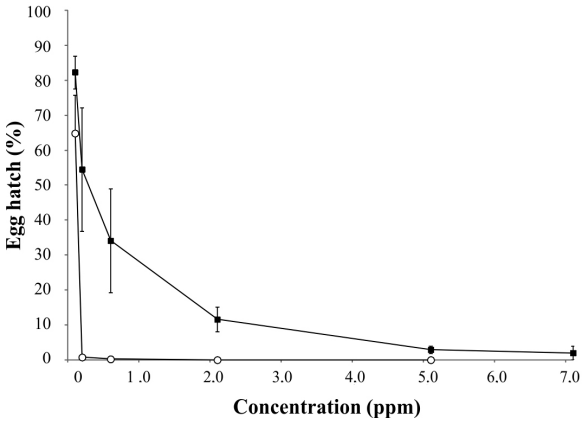

Figure 1. Effect of lufenuron on *Anastrepha fraterculus* male (black squares) and female (white circles) fertility (Experiments 1 and 2).

At the minimum concentration egg hatch was lower than 1% and inhibition was observed from the concentration of 2,000 ppm. For this reason it was not possible to estimate the EICs. At 7,000 ppm fecundity was severely affected and it was not possible to obtain more than 10 eggs in any collection. Experiment 3 showed that the effect of lufenuron was also evident in already inseminated females (Chi-square = 582.26; $p < 0.0001$) and this depended on the time the eggs were collected (Chi-square = 1374.43; $p < 0.0001$). The interaction was also significant (Chi-square = 682.05; $p < 0.0001$). For all concentrations, egg hatch was reduced as days passed (Table 2) while this did not occur for the control (0 ppm).

The greatest response was obtained at the fourth day where at concentrations of 2,000 and 5,000 ppm egg hatch inhibition was greater than 90%. As for the second trial, female fecundity was severely affected by lufenuron and at the higher concentrations it was not possible to collect enough number of eggs to assess egg hatch.

Table 2. Fertility of *Anastrepha fraterculus* females that were fed diets containing different concentrations of lufenuron after sexual maturation and insemination.

Concentration (ppm)	Egg collection day																				
	0				1				2				3				4				
0 (control)	89.9	±	3.1	a	82.2	±	3.3	a	61.0	±	22.5	a	85.0	±	5.0	a	70.0	±	20.0	a
100	76.5	±	6.6	a	87.7	±	4.0	a	80.3	±	7.7	a	55.0	±	16.6	ab	41.5	±	17.5	ab
500	83.3	±	6.1	a	79.1	±	11.3	a	72.7	±	12.1	a	42.9	±	19.8	ab	12.1	±	7.7	b
2.000	89.2	±	2.5	a	80.0	±	6.8	a	32.9	±	15.9	a	11.2	±	7.1	b	1.6	±	1.3	b
5.000	85.3	±	3.5	a	89.4	±	2.1	a	18.5	±	13.5	a	18.2								

Egg collection day is referred to the initiation of lufenuron exposure with day 0 referring to the day prior to the exposure. Values are expressed as percentage of egg hatch (mean ± standard error). Within each oviposition day (column). values with the same letter are equal (Tukey test; $p > 0.05$). On day 3 for 5000 ppm only one cage laid more than 10 eggs. On day 4. none of the cages at this concentration laid more than 10 eggs.

Effect of lufenuron on fecundity

In experiment 1, the treated sex was the male and no effect was found ($F = 2.03$; $df = 5,11$; $p > 0.05$). In Experiment 2, there was a marked reduction at the highest concentration (Table 3), but differences were not significant ($F = 1.64$; $df = 5,11$; $p > 0.05$), probably due to sample size. In Experiment 3, there was a significant effect of the concentration ($F = 7.64$; $df = 4$; $p < 0.0001$), no effect of the observation period ($F = 0.34$; $df = 4$; $p > 0.05$) and the interaction was significant ($F = 2.11$; $df = 16$; $p < 0.0166$). As the concentration increased, the fecundity decreased (Figure 2) and this was clear 72 h after the treatment.

Table 3. Effect of lufenuron on the fecundity of *Anastrepha fraterculus* males and females treated during the time of sexual maturation (from emergence up to 10 days-old).

Concentration (ppm)	Males			Females		
0 (control)	198.33	±	1.67	89.33	±	26.74
100	165.33	±	34.67	129.67	±	35.36
500	194.5	±	5.5	57.33	±	31.42
2,000	106.67	±	12.81	31	±	16
5,000	160.33	±	39.67	60.5	±	46.5
7,000	189.33	±	11.17	4.5	±	2.5

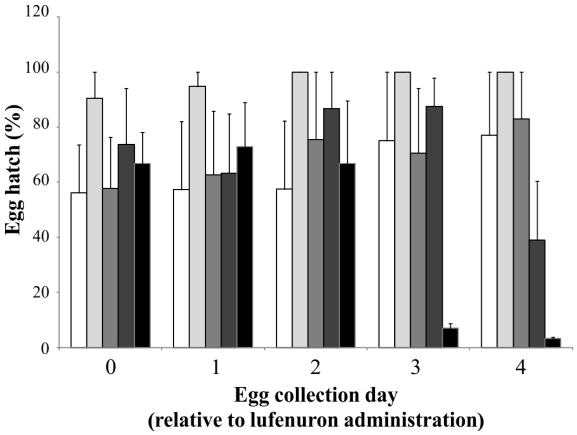

Figure 2. Effect of lufenuron on the fecundity of *Anastrepha fraterculus* females that were fed diets containing different concentrations (white 0 ppm; light grey 100 ppm; grey 500 ppm; dark grey 2,000 ppm and black 5,000 ppm) of lufenuron after sexual maturation and insemination (Experiment 3). Egg collection day is referred to the initiation of lufenuron exposure with day 0 referring to the day prior to the exposure.

Discussion

In this work we evaluated the sterilizing effect of lufenuron on *Anastrepha fraterculus*, a major fruit pest in several countries of South America. Egg hatch was significantly reduced when lufenuron was applied to the adult diet of either males or females during the time of sexual maturation. In addition, inseminated females, which were already laying viable eggs, reduced their fertility in approximately 48 h when provided a diet with this insect growth regulator. Lufenuron also had a significant effect on fecundity when applied to females.

The effect of lufenuron on fertility was in agreement with what has been observed for other fruit flies such as *C. capitata* (Casaña-Giner *et al.*, 1999), *Anastrepha ludens* (Loew), *Anastrepha obliqua* (macquart), *Anastrepha serpentina* (Wiedemann) and *Anastrepha striata* (Schiner) (Moya *et al.*, 2009), *B. dorsalis* and *B.latifrons* (Chang *et al.*, 2012). Moya *et al.* (2009) found a different response in four Anastrepha species; *A. obliqua* and *A. striata* were the most affected, *A. ludens* was affected to a lesser extent and *A. serpentina* was almost not affected by the treatment. This differential response was also seen in some *Bactrocera* species (Chang *et al.*, 2012) and could be related to the physiology of egg production, the permeability of the egg chorion or other biological traits such as the feeding behavior of the adults. In our case, and although the different experimental conditions impede from comparing the concentrations that we used compared to that used by Moya *et al.* (2009), it could be proposed that *A. fraterculus* response resembles that of *A. obliqua* and *A. striata*.

The effect of lufenuron depended on the sex that was treated. In males, it was evident at 500 ppm while in females, it occurred at lower concentrations (100 ppm). In fact, this was the lowest concentration evaluated and for this reason it was not possible to adjust females' data to a log-logistic model. At this concentration, egg hatch was 0.8% while for the control it was 64.9%. This pronounced drop in fertility shows that induction of sterility trough females requires less amount of product. It would be interesting to see if this can be related to a single or few food ingestions.

This differential effect of lufenuron in both sexes has already been found in *C. capitata* (Casaña-Giner *et al.*, 1999) where 3 h exposure to a diet with lufenuron significantly reduced egg hatch at 5,000 and 1,000 ppm for males and females respectively. Moya *et al.* (2009) also found a differential effect between sexes and also differences among species. Interestingly, they found no effect of lufernuron on males of *A. ludens*, *A. obliqua* and *A. striata* and some effect on *A. striata*. The differences between the sexes can be explained by a need in males to ingest more

amounts of the product in order to pass it to the females during mating and hence induce sterility. In that experiment flies were fed only for 24 h right after emergence and then were kept with no access to lufenuron suggesting that the time at which the males were exposed to the product may have been not appropriate. Moreover, the differences among species deserve special attention. As mentioned above, these differences could be due to biological and physiological differences among the species studied. For example, Moya *et al.* (2009) argue that the low or null susceptibility of *A. serpentina* could be related to the feeding behavior of this species which "feeds sporadically and ingest small amounts of food" (Moya *et al.*, 2009). In addition, they suggest that some decrease in intake could be occurring at high doses in *A. serpentina* and *A. striata* and propose a repellent effect of lufenuron; which again was not seen in the other species. They speculate that the feeding behavior could be the explanation as *A. ludens* and *A. obliqua* are markedly polyphagous and probably less discriminant. In all, evidence so far shows the need to incorporate the timing and duration of the exposure period in the experimental design and to consider the feeding habits of each species.

Fecundity was affected by lufenuron in those treatments were the females was the treated sex. Although this was statistically significant only in Experiment 3, the tendency was the same in Experiment 2. This is contrary to what was observed by Casaña-Giner *et al.* (1999) in *C. capitata*, Moya *et al.* (2009) in four *Anastrepha* species and Chang *et al.* (2012) in *C. capitata* and some *Bactreocera*. Again, the experimental design is the most likely explanation; in our case, *A. fraterculus* females were treated for longer periods before and after sexual maturation than in the previous works. The fact that fecundity has been shown to be affected in other insects such as *Lobesia botrana* Denis and Schiffermuller (Sáenz de Cabezón *et al.*, 2006) suggests that the dose at which each fertility and fecundity are affected is different and this could be the explanation of the differences found.

In all, we showed the sterilizing effect of lufenuron on *A. fraterculus* and we showed that fecundity is likewise affected. Previous works have shown the efficacy of chemoesterilization to reduce *C. capitata* populations in the field (Navarro-Llopis *et al.*, 2004; 2007; 2010; Alemany *et al.*, 2008) and recent studies reveal that this IGR is also able to sterilize other fruit fly species. As such, the use of this product to reduce fruit fly populations is feasible. Given that the way to treat wild flies with lufenuron is trough ingestion and the product is distributed in the field in baited traps, we envision that this technique could be more effective in those species in which specific attractants are known. Specifically it could be worth testing it in species from the genus *Bactrocera* in which males show a strong attraction to methyl-eugenol. This plant-derived compound is ingested by males and used as a precursor of the sex pheromone. Effective female attractants are also desired given the highest impact of lufenuron on this sex. Moreover, recent studies have shown the potential to integrate the use of IGRs with the SIT (Navarro-Llopis *et al.*, 2011) with promising results.

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