



AGRARIAN SCIENCES

Influence of wing bands and behavior of *Anastrepha fraterculus* (Diptera: Tephritidae) by the presence of *Megafreya sutrix* (Araneae: Salticidae)

PRISCILLA C. GOBBI, ADRISE M. NUNES, EDISON ZEFA & FLAVIO R.M. GARCIA

Abstract: The objective of this study was to evaluate the influence of wing bands and the behavior of *Anastrepha fraterculus* in the presence of *Megafreya sutrix*. The first experiment used specimens of *M. sutrix* (n = 40), 20 males and 20 females of *A. fraterculus* and *Musca domestica*. The second experiment used 20 individuals of *M. sutrix* (10 males and 10 females) and 120 of *A. fraterculus* (20 males and 20 females) for each treatment. Marks were made on the wings of the flies: a group with painted wing bands (n = 40) with their wings fully painted; another with highlighted wing bands (n = 40) and normal bands (n = 40). Recordings were made for 15 min or until the fly was preyed upon. The analyses were performed through observation of the recordings and Chi-square test with Yates correction for continuity. The values found for predation of *A. fraterculus* were significant when compared to *M. domestica* when evaluating the predation factor, showing that, *A. fraterculus* is less preyed than *M. domestica*. It was found that the wing patterns did not influence the predatory behavior of *M. sutrix*.

Key words: Interaction, fruit fly, predator, spider, biological control, behavior.

INTRODUCTION

Fruit flies (Diptera, Tephritidae) are considered the main pests that affect fruit production, as they cause severe damage to the production (Aluja 1994, Souza-Filho et al. 2003). The damage caused by these flies occurs due to the punctures made by the females during oviposition and the galleries opened by the larvae in the fruit pulp (Garcia et al. 2017). In the South region, the South American fruit fly *Anastrepha fraterculus* (Wiedmann 1930) (Diptera: Tephritidae) is the main responsible for losses in temperate fruit trees (Garcia & Norrbom 2011), representing approximately 95% of the species of *Anastrepha* caught in traps in the State of Rio Grande do Sul (Salles & Kovaleski 1990).

The behavioral study of fruit flies has contributed to the reduction of damages to fruit growing through studies aimed at control methods; however, an interesting aspect is that these flies have peculiar characteristics. The South American fruit fly adults have a yellow body with transparent wings that have two characteristic bands, one S-shaped in the central part and one inverted V-band at the apex (Alberti et al. 2009) and the formation of intraspecific and interspecific competition leks that allow their use in behavioral, biological and evolutionary studies (Neto et al. 2012).

Intraspecific competitions between males are common in polyphagous species of the genera *Anastrepha* and *Ceratitis*, such as *Anastrepha ludens* Loew 1873 (Robacker & Hart

1985), *A. fraterculus* and *C. capitata* (Segura et al. 2007, Whittier et al. 1994). These species establish mating leks in which they exhibit courtship and pheromone-releasing behaviors to attract females (Robacker & Hart 1985, Arita & Kaneshiro 1989, Whittier et al. 1992). Competitions between males for resources reveal an aggressive behavior, with the wings are close to the abdomen and approximately parallel to the ground, lifted and rolled rapidly raised in longitudinal axis of the body and a torsion of wings leaving the costal margin almost in contact with the surface of the substrate (Benelli et al. 2014a, b).

The behavior was also observed in interspecific relationships with other predatory arthropods such as the interaction between North American species, *Zonosemata vittigera* (Coquillet 1984) (Diptera: Tephritidae) and *Phidippus apacheanus* Chamberlin & Gertsch 1929 (Araneae: Salticidae) (Greene et al. 1988), *Salticus scenicus* (Clerck 1757) (Araneae: Salticidae) and *Rhagoletis zephyria* Snow 1894 (Diptera: Tephritidae); *C. capitata* and *Plexippus paykulli* Audouin 1826 (Araneae: Salticidae), species from the west coast of Africa and Southeast Asia, respectively (Mather & Roitberg 1987); *A. ludens* Loew 1873 and *Paraphidippus aurantius* F.O.P Cambridge 1901 (Araneae: Salticidae) and *Phidippus bidentatus* F.O.P Cambridge 1901 (Araneae: Salticidae) and *Phidippus audax* (Hentz 1845) (Araneae: Salticidae) distributed throughout North America (Rao & Díaz-Fleisher 2012, Aguilar-Argüello et al. 2015). In Brazil, *Megafreya sutrix* Holmberg, 1874 (Salticidae) is considered the most common fruit fly predator species in orchards (Garcia 2014).

All studies dealt with the predator vs. prey interaction in which the flies presented defensive behavior, which resembles the behavior developed by spiders (Salticidae) during mating, resulting in the escape of the

flies, however, this behavior is not present in the species *Musca domestica* (L. 1758), being observed constant catch by the salticids being an efficient control treatment based on the size and shape of the wings that both flies have, but with different patterns (Greene et al. 1987).

The present study hypothesized that the aggressive behavior also occurs in the South American fruit fly, *A. fraterculus*; and this behavior influences the predation by *M. sutrix*; that the presence of wing bands has no influence on predation and that the sex of spider's influence predation of *M. sutrix*. The objective of this study was to observe and analyze the interference of the wing bands and the behavior of *A. fraterculus* in the presence of the potential predator *M. sutrix* and verify the factor responsible for the non-predation of the flies.

MATERIALS AND METHODS

The experiments were carried out at the Laboratory of Ecology of Insects, Federal University of Pelotas (LAbEI/UFPel), under controlled conditions of temperature ($25 \pm 2^\circ\text{C}$), relative humidity ($70 \pm 10\%$) and photophase (12 h) in an acrylic arena adapted according to Rao & Díaz-Fleischer (2012), with 21 x 21 x 21 cm coated with a colorless acrylic cover containing a 2 mm hole which received a dark acrylic with 22 x 20 cm dimensions.

Experiment 1

Forty adult individuals of *M. sutrix*, 20 males and 20 females of *A. fraterculus* (n=40) and *M. domestica* (n=40) were used, totaling 80 interactions. In this experiment, we aimed to observe and evaluate the influence of behavior on predation by *M. sutrix*.

The rearing of *M. sutrix* was established from individuals collected in a non-commercial

orchard of *Citrus limon* (L.) (32° 3' 89" S, 52° 11' 97" W) in the municipality of Rio Grande (State of Rio Grande do Sul). Spiders were collected manually between 9:00 am and 10:00 am, during which time they were seen hunting more frequently. In the laboratory, the spiders were fed daily with 6 specimens of *Drosophila melanogaster* (Meigen 1830) from the laboratory rearing.

Each spider was fasted for two days before the interaction. The flies were randomly caught in the rearing cages and introduced into the arena, each fly was used once and each spider was used twice in each test, this number being two, with *A. fraterculus* (male and female) and *M. domestica* (male and female).

The introduction of spider and fly was performed by the top of the arena. First, the spider *M. sutrix* was placed on one side of the partition, and in the other, the *A. fraterculus* fly (male or female); the same was made with another spider and the *M. domestica* specimen (male or female). During the first minute, the spider and the fly were maintained in the arena, separated by the partition for acclimatization with the environment. After this period, the partition was removed and, interaction was filming and the predation time was annotation during to 15 minutes or until the predation of the fly. The choice of the latter was based on the size and shape of the wings that both flies have, however, with different patterns and behaviors (Mather & Roitberg 1987). The *M. domestica* specimens used as control, differ from tephritids because do not have the behaviors, alert signal, extension, supination and approximation.

Laboratory recordings were carried out to analyze the behavior of *A. fraterculus* in the presence of *M. sutrix*. The recordings were obtained in AVI (Audio Video Interleave) format, using Sony full HD PJ380 camcorder and photographed with a Nikon D3100 camera.

After each encounter, the spider was kept in quarantine for seven days, being fed *ad libitum*. Afterwards, it was again used after a two-day fast, in an encounter with a fly of the same species, but of opposite sex. The results for predation were evaluated through a Chi-square test $p = 0.05$ with Yates correction for continuity ($p = 0.05$; $df = 1$) and the behavioral interactions for *A. fraterculus* and *M. domestica* species, observed and counted for the events: extension and supination, fly escape, spider escape and disinterest of the spider.

Experiment 2

The second experiment was carried out to evaluate the effect of markings on the wings of males and females of *A. fraterculus* on the deterrence of spiders. The test was divided into treatments based on the type of marks made on the wings. First treatment, control, normal bands ($n = 40$), in which no type of mark was made on the wings of *A. fraterculus* specimens. Second treatment, in which the wing bands were painted using the CD Marker Faber-Castell 1.0 mm tip, in which the wings were fully painted ($n = 40$) and the third treatment, in which the bands were highlighted using CD Marker in order to strengthen the previously existing bands in the wings ($n = 40$).

For the second and third treatments, the flies were placed in a refrigerator at 5°C for 10 min to anesthetize them. The anesthesia allowed the immobilization of the flies without killing them, which allowed the wings to be painted and highlighted. For control treatment, the flies were refrigerated for 10 min and the wings were falsely painted with an empty CD Marker. The flies were left in recovery for 15 min prior to test.

The fly was placed in the arena separated from the spider by the dark acrylic partition, which was left for one minute for acclimation to the environment, then the partition was

removed and the time was counted for 15 min or until the fly was preyed upon (Mather & Roitberg 1987). After each encounter, the spider was kept unused in the experiment for a period of seven days, being fed *ad libitum*. Afterwards, it was again used after a two-day fast, in an encounter with a fly of the same species, but of opposite sex. The same was repeated for all three treatments, totaling 120 interactions. The results for predation were evaluated through the Chi-square test with Yates correction for continuity ($p = 0.05$; $df = 1$) to verify if the presence of bands influenced the predatory behavior of male and female spiders.

RESULTS

Both sexes of fruit flies, *A. fraterculus*, presented during interaction with the predator *M. sutrix* the behaviors of alert signal, escape by flight, extension, supination, approximation, walking, body cleaning. The alert signal behavior was evidenced when the fly perceived the presence of the spider in the environment, and this occurred in general right after the removal of the dark acrylic partition.

The alert signal was identified as the fly lifted its body imposingly and watched closely all movements made by the spider. The escape by flight was observed when the spider approached by cornering the fly or jumping on it and through flights of short distances, with trajectories of flight very close to the spider, like low flights.

The extension behavior, characterized by opening the wings perpendicular to the longitudinal axis of its body followed by a twist of wings leaving the costal margin of these almost in contact with the surface of the substrate (Benelli et al. 2014a, b), in turn, supination occurs when the wings are directed

forward perpendicular to the axis of the body, with its ventral face facing forward and the costal margin of the wing facing the back of the body with wave movements (Headrick & Goeden 1994).

The extension of the wings was almost always associated with the supination behavior, which consists of swinging the wings that are in extension, forwards and backwards, this movement may be asynchronous, in which the fly alternates the movement of the wings or synchronous, where the fly makes the movement of the wings together in the same direction (Headrick & Goeden 1994).

The South American fly by using the extension and supination behaviors together with the approximation behavior, caused most of the time intimidating behavior on the spider, followed by the retreat of the spider. The walking behavior was used to recognize the environment, especially when the fly was separated from the spider by the partition or until the moment the fly noticed the presence of the spider, reducing the frequency of this behavior.

The moments in which the fly was under reduced or absent stress condition, it was observed that sometimes it performed the "preening behavior", body cleansing (Khoo et al. 2000), with more frequent cleaning of the anterior legs and head. In this situation, the fly brings the legs to the proboscis and then rub them on the head and antennas or one leg in the other.

The *M. domestica* specimens used as controls differ from tephritids because they do not present the behaviors, alert signal, extension, supination and approximation.

Experiment 1

During the first experiment, it was observed that 65% of the females and 70% of the males of *M. domestica* were preyed upon; for *A. fraterculus*

the predation index was low, being 5% for females and 20% for males. When evaluating the rate of *A. fraterculus* individuals not preyed upon by *M. sutrix* using the Chi-square test with Yates correction for continuity ($p = 0.05$; $df = 2$), it was found that values were significant (females $\chi^2 = 0.11$, males $\chi^2 = 1.01$) when compared to those found for *M. domestica*.

The times until the occurrence of predation were counted, and through these values, it was verified that the individuals of *M. domestica* were more frequently preyed upon during the time interval of 2-3 min, reducing gradually until the end of the interaction period. This reduction over time allowed to verify that the specimens of *M. domestica* were usually preyed upon in the first spider attacks.

Individuals of *A. fraterculus* were preyed upon during all 15 min of interaction, with the highest number of individuals preyed upon in the interval of 7-10 min. This usually occurred when the flies were already under stress due to physical exhaustion after several exposures, supinations and escapes by flight.

The behavior displayed by *A. fraterculus* during the sequence of the movements of extension, supination and aggressive behavior, demonstrated that the flies of this species exhibit up intensely during the interactions. In addition, females showed greater aggressiveness than males (Table I). For *M. domestica*, extension and supination behaviors were not evidenced.

It was found that even some individuals of *A. fraterculus* that performed the combination of extension and supination also displayed escape by flight (Table I) to escape spider attacks and to be preyed upon by *M. sutrix*. The mean number of escapes by flight performed by *A. fraterculus* was 2.53 ± 1.25 escapes in a mean time interval of 7.89 ± 3.42 min. For *M. domestica*, mean escape by flight was 2.87 ± 2.36 escapes in the time interval of 10.17 ± 2.12 min.

Table I. Number of events occurring when not preyed upon in the interaction between *Anastrepha fraterculus* and *Musca domestica* with *Megafreya sutrix* in laboratory. For each species 20 females and 20 males were used, totaling 80 specimens.

Behavior	<i>Anastrepha fraterculus</i>		<i>Musca domestica</i>	
	♀	♂	♀	♂
Extension an supination	46	30	0	0
Fly Escape	24	14	15	8
Spider escape	1	0	0	0
Disinterest of the spider	9	10	2	3

The results show that *A. fraterculus* and *M. domestica* use escape by flight to avoid spider attacks and to avoid predation, however, the values for the species did not differ, demonstrating that this variable is not a relevant factor to avoid predation. However, it is observed that South American fruit fly performs flights at shorter intervals than *M. domestica*, which makes it possible for *A. fraterculus* to spend shorter time in the visual field of *M. sutrix* reducing the chances of spider attack.

During the observations, a single escape event for *M. sutrix* was evidenced in the presence of a female specimen of *A. fraterculus* (Table I). This event was observed during the extension and supination behavior of the fly. The same analysis was performed by Rao & Díaz-Fleischer (2012), however, no results were found for spider escape.

Experiment 2

The second step evaluated the effect of the presence or absence of marks in *A. fraterculus* wings through observations made during fly and spider interactions. The Chi-square test with Yates correction for continuity ($p = 0.05$; $df = 2$) indicated that treatments painted wing (females $\chi^2 = 0.64$, males $\chi^2 = 0.25$) and highlighted bands

(females $\chi^2 = 0.33$; males $\chi^2 = 0.1$) (Figure 1) had no influence on the number of predated individuals when compared to individuals of *A. fraterculus* without marks, normal wings (Fig. 1).

The supination behavior was evidenced for both sexes of the flies, observing asynchronous movements, in which the fly alternates the movement of the wings and synchronous movements, nevertheless, a greater number of asynchronous exhibitions for males and females were found, being these respectively 947 and 928 when compared to synchronous exhibitions that were 95 and 63, respectively, with a mean per individual of 22 asynchronous exhibitions for males and 21 for females and of 6.3 synchronous exhibitions for males and 5.25 for females.

An escape event for *M. sutrix* was observed in the first experiment and two escapes were observed in the second experiment, one of these interactions in *A. fraterculus* presenting highlighted bands and another one for painted bands. This behavior was only observed with exhibitions of wing extension followed by supination.

When evaluating the occurrence of predation in the interactions of female spiders in the presence of male and female flies of the treatments: painted bands, highlighted bands and normal bands and male spiders that interacted with male and female flies of the same treatments, using the Chi-square test with Yates correction for continuity ($p = 0.05$; $df = 1$), the results were significant for non-predation and these did not vary as to the sex of the spiders (Table II).

DISCUSSION

Experiment 1

The behavioral pattern observed for *A. fraterculus* was also described by Greene et al. (1987), Mather & Roitberg (1987) and Hasson (1995) for the species *Z. vittigera*, *R. zephyria* and *C. capitata*, which escaped predation when interacting with spiders of the family Salticidae. The authors reported that flies mimicked the stereotyped behavior of salticids by means of dark bands on their wings that resemble, in the

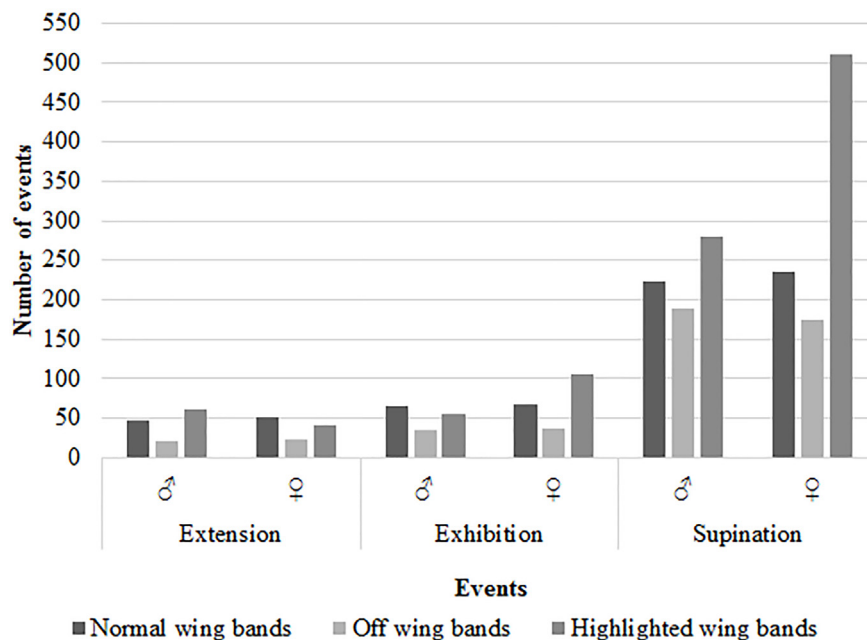


Figure 1. Number of events related to extension, exhibition and supination of *Anastrepha fraterculus* in different treatments.

Table II. Predation of the treatments *Anastrepha fraterculus* off bands, highlighted bands and normal wings by both sexes of *Megafreya sutrix* through the Chi-square test with Yates correction for continuity, probability 0.05.

	<i>Anastrepha fraterculus</i>					
	Painted wings		Highlighted wings		Normal wings	
Megafreya sutrix	Females	Males	Females	Males	Females	Males
Females	0.31	0.61	0.31	0.01	0.01	0.31
Males	0.11	0.11	0.31	0.11	0.01	0.61

authors' view, the legs of the spiders and their movements that resembled the spider displays in front of the presence of a sexual partner.

Corroborating the authors, Eisner (1985) described that the dark stains in the thorax of *Z. vittigera* were similar to the eyes of the spiders. However, it was observed for *A. fraterculus* that the low number of predated individuals is related to the aggressive behavior displayed during the interaction, since they do not present such stains in the thorax and even individuals that had their wings painted, that is, absence of wing bands, avoided predation. A similar result was found for interactions using *A. ludens* (Rao & Díaz-Fleischer 2012, Rao et al. 2014).

The results for predation demonstrate that *M. sutrix* more readily preyed upon the *M. domestica* specimens, as expected, since they do not have bands in the wings and the behaviors of tephritids, as suggested in the works of Greene et al. (1987, 1988), Mather & Roitberg (1987) and Rao & Díaz-Fleischer (2012).

The highest number of *M. domestica* individuals preyed upon by *M. sutrix* in the first minutes of interaction and the gradual reduction over time, corroborate with the results obtained by Mather & Roitberg (1987), which relate this phenomenon to the lack of mechanisms of defense similar to those found in Tephritidae. The authors also point out that the lack of defensive strategies may be one of the reasons for the greater number of predated individuals.

The cleaning behavior of body parts causes the release of compounds that would prevent predation. The authors report that mature males that produce 6-oxo-1-nonanol and females, on which such a compound was applied, are less preyed upon by geckos (Wee & Tan 2005).

Predation times were not tested by other authors for the different tephritid species. Nevertheless, Jackson (1990) associates non-predation with the precaution that the salticids have to carry out a successful attack, investing in the prey only when the probability of capture is high and decreasing when the prey is attentive.

Experiment 2

The results for the different wing band patterns tested (painted bands, highlighted bands and normal bands) showed no influence on the predation of *M. sutrix*, in this way, the fact that *A. fraterculus* is not preyed upon is not related to the pattern of wing marks but rather to the aggressive behavior (Rao & Díaz-Fleischer 2012, Rao et al. 2014) constituted by extension and supination events, as described by Headrick & Goeden (1994). The experiment demonstrated that exhibition performed by tephritids is an aggressive behavior, not an imitation of the sexual behavior of the spiders as described by Mather & Roitberg (1987), being sometimes necessary for the flies to escape by flight to avoid capture.

The trigger to start supination occurred when the fly visualized the opponent or when

the it began to move. The same was reported by Aguilar-Argüello et al. (2015) in studies with *P. audax* and *A. ludens*. When observing the type of movement carried out by the flies during supination, it was noticed that the South American fruit flies perform more asynchronous movements. Observation of movements during supination was not evaluated by other authors.

The escape behavior in Salticidae spiders was also evaluated by Rao & Díaz-Fleischer (2012) in the interaction of *A. ludens* with *P. aurantius* and *P. bidentatus*, but no results were observed for this behavior. Predation results for both male and female spiders in the presence of both sexes of *A. fraterculus* for treatments painted bands, highlighted bands and normal bands show that *M. sutrix* spiders have no preference for attacking male or female flies and do not distinguish them as for the treatments, all of which are similarly preyed upon.

CONCLUSIONS

The South American fruit fly *A. fraterculus* presents an aggressive behavior, which often prevents them from being preyed upon by *M. sutrix*, constituting the first report for this species. The aggressive behavior visualized is not associated with the presence of bands on the wings of the flies.

Predation is lower in individuals that perform extension associated with supination with greater repetition of events. The flies performed more asynchronous supination and the females were more aggressive than the males.

M. sutrix spiders have no preference or ease of predation for *A. fraterculus* females and males, nor does it distinguish wing bands in different treatments. The interactions between the Salticidae spiders and Tephritidae flies are

successful cases, in which a prey signals to the predator and avoids predation.

The present work found that the spiders of the Salticidae family still have limitations for *A. fraterculus* predations, but in natural environments it is important biological control agents for conservation since they may be important natural enemies of other organisms.

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PRISCILLA C. GOBBI

<https://orcid.org/0000-0002-0635-2440>

ADRISSE M. NUNES

<https://orcid.org/0000-0003-2566-2548>

EDISON ZEFA

<https://orcid.org/0000-0002-0317-7843>

FLAVIO R.M. GARCIA

<https://orcid.org/0000-0003-0493-1788>

Programa de Pós-Graduação em Entomologia, Instituto de Biologia, Universidade Federal de Pelotas, Departamento de Ecologia, Zoologia e Genética, Caixa Postal 354, Porto, 96010-900 Pelotas, RS, Brazil

Correspondence to: **Flávio Roberto Mello Garcia**

E-mail: flavio.garcia@ufpel.edu.br

Author contributions

FRMG, PCG and AMN conceived and planned the experiments. PCG carried out the experiments. AMN, EZ, FRMG and PCG contributed to the interpretation of the results. PCG and FRMG took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

