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SPECIAL ISSUE ARTICLE

Mating compatibility among different wild and laboratory strains of the Brazil-1 morphotype of *Anastrepha fraterculus* (Diptera: Tephritidae)

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Abstract In cases where a pest that is target of a Sterile Insect Technique (SIT) campaign is a member of a cryptic species complex, it is necessary to know in advance whether the sterile mass-reared males are sexually compatible with the wild females, otherwise the releases would result in failure to induce sterility in the target population. The South American fruit fly, commonly known as *Anastrepha fraterculus*, represents such a complex of cryptic species with at least 8 different morphotypes. From northern Argentina to southeastern areas of Brazil, the "Brazil-1" morphotype predominates and laboratory colonies have been established for its control through the use of SIT. Our goal was to assess the mating compatibility between different populations, including two wild ones from southern Brazil (Vacaria-WV; and Pelotas-WP) and laboratory strains derived from this morphotype. These included two bisexual laboratory strains (*Piracicaba*-PIRA; and *Vacaria*-VAC), and a recently developed genetic sexing strain, the GSS-89. Field cage tests with fertile flies demonstrated that PIRA flies present partial sexual incompatibility with all other strains, and therefore cannot be recommended for SIT field release. Also, males of the VAC strain, both fertile and sterile, mated randomly with WV and WP flies. No evidence of sexual isolation was found between the flies of the GSS and WV flies, but a certain level of incompatibility was shown between the fertile or sterile GSS males and WP females. Recommendations on the use of those strains through the SIT against the two southern Brazilian populations were made.

Key words field cages; fruit flies; genetic sexing strain; South American fruit fly; sterile insect technique

Introduction

In the genus *Anastrepha*, which is endemic to the Americas, the nominal species *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae), also known as the South American fruit fly, is present from Mexico to northern Argentina. It is an important pest in South American countries, causing direct damages to commercial fruit production by destroying the pulp and causing the premature fall of the attacked fruits, in addition to indirectly disrupting international trade (Rendon & Enkerlin, 2021). The list of host plants for this fruit fly comprises about 177 species belonging to 40 plant families, with the highest number of hosts being recorded in Brazil (121 species), followed by Argentina (40 species)

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(Oroño *et al.*, 2005; Hernandez-Ortiz *et al.*, 2019; Zucchi & Moraes, 2025).

Several studies using different approaches including morphometric analyses, biological and reproductive parameters, courtship behavior, chromosome karyotypes, DNA sequences, pheromone profiles and presence of *Wolbachia* have shown that, in reality, *A. frateculus* represents a complex of cryptic species, with at least eight morphotypes having been identified so far over its geographical distribution (Caceres *et al.*, 2009; Hernandez-Ortiz *et al.*, 2012; Vaníèková *et al.*, 2015; Dias *et al.*, 2016; Devescovi *et al.*, 2019; Hernandez-Ortiz *et al.*, 2019; Selivon *et al.*, 2022). The "Mexican" morphotype occurs from Mexico to Panamá, and it does not have as much economic importance in Central American countries. The "Venezuelan" morphotype is present in the Caribbean and lowlands of Venezuela. The "Andean" morphotype can be found in the highlands of Venezuela and Colombia, while the "Peruvian" morphotype is present in the Pacific coastal lowlands and Andean valleys of Peru and Ecuador. The "Ecuadorian" morphotype is distributed in the Andean highlands of Peru and Ecuador. From northern Argentina to southern and southeastern areas of Brazil, there is the "Brazil-1" morphotype, while the "Brazil-3" morphotype has been associated with coastal areas from southern, southeastern, and northeastern regions of Brazil, and the "Brazil-2" morphotype has been found in both coastal and plateau regions from the Southeast and Northeast of Brazil (Hernandez-Ortiz *et al.*, 2004; Selivon *et al.*, 2004; Hernandez-Ortiz *et al.*, 2012; Vaníèková *et al.*, 2015). Cases of sympatry mainly between Brazil-1 and Brazil-3, and between Brazil-2 and Brazil-3 morphotypes had been reported in some southeastern areas of Brazil, but with a hybridization frequency lower than 1% (Selivon *et al.*, 2022).

In the inland plateau of the Valley of Paraiba do Sul River, the three Brazilian morphotypes were found occurring in sympatry, but presenting different preferences for host fruits (Selivon *et al.*, 2022). In exotic oranges, only Brazil-2 larvae were found, while Brazil-1 and 3 individuals were detected infesting the native guavas. In general, the Brazil-1 morphotype appears to be more generalist than Brazil-2 and 3 morphotypes in terms of host fruits, but in that specific area, Brazil-1 seems to have been displaced after a competitive process against the Brazil-2 population, and larvae of the Brazil-3 morphotype have not been detected so far in oranges (Selivon *et al.*, 2022). In the context of Area-Wide Integrated Pest Management (AW-IPM), accurate knowledge of the species that is causing damage to the orchards of interest is of fundamental importance in choosing the best control methods.

Current consumer pressure for fruits free of pesticide residues has driven the search for methods that contribute to the production of healthier fruits, and a promising alternative for controlling the *A. fraterculus* complex would be the Sterile Insect Technique (SIT). This technique comprehends mass production, sterilization and inundative releases of the target pest as an autocidal, species-specific control method. After release into the field, the sterile males seek out or group together in leks (i.e., aggregation of males for the purpose of mating) to attract wild females to mate with, and if copulation is successful, the eggs laid by the females become unviable due to the dominant lethal mutations transferred by the sperm of the sterile males. By maintaining the induction of sterility over generations, the wild population will be suppressed and may eventually collapse (Dyck *et al.*, 2021). Many efforts by research groups from Argentina and Brazil, with support from the joint FAO/IAEA as well as national funding sources, have been made since 1996 to implement the SIT for *A. fraterculus*, and significant advances have been achieved mainly with regard to the mass rearing and sterilization of flies (Cladera *et al.*, 2014; Mastrangelo *et al.*, 2018; Kovaleski & Mastrangelo, 2021; Mastrangelo *et al.*, 2021).

For the SIT to be applied more efficiently against *A. fraterculus* populations, the ideal would be to release only sterile males into the field. This is because male-only releases prevent males from mating with sterile females, what distracts them from seeking and mating with wild females (McInnis *et al.*, 1994; Orozco *et al.*, 2013; Shelly & Manoukis, 2022). But for this to be possible, it is necessary to eliminate the females or distinguish the males during the production process in the mass-rearing facility. This can be achieved through the development of genetic sexing strains (GSSs), in which sex separation can be enabled via genetic mechanisms or mutations, such as pupal color or developmental rate (Franz *et al.*, 2021). Recently, the black pupae (*bp*) gene was used as a selectable marker for the development of a GSS of *A. fraterculus* from the Brazil-1 morphotype, named GSS-89. In this GSS, the black pupae phenotype was characterized by the black color of the female pupae, as well as the dark coloration and wing veins of adult females, while the males present the wild-type phenotype and emerge from brown pupae (Meza *et al.*, 2020). This strain is now being adapted for mass rearing in both Argentina and Brazil (Kovaleski & Mastrangelo, 2021), but little is known about the sexual compatibility of its sterile males with wild populations from those countries.

A prerequisite for area-wide application of the SIT against the *A. fraterculus* complex is to know what morphotype would be present in the target area, and whether

sterile, lab reared males would be sexually compatible with that population. If sterile males of the "wrong" morphotype or from a less competitive strain are released, this would fail to induce sterility into the target *A. fraterculus* population. Therefore, the aim of this study was to assess the mating compatibility among different wild and laboratory strains of the Brazil-1 morphotype of *A. fraterculus* to check if there would be incompatibility between any of them and if sterile males from at least one of the laboratory strains could be used for field control.

Materials and methods

Insect strains

Individuals from five different populations of *A. fraterculus* (described below), all derived from the Brazil-1 morphotype, were used in this study. The laboratory colonies used in this study were reared at 24–26 °C and 50%–80% RH under a 12 h light : 12 h dark cycle. Wild flies were maintained in small screened cages (30 cm × 30 cm × 30 cm), with adult diet (a mix of sugar, wheat germ and yeast Bionis® YE MF at 3 : 1 : 1) and water *ad libitum*, and multiplied in papaya (*Carica papaya*) (Gayle *et al.*, 2013) for one or two generations to provide sufficient flies for the tests.

The wild flies were collected as pupae from infested peaches (*Prunus persica* L. Batsch) and strawberry guava (*Psidium cattleianum* Sabine) at Pelotas (31°46′19″S, 52°20′34″W) and from infested guabiroba (*Campomanesia xanthocarpa* Berg) and pineapple guava (*Feijoa sellowiana* Berg) at Vacaria, Rio Grande do Sul state, where the largest peach and apple producers in Brazil are located, respectively. Most of the wild pupa lots used for the tests were received in November–December 2021, February–March 2022 and April–May 2023.

A laboratory strain representing long-term mass rearing, the *Piracicaba* strain (PIRA), was domesticated as described by Walder *et al.* (2014). It has been maintained at the Food Irradiation & Radioentomology Laboratory of the Center for Nuclear Energy in Agriculture of the University of São Paulo (CENA/USP) under continuous rearing with artificial conditions, without any introduction of wild flies (or "refreshment") for 133 generations until the tests were carried out.

The another bisexual *Vacaria* strain (VAC) used in this study had been established with wild flies from Vacaria in late 2015 and maintained since then at CENA/USP under semimass rearing conditions according to the rearing protocol of Mastrangelo *et al.* (2021). Flies from the 14th, 17th, and 25th generations of this colony were used for the tests. Pupae were irradiated 2 d before adult emergence under normoxia with 40 Gy of gamma rays from a *Gammabeam*-650® irradiator (MDS Nordion International Inc., Canada), with 2.02 Tbq (54.7 Ci) and a dose rate of 1.4 Gy/min at the beginning of the tests, to obtain males with 99% sterility and females fully sterile (Mastrangelo *et al.*, 2018). These conditions were used because the radiation dose required to render *A. fraterculus* males 99% sterile under hypoxia is much higher than that under normoxia (74 × 40 Gy, respectively) (Giustina *et al.*, 2021).

The *genetic sexing strain* (GSS) of *A. fraterculus* based on a pupal color dimorphism (i.e., adult males emerge from brown pupae, while females come from black pupae), named GSS-89, was developed from *bp* mutants found in a bisexual colony with flies originally from Vacaria (Meza *et al.*, 2020). It was imported to CENA/USP in 2020 and a stable colony, free of recombinants, has been maintained since middle 2021 after the implementation of a Filter Rearing System (FRS) (Franz *et al.*, 2021), through which all pupae are selected by color, as well as adults that are destined for the mother colony. It has been maintained under the same rearing conditions of the bisexual strains (Mastrangelo *et al.*, 2021), and flies from the 5th to 7th generations were used in the tests. A dose of 74 Gy of gamma rays from the same irradiator, but with the irradiation room cooled to 15 ± 3 °C, was used to sterilize brown pupae (48 h before emergence) under hypoxia (Giustina *et al.*, 2021) to provide sterile males for some of the sexual compatibility tests. These conditions were used since the dose that induces 99% sterility in males of this strain under normoxia has not yet been determined or published. All strains were kept isolated from each other. Quality control parameters of the strains were monitored following the procedures of FAO/IAEA/USDA (2019).

Mating compatibility between fertile flies of the different strains

Mating tests between mass-reared flies from CENA (*Vacaria* strain-VAC; *Piracicaba* strain-PIRA; the GSS-89-GSS) and wild flies from the southern regions of Vacaria (WV) and Pelotas (WP) were carried out in circular, nylon-screened field cages (3 m diameter × 2 m high). A potted citrus tree (*Citrus sinensis* L. cv. Bahia) was placed in the center of each field cage. The trees, ca. 2 m in height and a canopy of 1 m in diameter without flowers or fruits, were lightly pruned before the tests (FAO/IAEA/USDA, 2019).

Nine different pairwise combinations with fertile males and females from the five strains were evaluated: WV vs. PIRA; WP vs. PIRA; VAC vs. PIRA; PIRA vs. GSS; WV vs. VAC; WP vs. VAC; WV vs. GSS; WP vs. GSS; VAC vs. GSS.

In each field cage, 25 females and 25 males of each strain were released. The released laboratory flies were 9–10 d old, while 15- to 17-d-old wild flies were used (Petit-Marty *et al.*, 2004; Abraham *et al.*, 2011; Mastrangelo *et al.*, 2018). Forty-eight hours before the test, flies were marked individually with a small dot of water-based paint on the dorsal surface of the thorax (FAO/IAEA/USDA, 2019). On the day of the test, the males were released first into the cages to give them the opportunity to disperse, dead flies were replaced, and the females were released 20 min later. Observations were made from 8.00 to 11.00 am (Abraham *et al.*, 2011; Mastrangelo *et al.*, 2021) by one person per cage, and throughout the observation period, the mating pairs were collected in 30-mL glass vials for later identification. Each cage was considered as a replicate, and each pairwise combination was replicated at 8–12 times.

Mating compatibility between sterile and wild flies

To verify whether VAC sterile flies or GSS sterile males would be sexually compatible with wild flies, additional field cage tests with sterile flies from these strains and fertile WV and WP flies were set at the following combinations: VAC (♂ and ♀, both sterile) vs. WV (♂ and ♀); VAC (♂ and ♀, both sterile) vs. WP (♂ and ♀); GSS (sterile ♂) vs. WV (♂ and ♀); and GSS (sterile ♂) vs. WP (♂ and ♀).

In tests with sterile VAC and wild flies, 25 females and 25 males of each strain were released into the field cage. In the tests with the GSS, 25 females and 25 males of wild flies were release in each field cage together with 25 sterile GSS males (GSS females were not released). Laboratory and wild flies were released at the same ages as mentioned in the previous section, and were individually marked using the same methodology described previously. Observations were also made from 8:00 to 11:00 am by one person per cage, and the mating pairs were collected in 30-mL glass vials for later identification. Each cage was considered as a replicate, and each pairwise combination was replicated at 8–12 times.

Data analyses

The index of sexual isolation (ISI), male and female relative performance indices (MRPI and FRPI) and the relative isolation index (RII) were estimated (McInnis *et al.*, 1996; Cayol *et al.*, 1999; FAO/IAEA/USDA, 2019). Values for the isolation index (ISI) can range from -1 to $+1$, with values close to -1 or $+1$ indicating complete negative or positive assortative matings (or sexual isolation) while values closer to 0 indicate sexual compatibility. The relative isolation index (RII) provides an indication of mating compatibility between two strains. An RII value of 1 means random mating, while larger values indicate assortative mating. One of the disadvantages of the RII is that it is undefined if the number of matings is zero for at least one of the heterotypic mating combinations (♂ strain 1 + ♀ strain 2; ♂ strain 2 + ♀ strain 1). The male and female relative performance indices (MRPI and FRPI) range from -1 to $+1$, showing the proportion of males and females of each strain taking part in matings, and it can be used to help interpret values of ISI over 0.5 (Parker *et al.*, 2021).

The one-way analysis of variance *F*-test was applied for the sexual indices (ISI, RII, MRPI, FRPI) estimated from field cages with fertile flies at the 5% of significance (ANOVA) and, when significant differences were detected, the Tukey's honestly significance difference (HSD) test ($\alpha = 0.01$) was applied to compare the means. The mean percentages of heterotypic and homotypic mating pairs for each mating compatibility test obtained using fertile flies from each of the five different strains were compared using the Tukey's significant difference (HSD) test ($\alpha = 0.01$).

The one-sample *t*-test was used to verify if the mean values of the ISI, MRPI, and FRPI indices estimated from field tests with sterile flies significantly differed from 0, or 1 in the case of the RII ($\alpha = 0.01$). The mean percentages of heterotypic and homotypic mating pairs from field cages with wild flies and sterile VAC flies were also compared by the Tukey's test ($\alpha = 0.01$), while the percentages of mating pairs from cages with wild flies and sterile GSS males were compared separately by the Student's *t*-test ($\alpha = 0.01$). All analyses were performed with the statistical program SAS 9.4 (PROC GLM, SAS Institute, 2013).

Results

Mating compatibility between fertile flies of the different strains

The indices of mating compatibility and performance estimated from field cages containing only fertile flies from the different strains are presented in Table 1.

Table 1 Indices of sexual compatibility (means ± SE) for *Anastrepha fraterculus* flies from different populations maintained at CENA/USP (*Vacaria* strain = VAC; *Piracicaba* strain = PIRA; the GSS-89 = GSS; wild Vacaria = WV; and wild Pelotas = WP).

Population		Indices of compatibility			
		ISI[†]	RII[‡]	MRPI	FRPI
PIRA ×	WV	0.54 ± 0.3	13.5 ± 11.5	0.125 ± 0.13	0.33 ± 0.17 a
	WP	0.6 ± 0.17	–	−0.19 ± 0.52	0.21 ± 0.35 a
	VAC	0.52 ± 0.1	7.3 ± 0.7	−0.0096 ± 0.24	−0.24 ± 0.01 ab
	GSS	0.28 ± 0.26	–	−0.24 ± 0.28	−0.95 ± 0.05 b
ANOVA		$F = 0.35$; $P = 0.78$	$F = 22.6$; $P = 0.13$	$F = 0.25$; $P = 0.86$	$F = 18.7$; $P = 0.0019$
GSS ×	WV	0.17 ± 0.09	1.42 ± 0.35	0.18 ± 0.2 a[§]	−0.014 ± 0.13
	WP	0.29 ± 0.1	13.0 ± 8.02	−0.24 ± 0.11 b	−0.61 ± 0.24
	VAC	0.14 ± 0.2	2.54 ± 1.4	0.19 ± 0.1 ab	0.012 ± 0.2
VAC ×	WV	−0.0064 ± 0.12	1.2 ± 0.38	0.27 ± 0.05 a	0.038 ± 0.07
	WP	0.055 ± 0.16	2.34 ± 1.16	−0.045 ± 0.08 ab	0.063 ± 0.11
ANOVA		$F = 0.56$; $P = 0.69$	$F = 3.8$; $P = 0.02$	$F = 5.8$; $P = 0.003$	$F = 3.6$; $P = 0.02$

[†] Index of Sexual Isolation = ISI; Relative Isolation Index = RII; Male Relative Performance Index = MRPI; Female Relative Performance Index = FRPI (FAO/IAEA/USDA, 2019).

[‡] The RII was undefined in field cages in which no crosses were observed for one of the heterotypic combinations.

[§] Means (± SE) followed by the same letters in the columns do not differ significantly by the Tukey's test ($P > 0.01$).

Results revealed that males from the *Piracicaba* strain (PIRA) are partially compatible with females from the VAC strain and wild females from Vacaria (WV), but practically incompatible with wild females from Pelotas (WP) (Table 1). The mean ISI values estimated for the crosses between those three strains were all higher than "0.5," indicating sexual isolation. The relative isolation index (RII) from field cages with PIRA and those two strains were also far from "1," demonstrating the occurrence of assortative matings. PIRA and GSS were also partially compatible, with a mean ISI value closer to zero compared to the values of other combinations (although not significant), with plenty of copulations between GSS males and PIRA females (Fig. 1).

For the four pairwise combinations of matings between the PIRA and other strains, no significant differences were detected for the ISI, RII, and MRPI indices. However, the mean FRPI index value between PIRA and the GSS was more negative, indicating the preference of matings only with PIRA females (Table 1). No mating was observed between PIRA males and black GSS females. Finally, the GSS males preferred to mate with PIRA females in the field cages ($F = 20.5$; $P = 4.10^{-4}$) (Fig. 1).

In field cages with PIRA fertile flies, the percentages of matings for homotypic combinations (♂ WV × ♀ WV; ♂ WP × ♀ WP; and ♂ PIRA × ♀ PIRA) were high, with significant differences when compared to some cases of heterotypical couples: ♂ PIRA × ♀ PIRA *vs*. ♂ WV × ♀ PIRA and ♂ PIRA × ♀ WV ($F = 4.6$; $P = 0.008$); and ♂ VAC × ♀ VAC *vs*. ♂ VAC × ♀ PIRA ($F = 10.2$; $P = 0.024$) (Fig. 1). Matings between wild males or VAC males and PIRA females were observed, but the later preferred to mate with males of the same strain. Wild females had mated preferably with wild males, and no PIRA male managed to mate with a WP female ($F = 13.7$; $P = 0.014$) (Fig. 1).

Fertile flies from the VAC strain and the GSS-89 were compatible with WV flies, as the mean ISI and MRPI values were around zero (Table 1). There was no difference in mating percentages between the GSS, WV and VAC strains, and no mating incompatibility was found between VAC and WP either (Table 2).

A certain level of incompatibility, although not significant for the ISI index ($F = 0.56$; $P = 0.69$), was verified between the GSS and WP, as very few matings were observed between WP males and GSS females (Table 2). The MRPI and FRPI indices also indicated the greater participation of WP males and females in matings compared to the GSS flies (Tables 1 and 2). Although being marginally significant (at $P \leq 1\%$), the RII for the combination GSS × WP was far from "1" and much larger than the others, corroborating the existence of assortative mating between the two strains (Table 1). It is worth noting that, although there was no significant difference, the black GSS females also participated less in matings with WV and VAC males (Table 2).

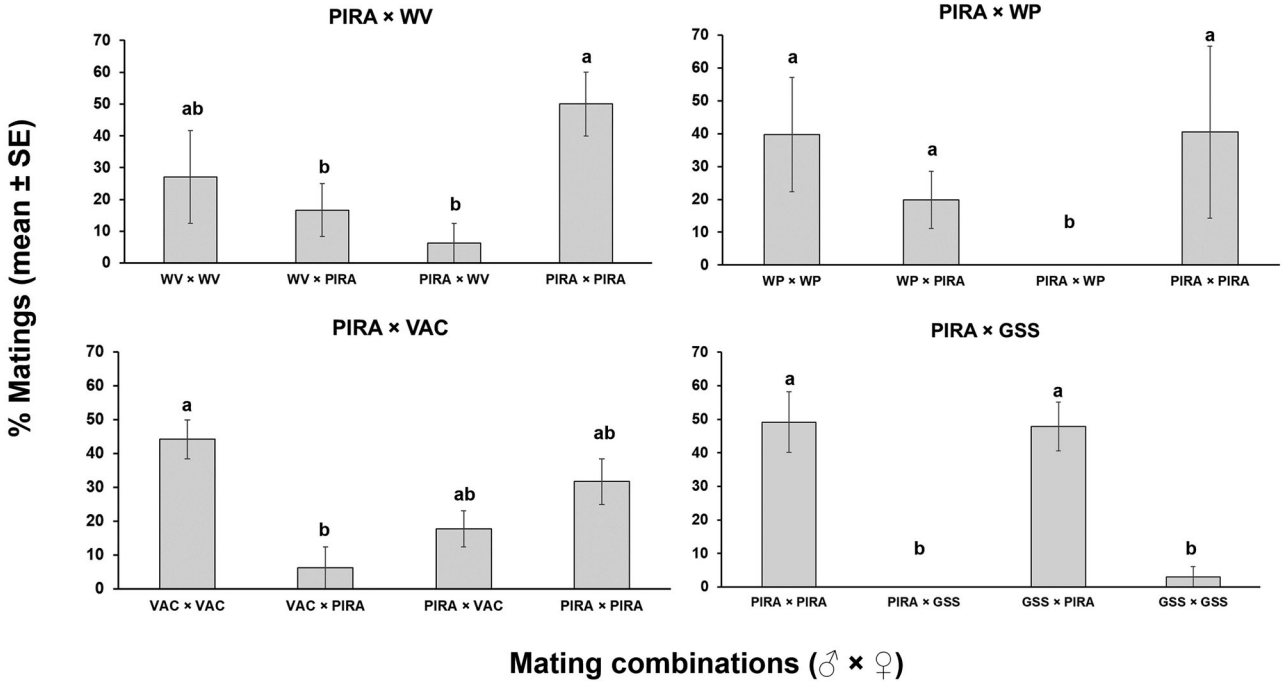

Fig. 1 Percentages of matings (means ± SE) for each mating compatibility test between fertile flies of the Piracicaba strain (PIRA) and flies from four other strains (wild Vacaria = WV; wild Pelotas = WP; *Vacaria* strain = VAC; and GSS-89 = GSS). Error bars represent standard errors from the percentages of matings. Different letters above the error bars indicate significant differences by Tukey's test ($P < 0.01$).

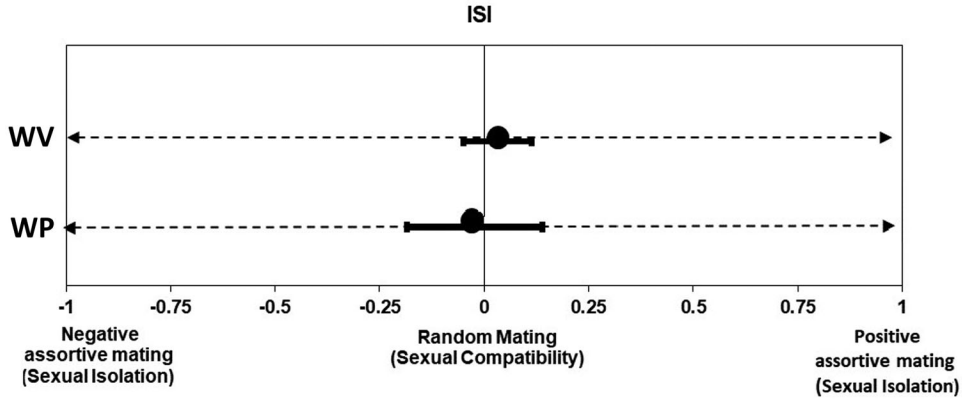

Fig. 2 Isolation Index (ISI) (means ± SE) estimates between sterile flies from the *Vacaria* strain (VAC) and wild flies from Vacaria (WV) and Pelotas (WP).

Mating compatibility between sterile and wild flies

The results from the field cage tests with sterile flies of the bisexual VAC strain and wild flies (WV and WP) are shown in Figs. 2–4 and Table 3. The mean ISI values did not differ significantly from "0," suggesting that wild females mated randomly with wild or sterile VAC males (Table 3). The mean relative isolation index (RII) was around 1.7 and did not differ significantly from "1," also indicating an acceptable level of sexual compatibility between strains. MRPI and FRPI values were close to "0," suggesting equal mating propensity in the strains. However, the FRPI estimated for the combination VAC (sterile) × WV differed significantly from "0" ($t = 9.03$;

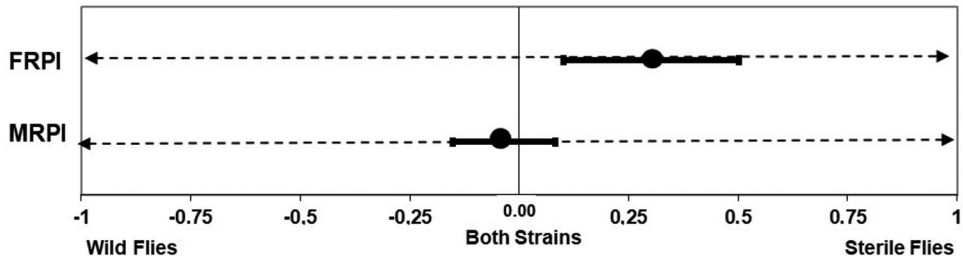

Fig. 3 Isolation Female and male relative performance indices (FRPI, MRPI) for sterile flies from the *Vacaria* strain (VAC) and wild flies from Vacaria (WV).

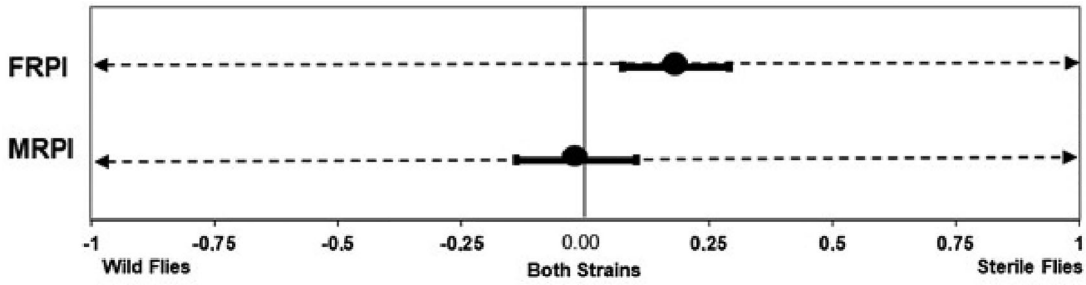

Fig. 4 Isolation Female and male relative performance indices (FRPI, MRPI) for sterile flies from the *Vacaria* strain (VAC) and wild flies from Pelotas (WP).

$P = 0.0029$), indicating that sterile VAC females participate more in matings (Table 3). In overall, these results showed that sterile VAC flies are still compatible with wild females from both regions.

In the field cage tests performed with sterile males from the GSS-89 and wild flies (WV and WP), no evidence of sexual isolation was found between the sterile GSS males of the GSS and WV flies, as the ISI and MRPI values were close zero the percentages of matings were around 50% (Table 3). However, with regard to matings with wild females from Pelotas (WP), the sterile GSS males had difficulty competing with fertile WP males for the females (Fig. 5). The percentage of matings between sterile GSS males and WP females was significantly lower ($F = 76.4$; $P < 10^{-3}$) and the ISI index was higher than "0" ($t = 4.7$; $P = 0.0053$), suggesting sexual isolation between the strains (Table 3).

Discussion

As an initial step for efficient application of an AW-IPM approach integrating the SIT against the South American fruit fly in Brazil, our study assessed the degree of sexual compatibility among individuals of several strains of *A. fraterculus*, including two southern Brazilian populations, two bisexual laboratory strains and a recently developed GSS. Although these populations belong to the Brazil-1 morphotype, or their colonies were originally established with individuals of this same morphotype, evidence of mating incompatibility was found between some of the strains.

The *Piracicaba* strain presented partial sexual incompatibility with all other strains tested in this study. The reasons for this may reflect its establishment. PIRA was the first *A. fraterculus* strain of the Brazil-1 morphotype to be domesticated and adapted to mass-rearing conditions in Brazil (Walder *et al.*, 2014). Its parental pupae came from a single host, "uvaia" fruits (*Eugenia pyriformis* Cambess.), the size of its founding population was very small (e.g., 262 parental females and only 42 F_1 females), no introduction of wild males (or "colony refreshment") was made since its establishment, and the colony had suffered at least 3 genetic bottlenecks over the years (Costa, personal communication).

Long-term rearing under laboratory conditions can provoke changes in tephritids after the selection for certain characters, such as rapid mating, courtship simplification, higher fecundity, earlier reproduction, lower longevity, reduced female discrimination, misalignment of periods of sexual activity and changes in the genetic diversity of colonies (Cayol, 2000; Briceño & Eberhard, 2002; Rull *et al.*, 2005; Gilchrist *et al.*, 2012; Meza

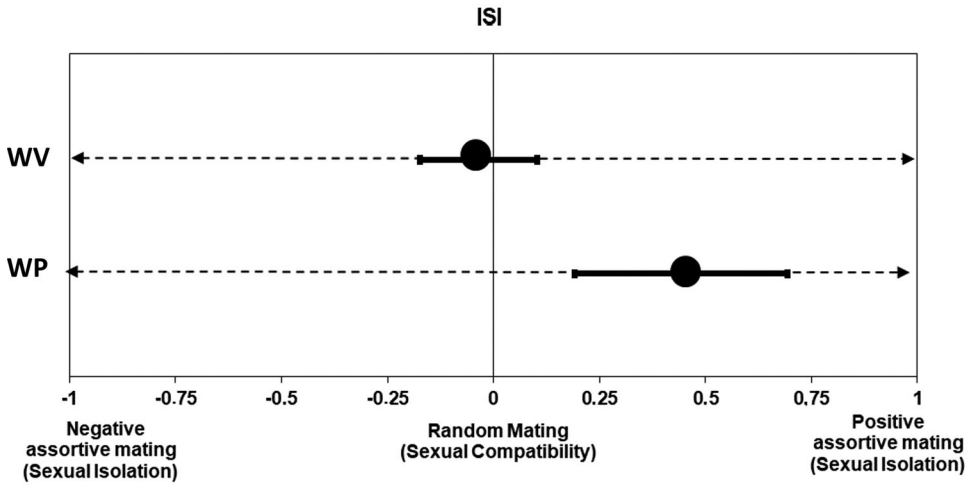

Fig. 5 Isolation Index (ISI) (means ± SE) between sterile males of the GSS-89 (GSS) and wild flies from Vacaria (WV) and Pelotas (WP).

et al., 2014; Parrreño *et al.*, 2014; Zygouridis *et al.*, 2014; Parker *et al.*, 2021; Gomes *et al.*, 2024). It is possible that the *Piracicaba* strain has accumulated enough genetic and/or behavioral changes over the years that caused her males to be discriminated against by females of other strains.

For mate selection in *A. fraterculus*, when a female approaches a lek, the males display a series of species-specific, stereotyped, courtship behaviors, which includes rapid wing movements, pheromone release and acoustic signals, with the aim of being chosen by the female (Gomez Cendra *et al.*, 2011; Segura *et al.*, 2018). When the male and female face each other at a shorter distance, several other interactions take place, such as wing signaling, mounting attempts, and even fights when one of the partners is not receptive (Calcagno & Vilardi, 2001). Considering the three Brazilian morphotypes known, it has been demonstrated that differences in the behavioral sequences that lead up to copulation are very important in the reproductive isolation among them (Roriz *et al.*, 2018), and evidence of isolation of the PIRA strain from other *A. fraterculus* populations had already been noted in previous studies.

For instance, Vera *et al.* (2006) verified partial sexual incompatibility and unequal participation of males within leks between Piracicaba and populations from Tucuman, Argentina, and La Molina, Peru. Also, Dias *et al.* (2016), using flies from the 50th generation of the *Piracicaba* strain, showed that the PIRA population was behaviorally, genetically, and morphologically different from four other populations in southern Brazil. As in our study, PIRA females had exhibited mate preference for conspecific males in field cages, and ISI values between pairs from PIRA and populations from the South of Brazil were above 0.5. PIRA also differed in the frequency of courtship displays and certain aspects of the calling song during courtship and mounting acoustic signal (i.e., presenting higher fundamental frequencies) (Dias *et al.*, 2016).

Further studies would be necessary to confirm whether those characteristics were maintained in the PIRA strain, or even whether it accumulated more behavioral or genetic differences in relation to other strains and morphotypes of the *A. frateculus* complex. Regardless, our results demonstrated once again the occurrence of partial prezygotic reproductive isolation of the *Piracicaba* strain with wild populations of southern Brazil, especially the population from Pelotas (Fig. 1), confirming that males of this strain should not be recommended for SIT programs in this part of Brazil.

In contrast, males of the *Vacaria* bisexual strain, both fertile and sterile, mated randomly with wild flies from Vacaria and Pelotas (Table 1, Figs. 2–5). The ISI, MRPI and FRPI values were always around zero, indicating sexual compatibility and equal mating propensity of wild and sterile flies. Previous studies with populations from Argentina or southern Brazil, belonging to the same Brazil-1 morphotype, also showed that they were not sexually isolated from each other (Rull *et al.*, 2012; Rull *et al.*, 2013; Dias *et al.*, 2016). Results from Dias *et al.* (2016) suggested full mating compatibility among the tested populations from southern Brazil (e.g., Vacaria, Pelotas, Bento Gonçalves, and São Joaquim). Mastrangelo *et al.*

Table 2 Percentages of matings (means ± SE) for each mating compatibility test with fertile flies from different strains of *Anastrepha fraterculus* (*Vacaria* strain = VAC; GSS-89 = GSS; wild Vacaria = WV; and wild Pelotas = WP).

	Strains	Mating combination (♂ × ♀)	Matings
GSS ×	WV	WV × WV	25.0% ± 6.5%
		WV × GSS	15.9% ± 4.7%
		GSS × WV	25.8% ± 5.6%
		GSS × GSS	33.4% ± 8.5%
		ANOVA, $F = 1.23$; $P = 0.32$	
	WP	WP × WP	54.4% ± 6.8% a[†]
		WP × GSS	6.2% ± 4.7% c
		GSS × WP	29.2% ± 8.7% ab
		GSS × GSS	10.7% ± 5.7% bc
		ANOVA, $F = 10.5$; $P < 10^{-3}$	
	VAC	VAC × VAC	23.4% ± 5.3%
		VAC × GSS	17.0% ± 5.9%
		GSS × VAC	26.0% ± 6.2%
		GSS × GSS	33.6% ± 6.5%
		ANOVA, $F = 1.4$; $P = 0.29$	
VAC ×	WV	VAC × VAC	17.1% ± 4.7%
		VAC × WV	19.3% ± 3.4%
		WV × VAC	31.0% ± 5.5%
		WV × WV	32.6% ± 6.6%
		ANOVA, $F = 4.8$; $P = 0.20$	
	WP	VAC × VAC	25.9% ± 2.7%
		VAC × WP	26.3% ± 4.3%
		WP × VAC	20.9% ± 5.8%
		WP × WP	26.8% ± 7.0%
		ANOVA, $F = 0.43$; $P = 0.74$	

[†] Means (± SE) followed by the same letters in the columns do not differ significantly by the Tukey's test ($P > 0.01$).

(2021) showed that sterile males from the *Vacaria* strain (from the 24th generation) were as effective at obtaining mates as wild males, with mean ISI and MRPI values close to zero, and a mean relative isolation index (RII) close to 1.0, also indicated random mating. The fact that the mother colony of this strain was kept under more relaxed conditions (Mastrangelo *et al.*, 2021) and underwent refreshment with wild males from the region of Vacaria at several different times may have contributed to the nonaccumulation of undesirable traits, avoiding the manifestation so far of different forms of pre- or postzygotic reproductive isolation with wild southern populations.

In the field cage tests with fertile couples, no mating incompatibility was found between the GSS-89 and wild flies or VAC, but the black GSS females participated in fewer matings with males of these strains (only up to 23% of matings or less). The black pupae phenotype of the GSS-89 is characterized not only by the black color of the pupae, but also by the very dark color and wing veins of the adult females compared to the wild type Brazil-1 phenotype (Meza *et al.*, 2020), and this could possibly impact visual recognition by wild males. It has previously been shown that morphological characteristics can serve as important visual cues in courtship processes of tephritds (Li *et al.*, 2023).

The black pupae (*bp*) mutation used as a selectable marker in the development of the GSS-89 acts through the absence of expression of the *ebony* gene. Under normal circumstances, this gene plays an important role in inhibiting black melanization in adult flies, but deletions at the *ebony* locus lead to excessive melanization, besides pleiotropic effects on both embryo viability and adult development of tephritids (Paulo *et al.*, 2024). The fact that the abundance of cuticular hydrocarbons can be influenced by the *ebony* gene (Massey *et al.*, 2019; Lamb *et al.*, 2020) may also have contributed to the greater number of unsuccessful mating attempts with black GSS females. From a practical standpoint, however, the smaller number of matings with black females of the GSS-89 is irrelevant, since they are not released into the field. What matters in the context of SIT is that GSS males remain compatible with wild females, and routine field cage testing should be maintained to monitor both the compatibility and competitiveness of the GSS males over generations.

Sterile males from the GSS-89 were as effective at obtaining mates as WV males, which was expected since it was developed from a population of Vacaria (Meza *et al.*, 2020). Sterile males of the brown-black pupa GSS Tapachula-7 of *A. ludens* have demonstrated comparable mating success to wild males or standard sterile males of a bisexual strain (Quintero-Fong *et al.*, 2018; Contreras-Navarro *et al.*, 2020). The males of a novel pupal color-based GSS of *A. ludens*, the GUA10, have shown even better mating indexes compared to males of the Tapachula-7 strain (Ramírez-Santos *et al.*, 2021).

Although no sexual incompatibility was observed between sterile VAC males and the WV and WP populations, and between sterile GSS males and WV, it may

Table 3 Percentages of matings (means ± SE) for each mating compatibility test between wild flies and sterile flies from a bisexual strain or a genetic sexing strain (*Vacaria* strain = VAC; GSS-89 = GSS; wild Vacaria = WV; and wild Pelotas = WP).

Strains		Mating combination (♂ × ♀)	Matings	Indices of Compatibility			
				ISI	RII	MRPI	FRPI
VAC (sterile)	× WV	WV × WV	19.1% ± 5.6%	0.03 ± 0.1	1.7 ± 0.69	−0.035 ± 0.12	0.30 ± 0.2
		WV × VAC	32.6% ± 3.4%				
		VAC × WV	15.8% ± 6.4%				
		VAC × VAC	32.5% ± 4.9%				
		ANOVA	$F = 4.6; P = 0.03$	$t = 0.39; P = 0.72$‡	$t = 1.07; P = 0.36$	$t = -0.29; P = 0.78$	$t = 9.03; P = 0.003$
	× WP	WP × WP	20.3% ± 5.2%	−0.02 ± 0.16	1.74 ± 0.7	−0.016 ± 0.12	0.19 ± 0.11
		WP × VAC	30.6% ± 6.6%				
		VAC × WP	20.5% ± 4.6%				
		VAC × VAC	28.7% ± 6.3%				
		ANOVA	$F = 0.89; P = 0.47$	$t = -0.13; P = 0.89$	$t = 1.03; P = 0.35$	$t = -0.14; P = 0.89$	$t = 1.7; P = 0.15$
GSS (sterile)	× WV	WV × WV	48.3% ± 6.9%	−0.03 ± 0.14	–	0.034 ± 0.14	–
		GSS × WV	51.7% ± 7.0%				
		ANOVA	$F = 0.12; P = 0.73$	$t = 1.0; P = 0.35$		$t = 0.25; P = 0.81$	
	× WP	WP × WP	75.6% ± 4.1% a†	0.44 ± 0.25	–	−0.44 ± 0.25	–
		GSS × WP	24.4% ± 4.6% b				
		ANOVA	$F = 76.4; P < 10^{-3}$	$t = 4.7; P = 0.0053$		$t = -4.7; P = 0.005$	

† Means (± SE) from percentages of matings followed by the same letters in the columns do not differ significantly by the Tukey's test in comparisons with sterile flies from Vacaria or by the Student's *t*-test in comparisons with sterile GSS males ($P > 0.01$).

‡ For the ISI, MRPI, and FRPI indices, the one-sample *t*-test was used to verify if the mean values (± SE) of them significantly differed from 0, or 1 in the case of the RII ($\alpha = 0.01$).

be that the relatively lower (but not significant) percentage of matings between sterile VAC males and WV or WP females (Table 3) was influenced in some way by the fact that VAC pupae were irradiated under normoxia. The radiological protective effects of oxygen-reduced atmosphere (hypoxia) to tephritid pupae during irradiation at low temperatures (< 20 °C) are well known and can even promote modest improvements in mating performance as in the case of *Anastrepha suspensa* (Loew, 1862) (Lopez-Martinez & Hahn, 2014; Dias *et al.*, 2021). However, higher doses are required under hypoxia to induce comparable reproductive sterility than in normoxia (Bakri *et al.*, 2021), and exposure times greater than 60 min have been avoided in the *Gammabeam*-650® irradiator used in this study to prevent the air compressors that keep the ^{60}Co capsules exposed from overheating or burning out. Since until now the sterilizing doses for males of the GSS-89 have only been determined under hypoxia (Giustina *et al.*, 2021), the irradiation room had to be momentarily cooled and the male GSS pupae irradiated under hypoxia with the recommended dose of 74 Gy. In future studies, it would be interesting to test whether both VAC and GSS males would be more or less competitive if irradiated under normoxia or hypoxia conditions.

Unlike the results observed with WV females, the percentage of matings between GSS males and WP females was significantly lower than the percentage of matings between wild males and females from Pelotas (Table 3). The tests with sterile GSS males served to confirm that exists a sexual incompatibility between the GSS and WP, as the ISI and RII indices from field cage tests with fertile flies from both strains were not so close to "0" and "1," respectively (Table 1). It is possible that WP females were able to discriminate against GSS males through divergence in courtship or male-produced pheromones, but additional studies would be needed to prove this. Differences in pheromone composition of flies from Pelotas compared to other southern populations (Bento Gonçalves and São Joaquim) and PIRA had already been identified (Brizova *et al.*, 2013). Also, despite the sexual compatibility observed between populations of Vacaria and Pelotas by Dias *et al.* (2016), the authors noticed that the flies from Pelotas mated faster than flies from Vacaria. These first maters also presented lower frequencies in two behavioral units of courtship behavior (e.g., fanning and spin).

In conclusion, for SIT applications, our results indicated the strains from which sterile males could be readily be used for control programs against the two wild southern Brazilian populations tested here. Regarding the GSS, however, our results also showed that at this time, sterile males from this strain could only be used effectively against the population from the largest apple-producing region in Brazil, Vacaria. In order for GSS males to be used in a SIT campaign in the peach-producing region of Pelotas, an introgression of genes from wild males from the target population into the genetic background of the GSS-89 would be recommended.

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Disclosure

All the authors confirm there is no conflict of interest.

References

Abraham, S., Goane, L., Rull, J., Cladera, J., Willink, E. and Vera, M.T. (2011) Multiple mating in *Anastrepha fraterculus* females and its relationship with fecundity and fertility. *Entomologia Experimentalis et Applicata*, 141, 15–24.

Bakri, A., Mehta, K. and Lance, D.R. (2021) Sterilizing insects with ionizing radiation. In *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management*. 2nd edn (eds. V.A. Dyck, J. Hendrichs & A.S. Robinson), pp. 355–398. CRC Press: Boca Raton, FL, USA.

Briceño, R.D. and Eberhard, W.G. (2002) Decisions during courtship by male and female medflies (Diptera, Tephritidae): correlated changes in male behavior and female acceptance criteria in mass-reared flies. *Florida Entomologist*, 85, 14–31.

Brizova, R., Mendonca, A.L., Vanickova, L., Silva, C.E.D., Tomcala, A., Paranhos, B.A.J. *et al*. (2013) Pheromone analyses of the *Anastrepha fraterculus* (Diptera: Tephritidae) cryptic species complex. *Florida Entomologist*, 96, 1107–1115.

Cáceres, C., Segura, D.F., Vera, M.T., Wornoayporn, V., Cladera, J.L., Teal, P. *et al*. (2009) Incipient speciation revealed in

Anastrepha fraterculus (Diptera, Tephritidae) by studies on mating compatibility, sex pheromones, hybridization and cytology. *Biological Journal of the Linnean Society*, 97, 152–165.

Calcagno, G. and Vilardi, J.C. (2001) Basic studies on Argentinean populations of *Anastrepha fraterculus* in support of pest control program: III. Preliminary analysis of mating behaviour by video recording. In *Working Material. Quality Assurance of Mass Produced and Released Fruit Flies for SIT Programmes*. IAEA.

Cayol, J.P., Vilardi, J.C., Rial, E. and Vera, M.T. (1999) New indices and methods to measure the sexual compatibility and mating performance of medfly (Diptera: Tephritidae) laboratory reared strains under field cage conditions. *Journal of Economic Entomology*, 92, 140–145.

Cayol, J.P. (2000) Changes in sexual behavior and life history traits of tephritid species caused by mass-rearing processes. In *Fruit Flies (Tephritidae): Phylogeny and Evolution of Behaviour* (eds. M. Aluja & A.L. Norrbom), pp. 843–860. CRC Press, Boca Raton, FL, USA.

Contreras-Navarro, Y., Pérez-Staples, D., Orozco-Dávila, D. and Díaz-Fleischer, F. (2020) Pre- and post-copulatory competitiveness of the genetic sexing strain Tapachula-7 of *Anastrepha ludens* (Diptera: Tephritidae). *Journal of Economic Entomology*, 113, 2163–2170.

Cladera, J., Vilardi, J., Juri, M., Paulin, L., Giardini, M., Gómez Cendra, P. *et al*. (2014) Genetics and biology of *Anastrepha fraterculus*: research supporting the use of the sterile insect technique (SIT) to control this pest in Argentina. *BMC Genetics*, 15, S12.

Devescovi, F., Conte, C.A., Augustinos, A., Martinez, E.I.C., Segura, D.F., Caceres, C. *et al*. (2019) Symbionts do not affect the mating incompatibility between the Brazilian-1 and Peruvian morphotypes of the *Anastrepha fraterculus* cryptic species complex. *Scientific Reports*, 9, 18319.

Dias, V., Silva, J.G., Lima, K.M., Pettinga, C.S.C.D., Hernández-Ortiz, V., Laumann, R.A. *et al*. (2016) An integrative multidisciplinary approach to understanding cryptic divergence in Brazilian species of the *Anastrepha fraterculus* complex (Diptera: Tephritidae). *Biological Journal of the Linnean Society*, 117, 725–746.

Dias, V.S., Cáceres, C., Parker, A.G., Pereira, R., Demirbas-Uzel, G., Abd-Alla, A.M.M. *et al*. (2021) Mitochondrial superoxide dismutase overexpression and low oxygen conditioning hormesis improve the performance of irradiated sterile males. *Scientific Reports*, 11, 20182.

Dyck, V.A., Hendrichs, J. and Robinson, A.S. (2021) *Sterile Insect Technique-Principles and Practice in Area-Wide Integrated Pest Management*, 2nd edn, p. 1200. CRC Press, Boca Raton, FL, USA.

FAO/IAEA/USDA (2019) *Product Quality Control for Sterile Mass-Reared and Released Tephritid Fruit Flies; Version 7.0*. IAEA: Vienna, Austria. pp. 148.

Franz, G., Bourtzis, K. and Caceres, C. (2021) Practical and operational genetic sexing systems based on classical genetic approaches in fruit flies, an example for other species amenable to large-scale rearing for the sterile insect technique. In *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management* (eds. V.A. Dyck, J. Hendrichs & A.S. Robinson), pp. 576–599. CRC Press: Boca Raton, FL, USA.

Gayle, S., McKinney, M., Follett, P. and Manoukis, N. (2013) A novel method for rearing wild tephritid fruit flies. *Entomologia Experimentalis et Applicata*, 148, 297–301.

Gilchrist, A.S., Cameron, E.C., Sved, J.A. and Meats, A.W. (2012) Genetic consequences of domestication and mass rearing of pest fruit fly *Bactrocera tryoni* (Diptera: Tephritidae). *Journal of Economic Entomology*, 105, 1051–1056.

Giustina, P.D., Mastrangelo, T., Ahmad, S., Mascarin, G. and Caceres, C. (2021) Determining the sterilization doses under hypoxia for the novel black pupae genetic sexing strain of *Anastrepha fraterculus* (Diptera, Tephritidae). *Insects*, 12, 308.

Gomes, I.V., Roriz, A.K.P., Araujo, A.S., Dias, V.S., Nascimento, A. and Joachim-Bravo, I.S. (2024) Sexual behaviour of *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae): Do laboratory domestication conditions influence male courtship behaviour? *Physiological Entomology*, 49, 99–109.

Gomez Cendra, P., Calcagno, G., Belluscio, L. and Vilardi, J.C. (2011) Male courtship behavior of the South American fruit fly, *Anastrepha fraterculus*, from an Argentinean laboratory strain. *Journal of Insect Science*, 11, 175.

Hernández-Ortiz, V., Gomez-Anaya, J.A., Sanchez, A., McPheron, B.A. and Aluja, M. (2004) Morphometric analysis of Mexican and South American populations of the *Anastrepha fraterculus* complex (Diptera: Tephritidae) and recognition of a distinct Mexican morphotype. *Bulletin of Entomological Research*, 94, 487–499.

Hernández-Ortiz, V., Bartolucci, A., Morales-Valles, P., Frias, D. and Selivon, D. (2012) Cryptic species of the *Anastrepha fraterculus* complex (Diptera, Tephritidae): a multivariate approach for the recognition of South American morphotypes. *Annals of the Entomological Society of America*, 105, 305–318.

Hernández-Ortiz, V., Barradas-Juanz, N. and Díaz-Castelazo, C. (2019) A review of the natural host plants of the *Anastrepha fraterculus* complex in the Americas. In *Area-Wide Management of Fruit Fly Pests* (eds. D. Perez-Staples,

F. Diaz-Fleischer, P. Montoya & M.T. Vera), pp. 90–114. CRC Press: Boca Raton, FL, USA.

Kovaleski, A. and Mastrangelo, T. (2021) Moscasul programme: first steps of a pilot project to suppress the South American fruit fly in Southern Brazil. In *Area-Wide Integrated Pest Management* (eds. J. Hendrichs, R. Pereira & M.J.B. Vreysen), pp. 215–230. CRC Press: Boca Raton, FL, USA.

Lamb, A.M., Wang, Z., Simmer, P., Chung, H. and Wittkopp, P.J. (2020) ebony affects pigmentation divergence and cuticular hydrocarbons in *Drosophila americana* and *D. novamexicana*. *Frontiers in Ecology and Evolution*, 8, 184.

Li, X.L., Li, D.D., Cai, X.Y., Cheng, D.F. and Lu, Y.Y. (2023) Reproductive behavior of fruit flies: courtship, mating, and oviposition. *Pest Management Science*, 80, 935–952.

López-Martínez, G. and Hahn, D. (2014) Early life hormetic treatments decrease irradiation-induced oxidative damage, increase longevity, and enhance sexual performance during old age in the Caribbean fruit fly. *PLoS ONE*, 9, e88128.

Massey, J.H., Akiyama, N., Bien, T., Dreisewerd, K., Wittkopp, P.J., Yew, J.Y. *et al*. (2019) Pleiotropic effects of ebony and tan on pigmentation and cuticular hydrocarbon composition in *Drosophila melanogaster*. *Frontiers in Physiology*, 10, 518.

Mastrangelo, T., Kovaleski, A., Botteon, V., Scopel, W. and Costa, M.d.L.Z. (2018) Optimization of the sterilizing doses and overflooding ratios for the South American fruit fly. *PLoS ONE*, 13, e0201026.

Mastrangelo, T., Kovaleski, A., Maset, B., Costa, M.D.L.Z., Barros, C., Lopes, L.A. *et al*. (2021) Improvement of the mass-rearing protocols for the South American fruit fly for application of the sterile insect technique. *Insects*, 12, 622.

Meza, J.S., Arredondo, J., Orozco, D. and Pérez-Staples, D. (2014) Disparity in sexual behaviour between wild and mass-reared Mexican fruit flies. *Physiological Entomology*, 39, 263–270.

Meza, J., Bourtzis, K., Zacharopoulou, A., Gariou-Papalexiou, A. and Cáceres, C. (2020) Development and characterization of a pupal-colour based genetic sexing strain of *Anastrepha fraterculus* sp. 1 (Diptera: Tephritidae). *BMC Genetics*, 21, 134.

McInnis, D.O., Tam, S., Grace, C. and Miyashita, D. (1994) Population suppression and sterility rates induced by variable sex ratio, sterile insect releases of *Ceratitis capitata* (Diptera: Tephritidae) in Hawaii. *Annals of the Entomological Society of America*, 87, 231–240.

McInnis, D.O., Lance, D.R. and Jackson, C.G. (1996) Behavioral resistance to the sterile insect technique by the Mediterranean fruit fly (Diptera: Tephritidae) in Hawaii. *Annals of the Entomological Society of America*, 89, 739–744.

Oroño, L.E., Ovruski, S.M., Norrbom, A.L., Schliserman, P., Colin, C. and Martin, C.B. (2005) Two new native host plant records for *Anastrepha fraterculus* (Diptera: Tephritidae) in Argentina. *Florida Entomologist*, 88, 228–232.

Orozco, D., Hernandez, M.R., Meza, J.S. and Quintero, J.L. (2013) Do sterile females affect the sexual performance of sterile males of *Anastrepha ludens* (Diptera: Tephritidae)? *Journal of Applied Entomology*, 137, 321–326.

Paulo, D.F., Nguyen, T.N.M., Ward, C.M., Corpuz, R.L., Kauwe, A.N., Rendon, P. *et al*. (2024) The genetic basis of the black pupae phenotype in tephritid fruit flies. *BioRxiv*, https://doi.org/10.1101/2024.06.07.597636.

Parker, A.G., Vreysen, M.J.B., Bouyer, J. and Calkins, C.O. (2021) Sterile insect quality control/assurance. In *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management* (eds. V.A. Dyck, J. Hendrichs, & A.S. Robinson), pp. 399–440. 2nd edition, CRC Press: Boca Raton, FL, USA.

Parreño, M.A., Scannapieco, A.C., Remis, M.I., Juri, M., Vera, M.T., Segura, D.F. *et al*. (2014) Dynamics of genetic variability in *Anastrepha fraterculus* (Diptera: Tephritidae) during adaptation to laboratory rearing conditions. *BMC Genetics*, 15, S14.

Petit-Marty, N., Vera, M.T., Calcagno, G., Cladera, J.L., Segura, D.F., Allinghi, A. *et al*. (2004) Sexual behavior and mating compatibility among four populations of *Anastrepha fraterculus* (Diptera: Tephritidae) from Argentina. *Annals of the Entomological Society of America*, 97, 1320–1327.

Quintero-Fong, L., Luis, J.H., Montoya, P. and Orozco-Dávila, D. (2018) *In situ* sexual competition between sterile males of the genetic sexing Tap-7 strain and wild *Anastrepha ludens* flies. *Crop Protection*, 106, 1–5.

Ramírez-Santos, E., Rendon, P., Gouvi, G., Zacharopoulou, A., Bourtzis, K., Cáceres, C. *et al*. (2021) A novel genetic sexing strain of *Anastrepha ludens* for cost-effective sterile insect technique applications: improved genetic stability and rearing efficiency. *Insects*, 12, 499.

Rendon, P. and Enkerlin, W. (2021) Area-wide fruit fly programmes in Latin America. In *Area-Wide Integrated Pest Management, Development and Field Application* (eds. J. Hendrichs, R. Pereira & M.J.B. Vreysen) pp. 161–195. CRC Press, Boca Raton, FL, USA.

Roriz, A.K.P., Japyassú, H.F. and Joachim-Bravo, I.S. (2018) Courtship in two morphotypes of the *Anastrepha fraterculus* (Diptera: Tephritidae) cryptic species complex and their implications for understanding mate recognition. *Journal of Insect Behavior*, 31, 535–551.

Rull, J., Brunel, O. and Mendez, M.E. (2005) Mass rearing history negatively affects mating success of male *Anastrepha ludens* (Diptera: Tephritidae) reared for sterile insect technique programs. *Journal of Economic Entomology*, 98, 1510–1516.

Rull, J., Abraham, S., Kovaleski, A., Segura, D.F., Islam, A., Wornoayporn, V. *et al*. (2012) Random mating and reproductive compatibility among Argentinean and southern Brazilian

populations of *Anastrepha fraterculus* (Diptera: Tephritidae). *Bulletin of Entomological Research*, 102, 435–443.

Rull, J., Abraham, S., Kovaleski, A., Segura, D.F., Mendoza, M., Clara Liendo, M. *et al*. (2013) Evolution of prezygotic and post-zygotic barriers to gene flow among three cryptic species within the *Anastrepha fraterculus* complex. *Entomologia Experimentalis et Applicata*, 148, 213–222.

SAS Institute Inc. (2013) *SAS Software Version 9.4*. Cary, NC.

Segura, D.F., Belliard, S.A., Vera, M.T., Bachmann, G.E., Ruiz, M.J., Jofre-Barud, F. *et al*. (2018) Plant chemicals and the sexual behavior of male tephritid fruit flies. *Annals of the Entomological Society of America*, 111, 239–264.

Selivon, D., Vretos, C., Fontes, L. and Perondini, A.L.P. (2004) New variant forms in the *Anastrepha fraterculus* complex. In *Proceedings of the 6th International Fruit Flies Symposium* (ed. B. Barnes), pp. 253–258. Irene: Isteg Scientific Publications.

Selivon, D., Perondini, A.L.P., Hernández-Ortiz, V., doVal, F.C., Camacho, A., Gomes, F.R.,*et al.* (2022) Genetical, morphological, behavioral, and ecological traits support the existence of three Brazilian species of the *Anastrepha fraterculus* complex of cryptic species. *Frontiers in Ecology and Evolution*, 10, 836608.

Shelly, T.E. and Manoukis, N.C. (2022) Mating competitiveness of *Bactrocera dorsalis* (Diptera: Tephritidae) males from a genetic sexing strain: Effects of overflooding ratio and released females. *Journal of Economic Entomology*, 115, 799–807.

Vaníèková, L., Hernández-Ortiz, V., Joachin-Bravo, I.S., Dias, V., Roriz, A.K.P., Laumann, R.A. *et al*. (2015) Current knowledge of the species complex *Anastrepha fraterculus* (Diptera: Tephritidae) in Brazil. *Zookeys*, 540, 211–237.

Vera, M.T., Caceres, C., Wornoayporn, V., Islam, A., Robinson, A.S., De La Vega, M.H. *et al*. (2006) Mating incompatibility among populations of the South American fruit fly *Anastrepha fraterculus* (Diptera: Tephritidae). *Annals of the Entomological Society of America*, 99, 387–397.

Walder, J., Morelli, R., Costa, K., Faggioni, K., Sanches, P., Paranhos, B. *et al*. (2014) Large scale artificial rearing of *Anastrepha sp*.1 aff. *fraterculus* (Diptera: Tephritidae) in Brazil. *Scientia Agricola*, 71, 281–286.

Zucchi, R.A. and Moraes, R.C.B. (2025) *Fruit Flies in Brazil—Anastrepha Species Their Host Plants and Parasitoids*. ESALQ/USP, Piracicaba, Brazil.

Zygouridis, N.E., Argov, Y., Nemny-Lavy, E., Augustinos, A.A., Nestel, D. and Mathiopoulos, K.D. (2014) Genetic changes during laboratory domestication of an olive fly SIT strain. *Journal of Applied Entomology*, 138, 423–432.

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