Received: 4 April 2022 | Revised: 13 July 2022 | Accepted: 14 July 2022

DOI: 10.1111/jen.13058

ORIGINAL CONTRIBUTION

JOURNAL OF APPLIED ENTOMOLOGY WILEY

Host-marking pheromone of the South American fruit fly *Anastrepha fraterculus* and cross-recognition by the Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae)

Diego Martins Magalhães[1] | Mirian Fernandes Furtado Michereff[1] | Adalecio Kovaleski[2] | Beatriz Aguiar Giordano Paranhos[3] | Dori Edson Nava[4] | Miguel Borges[1] | Raúl Alberto Laumann[1] | Maria Carolina Blassioli-Moraes[1]

[1]Laboratory of Semiochemicals, Brazilian Agricultural Research Corporation, EMBRAPA Genetic Resources and Biotechnology, Brasília, Brazil

[2]Laboratory of Entomology, Brazilian Agricultural Research Corporation, EMBRAPA Grape and Wine, Vacaria, Brazil

[3]Laboratory of Entomology, Brazilian Agricultural Research Corporation, EMBRAPA Semiarid, Petrolina, Brazil

[4]Laboratory of Entomology, Brazilian Agricultural Research Corporation, EMBRAPA Temperate Agriculture, Pelotas, Brazil

Correspondence
Diego Martins Magalhães, Laboratory of Chemical Ecology and Insect Behaviour, Department of Entomology and Acarology, Luiz de Queiroz College of Agriculture, University of São Paulo, 13418-900, Piracicaba-SP, Brazil.
Email: magalhaes.dmm@gmail.com

Funding information
Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: 152676/2016-3; Empresa Brasileira de Pesquisa Agropecuária, Grant/Award Number: 02.11.05.017.00.00

Abstract

Following oviposition, females of many fruit flies deposit on the fruit surface a host-marking pheromone (HMP) that can deter oviposition by conspecifics and heterospecifics, thereby minimizing overexploitation of hosts. We describe the oviposition behaviour of two economically important fruit flies (*Anastrepha fraterculus* and *Ceratitis capitata*) in agar spheres marked with methanolic and aqueous faeces extracts that contain the HMP. Laboratory bioassays revealed that *A. fraterculus* mated female produces an HMP that can be extracted from faeces, and causes a significant reduction in fruit infestation by both conspecifics and heterospecifics (cross-recognition). Furthermore, mated female faeces extract contains higher amounts of methyl (9*E*)-hexadecenoate, methyl hexadecanoate, methyl linoleate and methyl (9*Z*)-octadecenoate than male faeces extract. Our results provide evidence for the potential use of this ovipositional deterrent in crop protection and pest management programmes for *A. fraterculus* and *C. capitata*.

KEYWORDS
crop protection, faeces extracts, fruit flies, ovipositional deterrent, Tephritidae

1 | INTRODUCTION

Fruit flies (Tephritidae) are important pests that infest a wide range of host plants (White & Elson-Harris, 1992). Female adult flies cause direct damage by laying their eggs in fruits, where the larval stage takes place. Following oviposition, females deposit on the fruit surface a host-marking pheromone (HMP) that informs other conspecific females of a previously infested host, thereby minimizing overexploitation of resources (Roitberg & Prokopy, 1987). It is common to find more than one fruit fly species infesting the same fruit. Thus, the HMPs can also be recognized by members of other fruit fly species, a phenomenon known as cross-recognition (Aluja & Diaz-Fleischer, 2006; Prokopy et al., 1976), which reduces the competitive interactions among larvae in fruits (Malavasi et al., 1980).

Most of the available studies so far have isolated HMP from fruit flies' faeces using polar solvents, suggesting a polar character of this kind of pheromone (Aluja et al., 2003, 2009; Aluja & Diaz-Fleischer, 2006; Boller & Hurter, 1985; Cheseto et al., 2018; Edmunds et al., 2010; Kachigamba et al., 2012). Fruit flies store a substantial amount of HMP in the midgut, which can either be

released after egg laying through the ovipositor or with the faecal matter (Prokopy, Averill, et al., 1982; Scolari et al., 2021). Raw faeces material has been successfully used to deter oviposition behaviour in a few fruit fly species in both laboratory and field conditions (Aluja et al., 2009; Aluja & Boller, 1992; Cheseto et al., 2018; Kachigamba et al., 2012; Katsoyannos & Boller, 1980). The use of HMP as a potential management tool for fruit flies' control presents a sustainable alternative to conventional pesticides used today (Aluja et al., 2009; Aluja & Boller, 1992; Birke et al., 2020; Katsoyannos & Boller, 1980).

The South American fruit fly, *Anastrepha fraterculus* (Wiedemann), is native to the Neotropics (Hernández-Ortiz et al., 2015), whereas the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is an invasive pest native to the Afrotropical region (De Meyer et al., 2002). Both are considered the most important fruit fly species in Brazil in terms of economic importance, distribution and host plants spectrum (Zucchi, 2001, 2007). Direct wounds, such as ovipositor wounds and larvae damage to fruit pulp, affect the commercial value for *in natura* consumption of fruits or their industrial processing (Botton et al., 2016). In South America, *A. fraterculus* and *C. capitata* host range overlaps, and the larvae of these two species can be found sharing the same host (Devescovi et al., 2015; Malavasi et al., 1980; Ovruski et al., 2010; Silva et al., 2011). Host marking behaviour and pheromone extraction from faeces have been studied for *C. capitata* (Arredondo & Díaz-Fleischer, 2006; Kachigamba et al., 2012; Prokopy et al., 1978), while for *A. fraterculus* only host marking behaviour has already been documented (Prokopy, Malavasi, et al., 1982).

In the present study, the effects of *A. fraterculus*' faeces extracts on the oviposition behaviour of conspecifics and heterospecifics (*C. capitata*) were examined. Besides, faeces extracts were also analysed by HPLC and GC/GC-MS as a first step to identify their chemical profiles.

2 | MATERIALS AND METHODS

2.1 | Insects

The fruit flies were obtained from laboratory colonies from EMBRAPA Grape & Wine (28°24′32.0″S 50°53′23.9″W) and EMBRAPA Temperate Agriculture (31°40′55.3″S 52°26′45.9″W) (*A. fraterculus*) and EMBRAPA Semiarid (9°4′16.4″S 40°19′6.4″W) (*C. capitata*). Pupae were placed in a Petri dish containing a layer of moistened vermiculite, inside a 5.0 L plastic container with a screened lid. They were maintained in controlled environmental rooms at 26 ± 1°C and 65 ± 10% relative humidity, under a light regime of 14 h at EMBRAPA Genetic Resources and Biotecnology (41°43′51.4″S 47°54′10.5″W). Newly emerged adults were separated by sex and assigned to experimental treatments. Adults were fed ad libitum with a solid diet [sugar crystals, brewer's yeast and hydrolysed protein (3:1:1), adapted from Salles (1992)]. Water was provided in a plastic cup closed with a lid that was penetrated by a cotton wick.

2.2 | Faeces extraction

The putative HMP was extracted from faeces of laboratory-reared *A. fraterculus* using a slightly modified methodology described by Aluja et al. (2003). We evaluated raw faeces extracts from virgin and mated male and female fruit flies. Five-hundred flies were enclosed into 3.2 L glass chambers with a screened lid with ad libitum access to food and water. Adult flies were 8–10 days old at the beginning of the experiment. Once a week, during four consecutive weeks, flies were transferred to clean glass chambers, and food and water supplies were renewed. Weekly, dead flies were replaced by individuals of similar age to maintain a constant number of fruit flies per chamber. Dead flies, broken wings and legs and eggs were removed from the chambers, and the remaining material was washed with 5 ml of methanol and sonicated for 10 min. This procedure was repeated three times. The same chamber was then washed with 5 ml of distilled water and sonicated for 10 min, repeated thrice. A total of ~1500 couples for mated flies, ~1500 individuals for both virgin and mated females, and ~1500 individuals for virgin males were used, resulting in a 60-ml extract of each treatment. The methanolic and aqueous faeces extracts were concentrated on a rotary evaporator at 40°C to 30 ml each. The extractions were transferred to Falcon tubes and centrifuged for 15 min at 5000 rpm to remove solid residues. The resulting supernatant was concentrated again on a rotary evaporator to a final volume of 15 ml. The stock solution was kept at 4°C until used.

2.3 | Arena bioassays

The oviposition behaviour of *A. fraterculus* and *C. capitata* in response to faecal extracts was evaluated in closed arenas. For *A. fraterculus*, the arena consisted of a plastic container (22 cm ID × 26 cm high) with a voile fabric lid. In all bioassays, agar spheres (3 cm diameter) wrapped in Parafilm were used as an artificial oviposition substrate. The artificial oviposition substrate was used instead of real fruits to control for variation in fruit quality and chemistry, as these features may influence fruit fly oviposition behaviour (Aluja & Diaz-Fleischer, 2006; Papaj et al., 1992). The Parafilm was used to prevent the diffusion of faeces extracts (putative HMP) into the plain agar portion of the artificial oviposition substrate. The tested extracts were uniformly applied to the entire agar sphere wrapped surface with a glass micropipette (Hirschmann, Eberstadt, Germany). Marked spheres were left at room temperature for 30 min for solvent evaporation. In the dual-choice experiment, two agar spheres were marked with the faeces extracts and the other two with control solvent (methanol or distilled water, depending on the treatment). In the no-choice experiment, four agar spheres marked either with methanolic or aqueous faeces extracts or methanol or distilled water were placed in the arena. Marked spheres were placed equidistantly on the arena's floor (11 cm apart from each other). The position of treated agar spheres was randomized in each arena. Four mated *A. fraterculus* couples (10–20 days old) were released in the arena and remained

there for 24 h with unlimited access to water and food. Couples were used only once. After this period, the agar spheres were dissected and the laid eggs were counted.

In the dual-choice experiments, we tested: mated couples methanolic faeces extract (MCM) at 50 (500 µl), 10 (100 µl) and 1 (10 µl) insect equivalent dosage (IE) versus methanol (M); mated females methanolic faeces extract (MFM) at 50, 10 and 1 IE versus M; virgin female methanolic faeces extract (VFM) at 50 IE versus M; virgin male methanolic faeces extract (VMM) at 50 IE versus M; mated couples aqueous faeces extract (MCA) at 50 IE versus distilled water (DW); mated females aqueous faeces extract (MFA) at 50 IE versus DW; virgin females aqueous faeces extract (VFA) at 50 IE versus DW; and virgin males aqueous faeces extract (VMA) at 50 IE versus DW. In the no-choice experiment, we tested: MCM extract at 50 IE, MCA extract at 50 IE, M and DW. For each experimental setup, 25 replicates were run.

For *C. capitata*, the arena consisted of a plastic container (10 cm ID × 12 cm high) with a voile fabric lid. Although enough shreds of evidence indicate that female fruit flies accept artificial agar hosts as an oviposition substrate (Aluja & Diaz-Fleischer, 2006; Arredondo & Díaz-Fleischer, 2006; Díaz-Fleischer & Aluja, 2003), we previously tested agar spheres for *C. capitata* egg-laying and females did not oviposit in this substrate. It did not matter if the spheres were hung from the top lid or resting on the container's floor, in both cases, females laid eggs only on the voile lid on the top of the container. Hence, to test if *A. fraterculus* MCM extract reduces *C. capitata* egg-laying, we applied 500 µl of the extract directly on the voile lid, which was divided into two areas of the same size. Four *C. capitata* mated couples (10–20 days old) were released in the arena and remained there for 24 h with unlimited access to water and food. Couples were used only once. After this period, the laid eggs on the voile lid were counted. We tested *A. fraterculus* MCM extract at 50 IE versus M ($N = 25$).

2.4 | Faeces extracts preparation for high-performance liquid chromatography (HPLC), gas chromatography (GC), and GC–mass spectrometry (GC-MS) analyses

A 1-ml aliquot of the MCM, MFM, and VMM extracts was passed through a column of aluminium oxide (500 mg; Sigma Aldrich) and eluted with 2 ml of methanol. The obtained extracts were concentrated back to 1 ml under a gentle stream of charcoal filtered N_2. After this procedure, the oviposition deterrence effect of the extracts was tested as previously described, to verify for loss of biological activity.

2.5 | HPLC analysis

Reversed-phase HPLC analysis was performed on a Shimadzu LC-20AD (Shimadzu Corporation, Kyoto, Japan) coupled to a Photodiode Array Detector (Shimadzu SPD-M20A PDA) set at 220, 230 and 240 nm. A 10-µl injection was made on a Brownlee SSP C18 column (50 × 2.1 mm; Perkin Elmer). The column was eluted with acetonitrile +0.1% TFA (solvent A) and water +0.1% TFA (solvent B), with the following gradient: 98% A (5 min) – 75% A (15 min) – 100% A (15 min) – 98% A (5 min). The solvent flow rate was 1.0 L/min held constant. The oven temperature was maintained at 37°C. All samples were checked by triplicate injections.

2.6 | Vapour-phase extraction (VPE) for GC and GC-MS analysis

Methanolic faeces extracts (100 µl) were transferred to 2 ml conical glass vials and extracted using VPE as described by Moraes et al. (2008) with adaptations. A glass tube (3.2 mm ID × 4.5 OD and 7.0 cm length) packed with 100 mg Porapak Q (80/100 mesh, Supelco) with silanized glass wool plugs was used for the collection of the analytes. A gentle stream (0.2 ml/min) of charcoal filtered N_2 was passed over the extract in the conical vial placed in a heating block at 80°C. When the solvent was completely evaporated, the vial attached to a Porapak Q tube was moved to another heating block at 250°C for 1 min. The trapped volatiles were eluted from the Porapak Q tubes using hexane (500 µl). The eluates were stored in tightly capped vials at −20°C until required for analysis.

2.7 | GC and GC-MS analyses

Identifications were performed on an Agilent 5975-MSD quadrupole mass spectrometer coupled to a gas chromatograph (Agilent 7890A) equipped with a DB-5MS column (30 m × 0.25 mm ID, 0.25 µm film, Supelco, PA, USA), a splitless injector and helium as the carrier gas. Ionization was by electron impact (70 eV, source temperature at 230°C). The injector was at 250°C. The oven temperature was maintained at 50°C for 2 min, programmed at 5°C/min to 180°C, held for 0.1 min, then 10°C/min to 250°C, and held for 20 min. Data were collected with ChemStation software. Identifications were made by comparison of spectra with mass spectral library databases (NIST, 2008) and the use of retention indices (RI) and then confirmed by co-injection of the VPE samples with authentic standards. The retention indices were calculated by comparison to the retention times of a series of linear hydrocarbon alkanes (C_8–C_{40}) analysed with the same separation method. Compounds were also analysed on an Agilent 7890-A equipped with a flame ionization detector (FID) using the same non-polar column and temperature programme as described above. One microliter of each sample was injected on a splitless injector, with helium as the carrier gas. Data were collected with GC Open Lab. The relative abundance of the compounds was expressed as the integrated peak area of each identified compound divided by the total peak area ×100.

2.8 | Chemicals

Hexane for HPLC (>97% redistilled), methyl hexadecanoate, and (9Z)-tricosene were purchased from Sigma Aldrich (Steinheim, Germany). Octadecanol and methyl linoleate were purchased from Cayman Chemical (Michigan, USA). Methyl (9*E*)-hexadecenoate (10 mg/ml in heptane, 1 ml ampoule) was purchased from Supelco (Missouri, USA). Methyl oleate (99%), methyl linoleate (99%) and methyl (9Z)-hexadecenoate (10 mg/ml in heptane, 1 ml ampoule) were purchased from Sigma Aldrich (Missouri, USA).

2.9 | Data analyses

No-choice tests were analysed using a one-way analysis of variance (ANOVA). For dual-choice tests, a paired *t*-test was used to analyse differences in the oviposition on control and treated agar spheres. The number of laid eggs on agar spheres and voile lid was log-transformed to meet the assumptions of ANOVA and paired *t*-test. Untransformed means are presented. For biological activity in dual-choice tests, we also calculated a discrimination coefficient (DC): $C - T / C + T \times 100$, where C is the number of eggs laid into the control agar spheres, and T is the number of eggs laid into faeces extracts-treated agar spheres. DC is an index that can vary from −100 to +100. A DC value of 100 represents a complete inhibition of oviposition in the treated sphere/lid, while a DC of −100 indicates a complete absence of deterrent activity. DC = 0 represents no difference between treated and control agar spheres (Arredondo & Díaz-Fleischer, 2006; Boller & Hurter, 1985). The DC values from the individual replicates were used to calculate the mean DC value and the confidence interval (95% confidence level). A one-sample *t*-test (Z-test) was used to determine whether DC values were significantly different from zero, and ANOVA to determine whether there were any statistically significant differences among DC values from different doses. All analyses were carried out using R (v.3.1.2) (R Core Team, 2020).

3 | RESULTS

3.1 | Behavioural arena bioassays

Treating the agar spheres with methanolic extracts of *A. fraterculus* faeces significantly reduced the number of laid eggs by conspecific females in dual-choice tests (Figure 1). Mated couples methanolic faeces extracts (MCM) exhibited a biological activity, i.e. agar spheres treated with MCM were less preferred than spheres treated with methanol (Figure 1a). The effectiveness of MCM was observed at 50 (paired-t = −4.64, df = 24, $p < 0.001$), 10 (paired-t = −4.69, df = 24, $p < 0.001$) and 1 IE (paired-t = −4.85, df = 24, $p < 0.001$) (Figure 1a). Similar results were obtained for mated females methanolic faeces extracts (MFM) at 50 (paired-t = −7.52, df = 24, $p < 0.001$), 10 (paired-t = −4.42, df = 24, $p < 0.001$) and 1 IE (paired-t = −2.96, df = 24, p = 0.004) (Figure 1b). Virgin females (VFM) and males methanolic (VMM) faeces extracts displayed no significant difference compared with methanol treated spheres (paired-t = −0.48, df = 24, p = 0.62; paired-t = −0.08, df = 24, p = 0.93) (Figure 1c). Treating the agar spheres with aqueous extracts of *A. fraterculus* faeces did not reduce the number of laid eggs by conspecific females compared with distilled water treated spheres, showing no biological activity (Figure 1d).

All tested doses (1, 10 and 50 IE) of MCM and MFM revealed a biological activity on *A. fraterculus* oviposition behaviour, significantly

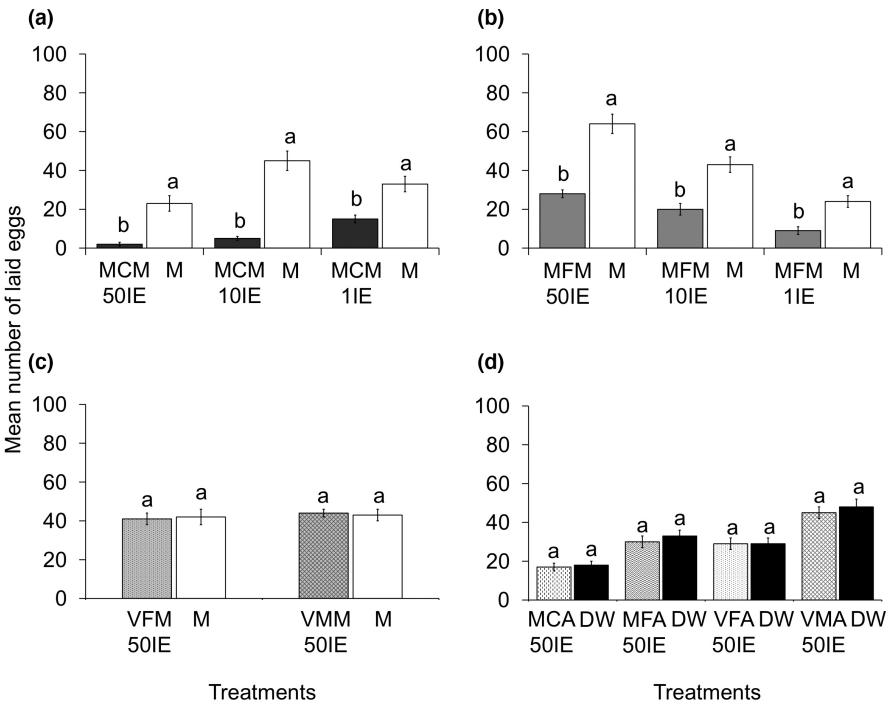

FIGURE 1 Mean number (±*SEM*) of *Anastrepha fraterculus* eggs laid on agar spheres treated with different faeces extracts and solvents control in dual-choice tests. (a) Mated couples methanolic faeces extracts (MCM) at 50, 10 and 1 insect equivalent dose (IE). (b) Mated females methanolic faeces extracts (MFM) at 50, 10, and 1 IE. (c) Virgin females and males methanolic faeces extracts (VFM and VMM, respectively) at 50 IE. (d) Aqueous faeces extracts at 50 IE. MCA: Mated couples aqueous faeces extracts. MFA: Mated females aqueous faeces extracts. VFA: Virgin females aqueous faeces extracts. VMA: Virgin males faeces extracts. M: Methanol. DW: Distilled water. Means with the same letter are not different (Tukey's test $p > 0.05$)

decreasing the number of eggs laid in agar spheres treated with these extracts (Table 1). The 50 and 10 IE dose of MCM extract exhibited the highest biological activity, with a mean DC of 83.52 ± 6.25 and 83.20 ± 6.68, respectively (t = 26.27, df = 24, p < 0.001; t = 24.41, df = 24, p < 0.001). The 1 IE dose of MCM extract showed the lowest biological activity, with a mean DC of 35.68 ± 9.93, but still effective in reducing oviposition behaviour (t = 7.06, df = 24, p < 0.001). The bioassays using MCM extracts showed an increase of HMP activity with increasing doses (Table 1). The MFM extract displayed the highest DC value at 1 IE dose (DC = 54.44 ± 10.29; t = 10.38, df = 24, p < 0.001) and the lowest DC value at 50 IE dose (DC = 37.15 ± 8.60; t = 8.48, df = 24, p < 0.001). Despite presenting different DC values, MFM extracts for 50, 10 and 1 IE did not have a significantly different effect among each other (F = 2.08, df = 2, p = 0.13), i.e. all doses were equally effective in deterring female oviposition behaviour. The other treatments (VFM, VMM, MCA, MFA, VFA and VMA) exhibited no effect on oviposition, with a mean DC value lower than 6.00 (p > 0.05).

Altogether, there were significant differences among treatments in the mean number of eggs laid on agar spheres in no-choice tests (Figure 2). Female *A. fraterculus* laid significantly fewer eggs on agar spheres treated with MCM at 50 IE dose than the other treatments (F = 39.07, df = 3, p < 0.001). On each treatment, there was no difference in the mean number of eggs laid on agar spheres meaning that there was no bias of oviposition preference regarding the position of the spheres in the arena (Figure 2).

Female *C. capitata* laid significantly more eggs in the voile lid area treated with methanol than the area treated with *A. fraterculus* MCM at 50 IE dose (paired-t = 3.83, df = 24, p < 0.001) (Figure 3), indicating that *A. fraterculus* putative HMP has a heterospecific effect. The mean DC value of MCM at 50 IE dose was 43.47 ± 3.86 (t = 11.47, df = 24, p < 0.001; Table 1).

3.2 | Chemical analyses

The HPLC analysis did not show differences in the chemical profile among MCM, MFM and VMM faeces extracts that could justify the responses observed in the behavioural assays (Supporting Information). Therefore, the same extracts were submitted to VPE and analysed by GC and GC-MS. The extracts analysed by GC–MS presented qualitative differences among the treatments, and 13 compounds were identified: (1) anastrephin (RI = 1590), (2) epianastrephin (RI = 1604), (3) methyl (9*E*)-hexadecenoate (RI = 1902), (4) methyl hexadecanoate (RI = 1925), (5) 10-heneicosene (RI = 2079), (6) octadecanol (RI = 2088), (7) methyl linoleate (RI = 2094), (8) methyl (9*Z*)-octadecenoate (RI = 2105), (9) methyl octadecanoate (RI = 2126), (10) octadecyl acetate (RI = 2206), (11) (9*Z*)-tricosene (RI = 2279), (12) eicosanol (RI = 2292), and (13) eicosyl acetate (RI = 2407) (Figure 4). The compounds anastrephin and epianastrephin are components of the male sex pheromone (Brizová et al., 2013), and they were not identified in MFM extract. On the other hand, methyl (9*E*)-hexadecenoate and methyl (9*Z*)-octadecenoate were produced in higher quantities by females and only in trace amounts by virgin males (Table 2). Despite being identified in all extracts, methyl hexadecanoate and methyl linoleate were also found in higher quantities in MCM and MFM extracts compared with VMM extract (Table 2).

4 | DISCUSSION

Fruit flies in the genera *Anastrepha*, *Ceratitis* and *Rhagoletis* mark host fruits by dragging the aculeus and depositing HMP on the fruit surface after oviposition (Prokopy et al., 1978; Roitberg & Prokopy, 1987; Scolari et al., 2021). The present study provides clear evidence that *A. fraterculus* mated female produces an HMP that can be extracted from faeces and causes a significant reduction in fruit infestation by conspecifics and heterospecifics. This type of pheromone has been successfully isolated from faeces of different Tephritidae species (Aluja et al., 2003, 2009; Aluja & Diaz-Fleischer, 2006; Boller & Hurter, 1985; Cheseto et al., 2018; Kachigamba et al., 2012). Only mated *A. fraterculus* females produced biologically active HMP. Males and virgin females did not seem to produce detectable amounts of the pheromone. Similarly, *R. cerasi* and *C. rosa* young males and females produce none or only traces of active HMP (Boller & Hurter, 1985; Cheseto et al., 2018; Stadler et al., 1992). These results suggest a reproductive status-dependent pheromone production for *A. fraterculus* female that seems to be produced upon mating. Age-dependent pheromone release has been demonstrated in *R. cerasi* and *C. rosa* (Cheseto et al., 2018; Stadler et al., 1992); however, it does not seem to be the case for *A. fraterculus* because faeces extracts were collected from 8–10-day-old to 38–40-day-old virgin females, and no biological activity was obtained using them, i.e. the HMP was not detected.

Anastrepha fraterculus putative HMP in faeces extracts was found to be soluble in methanol. Here we report on sequential methanolic extractions followed by aqueous extractions. The aqueous extracts did not display any biological activity on *A. fraterculus* oviposition behaviour, indicating that methanol is an effective solvent for the type of compounds comprising the HMP blend. So far, the HMPs currently identified are complex polar compounds with low volatility, considerable persistence and solubility in polar solvents, such as *N*-[15(β-glucopyranosyl)-oxy-8-hydroxypalmitoyl]-taurine found in *R. cerasi* (Hurter et al., 1987), *N*-(2,14-dimethyl-1-oxopentadecyl)-glutamic acid in *A. ludens* (Aluja et al., 2003; Edmunds et al., 2010), glutathione in *C. cosyra* (Cheseto et al., 2017), and glutamic acid in *C. rosa* (Cheseto et al., 2018). Fruit flies HMPs are water and methanol soluble, but methanol was proved to be a more efficient solvent than water (Díaz-Fleischer et al., 2004). Moreover, previous studies reported that methanol does not affect fruit flies' behaviour once evaporated (Aluja et al., 2009; Arredondo & Díaz-Fleischer, 2006; Díaz-Fleischer et al., 2004).

Many Tephritidae flies tend toward uniformity of egg dispersion among the available host fruits to achieve optimal utilization of resources for larval development. This phenomenon was postulated

Respondent species	Treatment	Control	Dose (IE)[a]	DC ± CI	*p* value[b]
Anastrepha fraterculus	MCM	M	50	83.52 ± 6.25	$p < 0.001$
	MCM	M	10	83.20 ± 6.68	$p < 0.001$
	MCM	M	1	35.68 ± 9.93	$p < 0.001$
	MFM	M	50	37.15 ± 8.60	$p < 0.001$
	MFM	M	10	43.76 ± 10.08	$p < 0.001$
	MFM	M	1	54.44 ± 10.29	$p < 0.001$
	VFM	M	50	3.22 ± 4.19	$p = 0.15$
	VMM	M	50	0.47 ± 6.19	$p = 0.89$
	MCA	DW	50	5.79 ± 7.28	$p = 0.13$
	MFA	DW	50	2.01 ± 6.89	$p = 0.81$
	VFA	DW	50	0.87 ± 7.93	$p = 0.85$
	VMA	DW	50	2.13 ± 4.53	$p = 0.89$
Ceratitis capitata	MCM	M	50	43.47 ± 7.41	$p < 0.001$

Abbreviations: MCA, mated couples aqueous faeces extracts; MCM, mated couples methanolic faeces extracts; MFA, mated females aqueous faeces extracts; MFM, mated females methanolic faeces extracts; VFA, virgin females aqueous faeces extracts; VFM, virgin females methanolic faeces extracts; VMA, virgin males aqueous faeces extracts; VMM, virgin males methanolic faeces extracts.

[a]Insect equivalent.

[b]One-sample *t*-test was used to determine whether DC values were significantly different from zero.

TABLE 1 Discrimination coefficient (DC) (mean ± confidence interval, CI) of *Anastrepha fraterculus* and *Ceratitis capitata* against agar spheres treated with methanolic and aqueous faeces extracts of *A. fraterculus*

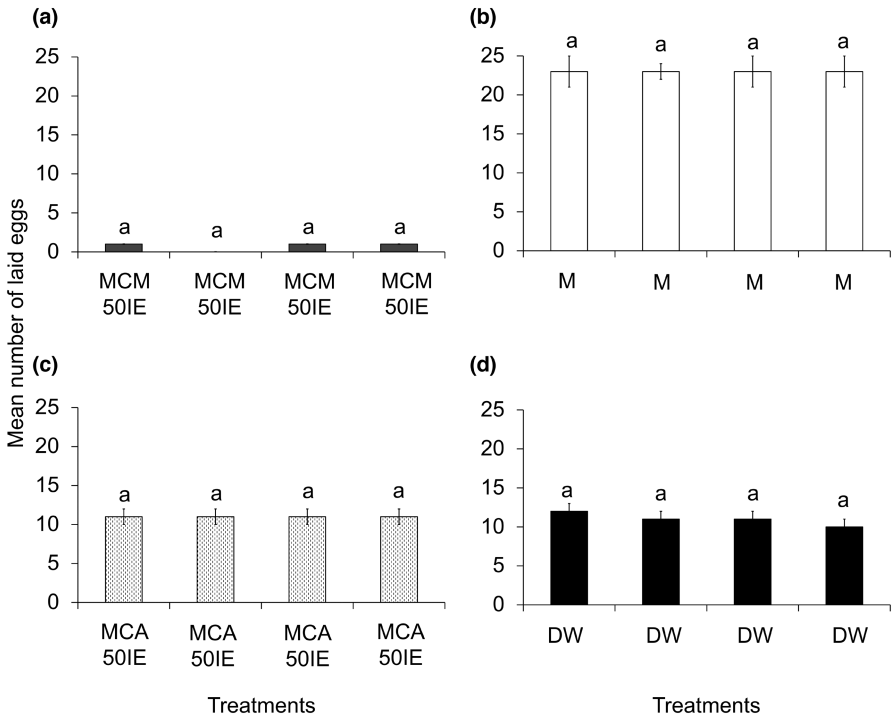

FIGURE 2 Mean number (±*SEM*) of *Anastrepha fraterculus* eggs laid on agar spheres treated with different faeces extracts and solvent control in no-choice tests. (a) Mated couples methanolic faeces extracts (MCM) at 50 insect equivalent dose (IE). (b) Methanol (M). (c) Mated couples aqueous faeces extracts (MCA) at 50 IE. (d) Distilled water (DW). Means with the same letter are not different (ANOVA $p > 0.05$)

to be the ecological significance of HMP (Prokopy et al., 1976). In the no-choice experiment, such a pattern was evident once all agar spheres were equally infested (there were no significant differences in the mean number of eggs per agar sphere). Usually, *A. fraterculus* lay one egg per oviposition bout, but more than one egg can be found per clutch (Nascimento & Oliveira, 1996). *Anastrepha ludens* females are more likely to lay eggs in lightly infested fruits than in heavily infested fruits. However, when uninfested fruits are not available, females exploit already occupied fruits (Papaj & Aluja, 1993). It is also true for *A. fraterculus*, as agar spheres treated with MCM and MFM extracts might be equivalent to a heavily infested fruit and over time, as females oviposit in control agar spheres (methanol-treated), these

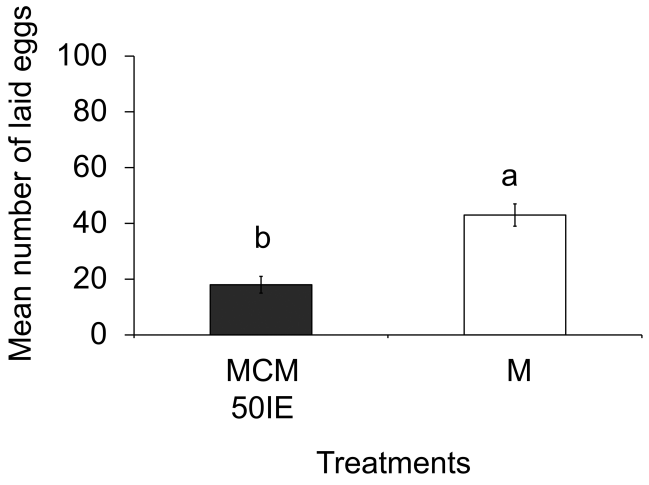

FIGURE 3 Mean number (± *SEM*) of *Ceratitis capitata* eggs laid on voile lids treated with *Anastrepha fraterculus* mated couples faeces extracts (MCM) at 50 insect equivalent dose (IE) and methanol (M) in dual-choice tests. Means with the same letter are not different (paired *t*-test $p > 0.05$)

became lightly infested and more preferred as oviposition hosts. In the no-choice tests, when all agar spheres were treated with MCM extract, the mean number of laid eggs were lower than that of methanol-treated agar spheres (control), clearly demonstrating that faeces extracts contained an HMP that reduces fruit infestation.

The discrimination coefficient values showed an increase of biological activity increasing the dose for MCM extract, similar to other studies using raw faeces extracts of other fruit fly species (Arredondo & Díaz-Fleischer, 2006; Boller & Hurter, 1985). Most previous studies used raw faeces extracts from mated couples fruit flies (Aluja et al., 2003, 2009; Aluja & Diaz-Fleischer, 2006; Boller & Hurter, 1985), and our results corroborate the effectiveness of these kinds of extracts. However, faeces extract from mated females only (MFM) showed a different pattern, in which all doses had similar biological activity. Kachigamba et al. (2012) also used MFM extracts and showed that *C. cosyra* females increased host discrimination as the concentration of faeces extracts increased, in a similar fashion as MCM extracts. Such differences could be possibly related to different bioassay methodologies (artificial vs. natural fruits), fruit fly

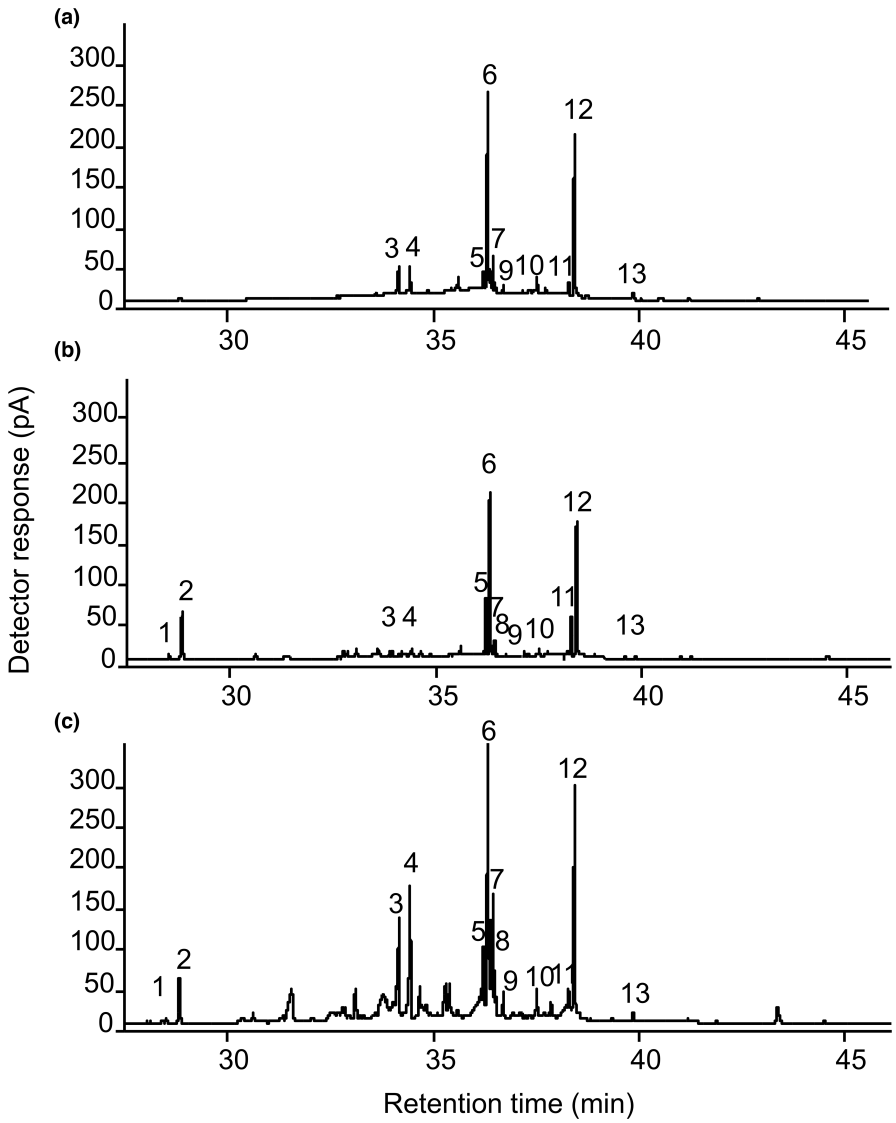

FIGURE 4 GC-FID chemical profiles of *Anastrepha fraterculus*' methanolic faeces extracts submitted to vapour phase extraction. (a) Mated females methanolic extract (MFM). (b) Virgin males methanolic extract (VMM). (c) Mated couples methanolic extract (MCM). Compounds: (1) anastrephin, (2) epianastrephin, (3) methyl (9*E*)-hexadecenoate, (4) methyl hexadecanoate, (5) 10-heneicosene, (6) octadecanol, (7) methyl linoleate, (8) methyl (9*Z*)-octadecenoate, (9) methyl octadecanoate, (10) octadecyl acetate, (11) (9*Z*)-tricosene, (12) eicosanol and (13) eicosyl acetate

TABLE 2 Relative abundance (%) of compounds in *Anastrepha fraterculus* methanolic faeces extracts submitted to vapour-phase extraction

Compounds	RI[a]	Percentage (%)		
		MFM	VMM	MCM
Anastrephin[b]	1590	–	Present	Present
Epianastrephin[b]	1604	–	Present	Present
Methyl (9*E*)-hexadecenoate	1902	4.9	0.7	7.9
Methyl hexadecanoate	1925	6.8	3.6	15.9
10-Heneicosene	2079	2.7	12.4	4.9
Octadecanol	2088	34.6	33.4	27.7
Methyl linoleate	2094	4.4	2.3	6.9
Methyl (9Z)-octadecenoate	2105	1.4	0.3	2.5
Methyl octadecanoate	2126	1.6	0.7	2.4
Octadecyl acetate	2206	3.8	2.3	2.7
(9Z)-Tricosene	2279	2.5	9.9	3.7
Eicosanol	2292	35.1	33.9	24.4
Eicosyl acetate	2407	2.1	0.5	1.0

Abbreviations: MCM, mated couples methanolic extract; MFM, mated female methanolic extract; VMM, virgin male methanolic extract.

[a]Retention indices calculated from the retention times on a DB-5 column.

[b]These compounds were not included in the relative abundance because they are components of male sex pheromone.

species and the amount of active pheromonal components present in the extracts.

We decided to start the chemical analysis of faeces extracts using HPLC based on previous studies and on the fact that HMP molecules usually have low volatility. Nonetheless, we could not spot any qualitative or quantitative differences among the extracts (MCM, MFM and VMM). The chromatograms showed no differences regarding the chemical profile and the wavelength of the peaks. The HMP of *A. ludens*, the compound *N*-(2,14-dimethyl-1-oxopentadecyl)-glutamic acid, was identified by GC-MS with ionization by electron impact (Aluja et al., 2009). Therefore, we submitted the faeces extracts to a vapour-phase extraction (VPE) and analysed them by GC/GC–MS. Using these techniques, we could detect qualitative and quantitative differences among the extracts. The compounds methyl (9*E*)-hexadecenoate, methyl hexadecanoate, methyl linoleate and methyl (9Z)-octadecenoate were found in higher abundance in females' faeces extracts (MFM and MCM); besides, the compounds methyl (9*E*)-hexadecenoate and methyl (9Z)-octadecenoate were found only in trace amounts in male faeces extract and seem to be a female-specific component. These kinds of compounds have never been reported in the faeces of Tephritidae flies, but long linear and non-saturated hydrocarbons, and their alcohols and esters, have already been described in faeces of other Diptera species, such as *Drosophila melanogaster* (Keesey et al., 2016). In *D. melanogaster*, such compounds led to increased feeding and aggregation behaviour. As methanol can accidentally cause the formation of chemical derivatives, such as methyl esters from carboxylic groups (Maltese et al., 2009), we performed the same extraction procedure, but using diethyl ether. We found similar chemical profiles using both solvents (methanol and diethyl ether), indicating that the fatty acid methyl esters identified were not methylation by-products (data not shown). Caution, therefore, is needed to prevent the formation of artefacts due to interactions with solvents during the extraction and storage of samples that may lead to incorrect chemical characterization and biological conclusions (Sauerschnig et al., 2018). To make sure the identified compounds were faeces-related, we analysed the artificial diet content using methanolic extraction and VPE. Our chemical analyses showed that diet did not present any of the compounds found in faeces extracts (Supporting Information). While it is clear that faeces extracts contain HMP components, it still is open for debate whether the identified compounds are HMP related. The chemical nature of the already identified HMP from other Tephritidae species differs from the kind of compounds we identified here (Aluja et al., 2003; Cheseto et al., 2017, 2018; Edmunds et al., 2010; Hurter et al., 1987). Potentially, we could have overlooked other compounds present in faeces extracts, such as long-chain fatty acids, as VPE technique used here does not cover such molecules. Further studies using synthetic standards should evaluate the role of the identified compounds in *A. fraterculus* oviposition behaviour.

Deposition of HMP following egg-laying provides important information not only to conspecific females but also to females from other species. However, there is considerable variation in heterospecific pheromone recognition. Prokopy et al. (1976), studying different species in the *Rhagoletis* genus, proposed that species from different intrageneric groups do not recognize each other's HMP, whereas species within the same group have different recognition degrees. This prediction was not supported in various *Anastrepha* species, in which species from different groups (*fraterculus* species group: *A. ludens* and *A. oliqua* vs. *serpentina* species group: *A. serpentina*) recognized each other's HMP (Aluja & Diaz-Fleischer, 2006). Our results showed that species from different genera are also able to recognize heterospecifics HMP, such as *C. capitata* that discriminated against hosts marked by *A. fraterculus* faeces extracts. We have not tested if complete cross-recognition exists in *A. fraterculus* and *C. capitata*, but given that in nature these two species compete for resources and their host range overlaps widely (Devescovi et al., 2015), and that under laboratory conditions they avoid laying eggs in fruits recently infested by heterospecific females (Liendo, 2013), it is reasonable to believe that *A. fraterculus* might also discriminate against *C. capitata* HMP. Complete interspecific cross-recognition has previously been reported to *A. ludens*, *A. obliqua* and *A. sepentina* (Aluja & Diaz-Fleischer, 2006). Interspecific recognition of HMP also occurs in *C. capitata*, *C. fasciventris*, *C. rosa* and *C. cosyra* (Kachigamba

et al., 2012), and *R. pomonella*, *R. mendax* and *R. cornivora* (Prokopy et al., 1976). Although females benefit from recognizing both conspecific and heterospecific HMP, avoiding oviposition in the presence of the former seems to be more advantageous.

Evidence from at least three genera within the Tephritidae family suggests that HMP is a promising tool in the management of fruit flies. Field experiments spraying faeces extracts and synthetic formulations of HMP caused a significant reduction in fruit infestation (Aluja et al., 2009; Aluja & Boller, 1992; Birke et al., 2020; Edmunds et al., 2010; Katsoyannos & Boller, 1980). Considering aspects of applicability and production cost of control methods, the phenomenon of cross-recognition allows the use of just one HMP against many fruit fly species. Future studies should focus on the identification of the bioactive compounds that comprise *A. fraterculus* HMP and field trials to evaluate its effectiveness outside laboratory conditions in a more realistic scenario.

AUTHOR CONTRIBUTION

DMM and MCBM conceived the research. DMM, MFFM and MCBM conducted the experiments. AK, BAGP and DEN contributed material. DMM and MCBM analysed data and conducted statistical analyses. DMM and MCBM wrote the manuscript. DMM and MCBM secured funding. All authors read and approved the manuscript.

ACKNOWLEDGEMENTS

This work received financial support from the National Council for Scientific and Technological Development (CNPq) through a grant to DMM (process number 152676/2016-3) and the Brazilian Corporation of Agricultural Research (EMBRAPA, project number 02.11.05.017.00.00).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available at https://doi.org/10.48432/1B5ZDD.

ORCID

Diego Martins Magalhães https://orcid.org/0000-0001-9757-3726
Mirian Fernandes Furtado Michereff https://orcid.org/0000-0002-0032-7746
Adalecio Kovaleski https://orcid.org/0000-0003-4518-5777
Beatriz Aguiar Giordano Paranhos https://orcid.org/0000-0001-6814-3831
Dori Edson Nava https://orcid.org/0000-0002-4956-526X
Miguel Borges https://orcid.org/0000-0003-3364-9677
Raúl Alberto Laumann https://orcid.org/0000-0003-3531-2613
Maria Carolina Blassioli-Moraes https://orcid.org/0000-0001-7569-9985

REFERENCES

Aluja, M., & Boller, E. F. (1992). Oviposition deterring pheromone of *Rhagoletis cerasi*: Field deployment of synthetic pheromone as a novel cherry fruit fly management strategy. *Entomologia Experimentalis et Applicata*, *65*, 141–147. https://doi.org/10.1111/j.1570-7458.1992.tb01637.x

Aluja, M., & Diaz-Fleischer, F. (2006). Foraging behavior of *Anastrepha ludens*, *A. obliqua* and *A. serpentina* (Diptera: Tephritidae) in response to feces extracts containing host marking pheromone. *Journal of Chemical Ecology*, *32*, 367–389. https://doi.org/10.1007/s10886-005-9007-6

Aluja, M., Diaz-Fleischer, F., Boller, E. F., Hurter, J., Edmunds, A. J. F., Hagmanna, L., Patrian, B., & Reyes, J. (2009). Application of the host marking pheromone of *Anastrepha ludens* significantly reduces fruit infestation by *a. obliqua* in tropical plum and mango backyard orchards. *Journal of Economic Entomology*, *102*, 2268–2278. https://doi.org/10.1603/029.102.0632

Aluja, M., Diaz-Fleischer, F., Edmunds, A. J. F. & Hagmann, L. (2003). *Isolation, structural determination, synthesis, biological activity and application as control agent of the host marking pheromone (and derivatives thereof) of the fruit flies of the type Anastrepha (Diptera: Tephritidae)* (U.S. Patent No. US6555120B1). https://patents.google.com/patent/US6555120B1/en

Arredondo, J., & Díaz-Fleischer, F. (2006). Oviposition deterrents for the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae) from fly faeces extracts. *Bulletin of Entomological Research*, *96*, 35–42. https://doi.org/10.1079/BER2005399

Birke, A., López-Ramírez, S., Jiménez-Mendoza, R., Acosta, E., Ortega, R., Edmunds, A., & Aluja, M. (2020). Host marking pheromone and GF120TM applied in a push-pull scheme reduce grapefruit infestation by *Anastrepha ludens* in field-cage studies. *Journal of Pest Science*, *93*, 507–518. https://doi.org/10.1007/s10340-019-01155-z

Boller, E. F., & Hurter, J. (1985). Oviposition deterring pheromone in *Rhagoletis cerasi*: Behavioral laboratory test to measure pheromone activity. *Entomologia Experimentalis et Applicata*, *39*, 163–169. https://doi.org/10.1111/j.1570-7458.1985.tb03558.x

Botton, M., Arioli, C. J., Machota, R., Jr., Nunes, M. Z., & da Rosa, J. M. (2016). Moscas-das-frutas na fruticultura de clima temperado: situação atual e perspectivas de controle através do emprego de novas formulações de iscas tóxicas e da captura massal. *Agropecuária Catarinense*, *29*, 103–108.

Břízová, R., Mendonça, A. L., Vaníčková, L., Lima-Mendonça, A., da Silva, C. E., Tomčala, A., Paranhos, B. A. J., Dias, V. S., Joachim-Bravo, I. S., Hoskovec, M., Kalinová, B., & do Nascimento, R.R.. (2013). Pheromone analyses of the Anastrepha fraterculus (Diptera: Tephritidae) cryptic species complex. *Florida Entomologist*, *96*, 1107–1115. https://doi.org/10.1653/024.096.0351.

Cheseto, X., Kachigamba, D. L., Bendera, M., Ekesi, S., Ndung'u, M., Beck, J. J., & Torto, B. (2018). Identification of glutamic acid as a host marking pheromone of the African fruit fly species *Ceratitis rosa* (Diptera: Tephritidae). *Journal of Agricultural and Food Chemistry*, *66*, 9933–9941. https://doi.org/10.1021/acs.jafc.8b04481

Cheseto, X., Kachigamba, D. L., Ekesi, S., Ndung'u, M., Teal, P. E. A., Beck, J. J., & Torto, B. (2017). Identification of the ubiquitous antioxidant tripeptide glutathione as a fruit fly semiochemical. *Journal of Agricultural and Food Chemistry*, *65*, 8560–8568. https://doi.org/10.1021/acs.jafc.7b03164

De Meyer, M., Copeland, R. S., Wharton, R. A., & McPherson, B. A. (2002). On the geographic origin of the medfly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). In *Proceedings of 6th international fruit fly symposium* (pp. 45–53). Isteg Scientific Publications.

Devescovi, F., Liendo, M. C., Bachmann, G. E., Bouvet, J. P., Milla, F. H., Vera, M. T., & Segura, D. F. (2015). Fruit infestation patterns by *Anastrepha fraterculus* and *Ceratitis capitata* reveal that cross-recognition does not lead to complete avoidance of interspecific competition in nature. *Agricultural and Forest Entomology*, *17*, 325–335. https://doi.org/10.1111/afe.12111

Díaz-Fleischer, F., & Aluja, M. (2003). Influence of conspecific presence, experience, and host quality on oviposition behavior and clutch size determination in *Anastrepha ludens* (Diptera: Tephritidae). *Journal of Insect Behavior*, *16*, 537–554. https://doi.org/10.1023/A:1027307424150

Díaz-Fleischer, F., Aluja, M., Hurter, J., Enkerlin, W., & Boller, E. (2004). Propriedades físico-químicas de la feromona marcadora de hospedero (FMH) de tres espécies de moscas de la fruta del género *Anastrepha* (Diptera: Tephritidae). *Folia Entomológica Mexicana*, *43*, 43–53.

Edmunds, A. J. F., Aluja, M., Díaz-Fleischer, F., Patrian, B., & Hagmanna, L. (2010). Host marking pheromone (HMP) in the Mexican fruit fly *Anastrepha ludens*. *Chimia*, *64*, 37–42. https://doi.org/10.2533/chimia.2010.37

Hernández-Ortiz, V., Canal, N. A., Tigrero-Salas, J. O., Ruíz-Hurtado, F. M., & Dzul-Cauich, J. F. (2015). Taxonomy and phenotypic relationships of the *Anastrepha fraterculus* complex in the Mesoamerican and Pacific neotropical dominions (Diptera, Tephritidae). *ZooKeys*, *540*, 95–124. https://doi.org/10.3897/zookeys.540.6027

Hurter, J., Boiler, E. F., Stadler, E., Blattman, H., Buser, R., Bosshard, N. U., Damm, L., Kozlowski, M. W., Schoni, R., Raschdorf, F., Dahinden, R., Schlumpf, E., Fritz, H., Richter, W. J., & Schrexber, J. (1987). Oviposition-deterring pheromone in *Rhagoletis cerasi* L.: Purification and determination of the chemical constitution. *Experientia*, *43*, 157–164. https://doi.org/10.1007/BF01942834

Kachigamba, D. L., Ekesi, S., Ndung'u, M. W., Gitonga, L. M., Teal, P. E., & Torto, B. (2012). Evidence for potential of managing some African fruit fly species (Diptera: Tephritidae) using the mango fruit fly host-marking pheromone. *Journal of Economic Entomology*, *105*, 2068–2075. https://doi.org/10.1603/EC12183

Katsoyannos, B. I., & Boller, E. F. (1980). 2nd field application of oviposition-deterring pheromone of the European cherry fruit fly, *Rhagoletis cerasi* (Diptera, Tephritidae). *Journal of Applied Entomology*, *89*, 278–281. https://doi.org/10.1111/j.1439-0418.1980.tb03467.x

Keesey, I. W., Koerte, S., Retzke, T., & Haverkamp, A. (2016). Adult frass provides a pheromone signature for *Drosophila* feeding and aggregation. *Journal of Chemical Ecology*, *42*, 739–747. https://doi.org/10.1007/s10886-016-0737-4

Liendo, M. C. (2013). *Interacciones competitivas entre dos especies de mosca de la fruta: Ceratitis capitata y Anastrepha fraterculus (Diptera: Tephritidae)* [PhD Thesis]. Universidad de Buenos Aires.

Malavasi, A., Morgante, J. S., & Zucchi, R. A. (1980). Biologia de "Moscas-das-frutas" (Diptera, Tephritidae). I: Lista de hospedeiros e ocorrência. *Revista Brasileira de Biologia*, *40*, 9–16.

Maltese, F., van der Kooy, F., & Verpoorte, R. (2009). Solvent derived artifacts in natural products chemistry. *Natural Product Communications*, *4*, 447–454. https://doi.org/10.1177/1934578X0900400326

Moraes, M. C. B., Birkett, M. A., Gordon-Weeks, R., Smart, L. E., Martin, J. L., Pye, B. J., Bromilow, R., & Pickett, J. A. (2008). *Cis*-Jasmone induces accumulation of defence compounds in wheat, *Triticum aestivum*. *Phytochemistry*, *69*, 9–17. https://doi.org/10.1016/j.phytochem.2007.06.020

Nascimento, J. C., & Oliveira, A. K. (1996). Embryogenesis in *Anastrepha fraterculus* (Diptera: Tephritidae). *Interciencia*, *21*, 158–165.

NIST (2008). Software NIST/EPA/NIH Mass Spectral Library. https://chemdata.nist.gov/dokuwiki/doku.php?id=chemdata:downloads:start

Ovruski, S. M., Schliserman, P., van Nieuwenhove, G. A., Bezdjian, L. P., Núñez-Campero, S., & Albornoz-Medina, P. (2010). Occurrence of *Ceratitis capitata* and *Anastrepha fraterculus* (Diptera: Tephritidae) on cultivated, exotic fruit species in the highland valleys of Tucuman in Northwest Argentina. *Florida Entomologist*, *93*, 277–282. https://doi.org/10.1653/024.093.0219

Papaj, D. R., & Aluja, M. (1993). Temporal dynamics of host-marking in the tropical tephritid fly: The role of host quality. *Behavioral Ecology*, *7*, 235–242. https://doi.org/10.1111/j.1365-3032.1993.tb00600.x

Papaj, D. R., Averill, A. I., Prokopy, R. J., & Wong, T. T. Y. (1992). Host-marking pheromone and use of previously established oviposition sites by the Mediterranean fruit fly (Diptera: Tephritidae). *Journal of Insect Behavior*, *5*, 583–598. https://doi.org/10.1007/BF01048006

Prokopy, R. J., Averill, A. L., Bardinelli, C. M., Bowdan, E. S., Cooley, S. S., Crnjar, R. M., Dundulis, E. A., Roitberg, C. A., Spatcher, P. J., Tumlinson, J. H., & Weeks, B. L. (1982). Site of production of an oviposition-deterring pheromone component in *Rhagoletis pomonella* flies. *Journal of Insect Physiology*, *28*, 1–10. https://doi.org/10.1016/0022-1910(82)90016-6

Prokopy, R. J., Malavasi, A., & Morgante, J. S. (1982). Oviposition-deterring pheromone in *Anastrepha fraterculus* flies. *Journal of Chemical Ecology*, *8*, 763–771. https://doi.org/10.1007/BF00988317

Prokopy, R. J., Reissig, W. H., & Moericke, V. (1976). Marking pheromone deterring repeated oviposition in *Rhagoletis* flies. *Entomologia Experimentalis et Applicata*, *20*, 170–178. https://doi.org/10.1111/j.1570-7458.1976.tb02630.x

Prokopy, R. J., Ziegler, J. R., & Wong, T. T. Y. (1978). Deterrence of repeated oviposition by fruit marking pheromone in *Ceratitis capitata* (Diptera: Tephritidae). *Journal of Chemical Ecology*, *4*, 55–63. https://doi.org/10.1007/BF00988260

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.r-project.org/

Roitberg, B. D., & Prokopy, R. J. (1987). Insects that mark host plants. *Bioscience*, *37*, 400–406. https://doi.org/10.2307/1310563

Salles, L. A. B. (1992). Metodologia de criação de *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae) em dieta artificial em laboratório. *Anais da Sociedade Entomológica do Brasil*, *21*, 479–486. https://doi.org/10.37486/0301-8059.v21i3.814

Sauerschnig, C., Doppler, M., Bueschl, C., & Schuhmacher, R. (2018). Methanol generates numerous artifacts during sample extraction and storage of extracts in metabolomics research. *Metabolites*, *8*, 1. https://doi.org/10.3390/metabo8010001

Scolari, F., Valerio, F., Benelli, G., Papadopoulos, N. T., & Vanícková, L. (2021). Tephritid fruit fly semiochemicals: Current knowledge and future perspectives. *Insects*, *12*, 408. https://doi.org/10.3390/insects12050408

Silva, L. N., Santos, M. S., Dutra, V. S., Araujo, E. L., Costa, M. A., & Silva, J. G. (2011). First survey of fruit fly (Diptera: Tephritidae) and parasitoid diversity among Myrtaceae fruit across the state of Bahia, Brazil. *Revista Brasileira de Fruticultura*, *33*, 757–764. https://doi.org/10.1590/S0100-29452011000300009

Stadler, E., Ernst, B., Hurter, J., Boller, E., & Kozlowski, M. W. (1992). Tarsal contact chemoreceptors of the cherry fruit fly, *Rhagoletis cerasi*: Specificity, correlation with oviposition behaviour, and response to the synthetic pheromone. In S. B. J. Menken & P. Harrewijin (Eds.), *Proceedings of the 8th international symposium of insect–plant relationships* (pp. 143–146). Kluwer Academic Publishers.

White, I. M., & Elson-Harris, M. M. (1992). *Fruit flies of economic significance: Their identification, and bionomics*. CAB International.

Zucchi, R. A. (2001). Mosca-do-mediterrâneo, *Ceratitis capitata* (Diptera: Tephritidae). In E. F. Vilela, R. A. Zucchi, & F. Cantor (Eds.), *Pragas Introduzidas no Brasil* (p. 173). Holos.

Zucchi, R. A. (2007). Diversidad, distribución y hospederos del género *Anastrepha* en Brasil. In V. Hernández-Oritz (Ed.), *Mosca de la fruta en Latinoamérica (Diptera: Tephritidae): diversidad, biología y manejo* (pp. 77–100). S y G Editores.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Magalhães, D. M., Michereff, M. F. F., Kovaleski, A., Paranhos, B. A. G., Nava, D. E., Borges, M., Laumann, R. A., & Blassioli-Moraes, M. C. (2022). Host-marking pheromone of the South American fruit fly *Anastrepha fraterculus* and cross-recognition by the Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae). *Journal of Applied Entomology*, *146*, 1087–1097. https://doi.org/10.1111/jen.13058