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Oviposition behaviour and larval development of *Anastrepha fraterculus* from Argentina in citrus

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Abstract

Citrus peel physicochemical attributes are considered the main components conferring partial or even total resistance to fruit fly (Diptera: Tephritidae) infestation. Fruit fly females adapt their ovipositional strategies to overcome such resistance. Here, we explored the effects of citrus species (Rutaceae) on the ovipositional behaviour of the South American fruit fly, *Anastrepha fraterculus* (Wiedemann), and on its immature development. Particularly, we investigated the effects of (1) citrus species on oviposition behaviour and immature development, (2) citrus species on oviposition preference and on the location of the eggs at different depth in the citrus peel, and (3) harvest season and post-harvest storage time on oviposition behaviour and immature development in lemon. Citrus species influenced ovipositional behaviour and affected survival of immature stages. Females laid eggs in lemon [*Citrus limon* (L.) Burm.], orange [*Citrus sinensis* (L.) Osbeck], and grapefruit (*Citrus paradisi* Macfadyen). In orange and lemon, larvae were found dead close to the oviposition areas, suggesting chemically mediated resistance mechanisms. Under choice conditions, females preferred grapefruit over lemon and bigger clutches were found in the layers where embryonic development is favoured. Unsuitability of lemon as a medium to complete development was neither affected by harvest season nor by storage time of the fruit after harvest. The physical and chemical characteristics of the peel were distinctive to each citrus species and may have affected the specific levels of resistance of these citrus species to infestation by *A. fraterculus*.

Introduction

Within insect–plant interactions, host location, host recognition, oviposition, and the capacity of the host to sustain immature development determine the suitability of a given plant as a host. In holometabolous insects, in which the larvae cannot move from host to host, adult female decisions are crucial for offspring survival. When the host is a crop and the insect is a pest, the outcome of such interaction has applied implications. One typical example are true fruit flies (Diptera: Tephritidae), which represent a serious threat in fruit-producing regions. Their impact is attributed to the damage caused by larval activity in the fruit and the restrictions to access pest-free markets (Malavasi et al., 1994). Understanding insect–plant relationships for each fruit fly species and possible host plant species is fundamental to determine the correct host status of a given commercial fruit for a given fruit fly species (Aluja & Mangan, 2008) and, consequently, to assess the risk of pest introduction during trade.

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The South American fruit fly, *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae), is native to South America with a distribution range from the southern USA to Argentina (Hernández-Ortiz & Aluja, 1993; Malavasi et al., 2000). It infests various families of fruit species with more than 80 host species reported (Norrbom & Kim, 1988; Norrbom, 2004) and there is ample evidence indicating that it comprises a complex of cryptic species (Steck, 1998; Hernández-Ortiz et al., 2004), with several morphotypes (Hernández-Ortiz et al., 2012) and records of differential host use (Aluja et al., 2003). In Argentina, only one morphotype is present [referred to as *A.* sp.1 aff. *fraterculus* (Yamada & Selivon, 2001) and also as Brazilian 1 morphotype (Hernández-Ortiz et al., 2012)] and it has been reported to naturally infest certain citrus species (Putruelle, 1996; Ovruski et al., 2003; Segura et al., 2006; Oroño et al., 2008).

Citrus fruits (Rutaceae) are mostly reported as poor hosts or non-preferred host for fruit flies (Back & Pemberton, 1915; Papachristos & Papadopoulos, 2009; Muthuthantri & Clarke, 2012). Peel physicochemical attributes are considered as the main components that confer partial or even total resistance to fruit flies (Back & Pemberton, 1915; Greany et al., 1983; Spitler et al., 1984; Papachristos et al., 2008). The gum secretion and the hardened calluses that drown the eggs and larvae (Bodenheimer, 1951; Spitler et al., 1984), and the toxic effect of flavedo chemical substances, mainly the essential oils (Back & Pemberton, 1915; Greany et al., 1983; Salvatore et al., 2004; Papachristos et al., 2008), are proposed as the major resistance mechanisms. The peel elasticity and the thickness also contribute to resistance (Papachristos & Papadopoulos, 2009; Muthuthantri, 2013) and interfere with the ovipositional behaviour of the female (Greany, 1989; Leyva et al., 1991; Birke et al., 2006). If the albedo is thick, the larvae can avoid the toxicity of the flavedo in their way to the pulp (Greany, 1989; Leyva et al., 1991; Birke et al., 2006). This has been reported for *Ceratitis capitata* (Wiedemann) (Papachristos et al., 2008) and for *Anastrepha ludens* (Loew), the long ovipositor has been accounted for its capacity to develop in citrus (Birke et al., 2006). In contrast, *Anastrepha suspensa* (Loew) only lays its eggs in the flavedo layer of the grapefruit (*Citrus paradisi* Macfadyen) peel (Greany et al., 1983; Eskafi, 1988; Rössler & Greany, 1990) and cannot avoid toxic essential oils with concomitantly high egg and larval mortalities (Ortu, 1978; Greany et al., 1983, 1985; Styer & Greany, 1983).

Maturation and post-harvest storage time lead to physical and chemical changes that affect fruit immunity and concerns plant regulatory organizations. Particularly in citrus, the chemical composition of the peel changes quantitatively and qualitatively during maturation, greatly affecting its toxic properties (Attaway et al., 1967, 1968; Greany et al., 1983; Greany, 1989; Flamini & Cioni, 2010). Changes in the chemical composition also occur when the fruit is removed from the plant and stored before commercialization. This has been associated with a decrease in the amount of aldehydes, alcohols, and coumarins present in extracts of lemon, *Citrus limon* (L.) Burm., peel (Salvatore et al., 2004).

Given the quarantine and therefore economic relevance of the exact host determination, there are several protocols for determining host status (Couey & Chew, 1986; Cowley et al., 1992; APPPC (Asia and Pacific Plant Protection Commission), 2005; Follett & Neven, 2006; Aluja & Mangan, 2008). Although recent studies argue that the best approach is to work under field conditions (Aluja & Mangan, 2008), laboratory tests are useful when their aim is to determine the mechanisms involved in host resistance to pest infestation. In addition, laboratory tests are useful to explore if the resistance offered by the plant is lost by chemical and physical changes after harvest (Salvatore et al., 2004; Mangan & Moreno, 2012).

In the case of lemon and *A. fraterculus*, development was not completed in field and laboratory infestation trials (Gastaminza et al., 2007). Moreover, field surveys and packing house inspections found no evidence of naturally occurring infestations (Augier et al., 2007). Those results were taken as evidence for the non-host status of this citrus species. In spite of this, the mechanisms involved are still poorly understood. In particular, it was not assessed whether females lay eggs in this fruit and, if oviposition occurs, what depth of the peel is preferred.

Considering that in Argentina *A. fraterculus* is present in areas of citrus production, our objective was to analyse the insect–plant relationship between three citrus species and this fruit fly. Particularly, we investigated the effect of: (1) citrus species on oviposition behaviour and immature development, (2) citrus species on oviposition preference and on the location of the eggs at different depth in the citrus peel, and (3) harvest season and post-harvest storage time on oviposition behaviour and immature development in lemon. In all cases, we determined the chemical composition of the citrus peel.

Materials and methods

Insects

Adult females of *A. fraterculus* were obtained from a colony established at the Agriculture Zoology laboratories of Estación Experimental Agroindustrial Obispo Colombres (EEAOC), Tucumán, Argentina. This colony was initiated in 1997 with pupae obtained from infested guavas

(*Psidium guajava* L.), collected in the vicinity of Tafí Viejo, Tucumán province (northwest Argentina) (Jaldo, 2001). At the beginning of the trials, it had ca. 100 generations under artificial rearing. Rearing follows the procedures described in Jaldo et al. (2001) and Vera et al. (2007) with oviposition occurring through a cloth covered with a thin silicon layer. To ensure that most of the experimental females were already inseminated, we took females from the rearing cages during their peak of oviposition and from which egg hatchability was confirmed to be above 80%.

Plant material

Species and varieties. The citrus species used were lemon [C. limon (L.)] cv. Eureka, grapefruit (*C. paradisi* Macfadyen) cv. Foster seedless, and sweet orange [*Citrus sinensis* (L.) Osbeck] cv. Valencia, Lemon was harvested from two localities: the experimental field at EEAOC (26°47′15.45″S, 65°11′23.72″W) in Las Talitas, Tucumán (experiments 1 and 2), and the commercial orchard El Rodeo in Burruyacú Department, Tucumán (26°39′28.56″ S, 64°55′25.36″W) (experiment 3). For the latter experiment, fruit was harvested when it was light green at two different times (referred to as summer and winter fruit). Grapefruit was harvested from the experimental field at EEAOC. Sweet orange was harvested from the commercial orchard El Carmen, in San Isidro de Lules Department (26°55′16″S, 65°19′33″W), Tucumán. In all cases, fruits were randomly selected from various plants. Special caution was taken to avoid collecting damaged fruit or fruit with symptoms of illness or pests. The number of harvested fruits and the time they were stored depended on the experiment.

Fruit peel characteristics. To provide an assessment of fruit suitability for immature fly development, we assessed physical parameters of the peel from fresh fruit of the three citrus species. The thickness of the flavedo and albedo layers, and the number of oil glands in the flavedo were recorded. Flavedo and albedo thickness were measured with a Vernier calliper upon the equatorial diameter. The density of the oil glands in each citrus fruit species was determined by counting the oil glands in a 1-cm^2 portion of the peel using a stereoscopic magnifying glass. Three counts were made per fruit and the values were averaged. These recordings were performed for experiments 1 and 2.

Extraction and chemical characterization of ether extracts. Another group of 10 fruits was used to extract the compounds from the peel for its chemical characterization. One day after harvest, the fruit was washed with tap water and dried at room temperature. The flavedo was removed from the peel with a metal grater and placed in a glass Erlenmeyer flask. Peel compounds were extracted by immersion in ethyl ether. The flask was covered with a cotton plug and was shaken for 40 min. Extracts were filtered and the solvent was evaporated using a rotary evaporator at room temperature.

Chemical characterization of the extracts was performed at the Laboratory for Research and Analytical Services (LISA) of the Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán (FBQF, UNT, Tucumán, Argentina). The ether extracts were analysed by gas chromatography (GC) using an Ultra Trace gas chromatograph with DB-1 column-MS 25 × 0.25 mm i.d., temperature ramp of 60 to 300 °C (3 °C per min), and an injection temperature of 270 °C. The mass spectrometer (MS) used was a Polaris Q, EI (+) 70 eV (ThermoElectron, Austin, TX, USA),) with an ion trap analyzer as detector. Individual peaks were identified by the retention time and retention rates. Two or three injections were performed for each extract. The results were processed to obtain the percentage of the area in the spectrogram occupied by each compound and this value was averaged in each extract. Identification of the components was performed by comparison of their retention index (RI) with reference to a homologous series of *n*-alkanes (C9-C25), by comparing their mass spectra with those reported in literature, and by computer matching with the Adams (2001) library.

Experiments

All experiments were performed in the laboratory at 25 ± 5 °C, 65 ± 5% r.h, and L12:D12 photoperiod. Gravid females were at their peak of the oviposition period. Experimental cages consisted of 15-l transparent plastic cages which were provided with the standard adult diet containing sugar, hydrolysed yeast (MP Biomedicals, Santa Ana, CA, USA), hydrolysed maize (Gluten Meal; ARCOR, Tucumán, Argentina), and vitamin E (Parapharm, Buenos Aires, Argentina) (Jaldo et al., 2001). Before being placed in the cages, each fruit was washed with tap water. For experiments 1 and 2, fruits were used 24 h after harvest; for experiment 3, various time intervals after harvest were evaluated. In all cases, fruit was removed from the cages 24 h after the beginning of the exposure to the females.

Experiment 1. The effect of citrus host (lemon, sweet orange, or grapefruit) on oviposition behaviour and immature development was determined in a no-choice experiment. Six fruits of a given species and 120 females were placed in each experimental cage and six cages (replicates) were set up for each fruit species. On the following day, the fruits were removed from the cages. Fruit coming from the six replicates for each citrus species

were grouped and randomly assigned to one of two groups.

In the first group, fruits were placed into a plastic tray and covered with a voile fabric and stored in a chamber at 26 °C and 60% r.h. for 5–7 days to allow embryonic development. After this period, fruit was dissected with a scalpel and the flavedo was removed from the peel. With the aid of a stereomicroscope, the number of successful oviposition events (clutch) per fruit, the number of eggs per clutch (clutch size), the number of egg shells or chorions (embryo completed development and the larvae initiated its way to the pulp), and the number of turgid eggs that failed to hatch (non-fertilized eggs or with dead embryos) were registered. Because the chorion of *A. fraterculus* is translucent, it was not possible to assess egg hatchability accurately in the albedo region. Some fruit were in poor conditions, with fungal infection, and were not evaluated.

In the second group, fruits were weighed and placed individually in plastic containers with sterilized sand as pupation substrate. Each container was covered with a voile fabric and incubated for 21 days. The sand was sieved and the number of pupae recovered was recorded every 7 days.

Experiment 2. Oviposition preference for lemon and grapefruit and the location of the eggs within the citrus peel were assessed in choice and no-choice experiments. In each experimental cage (as described above), two fruits were placed in opposite corners. In the choice experiment, one lemon and one grapefruit were placed in the cage; in the no-choice experiment, two fruits belonging to the same citrus species were placed. In both cases, 10 females were released in each cage. Exposure time was 48 h. Ten experimental cages (independent replicates) of each situation (choice and no-choice for each fruit species) were set up. After exposure, the fruits were removed from the cages and assigned to one of three groups. The first group was used to assess oviposition preference and involved the choice cages. To prevent development and allow easy identification of the eggs, fruits were maintained at 2–5 °C. On the following days, the number and location of the successful ovipositions within the peel (inside the essential oil glands or in the space between glands for the flavedo or in the albedo), as well as clutch size were registered using a stereomicroscope. This procedure was carried out in three randomly selected squares of the peel (3 × 3 cm). Data obtained from the three areas were summed to obtain the variables to be analysed. The second group of fruits, obtained from the no-choice experiments, was used to assess hatching. Fruits were stored in a chamber at 25 ± 2 °C and 70% r.h. to allow embryonic development. After 7 days of incubation, the number of chorions and turgid eggs was determined until 10 eggs were counted for each of the three peel layers. Hatch percentage was estimated by dividing the number of chorions on the total number of eggs. The third group, also from the no-choice experiments, was placed on individual sandboxes as described in experiment 1 to allow larval development. After incubation, the number of pupae per fruit was recorded.

Experiment 3. The effect of harvest season and post-harvest storage time in lemon cv. Eureka on oviposition and immature development was determined in a no-choice experiment. Methodology was similar as used in experiment 1. Two harvest periods (summer and winter) and five post-harvest storage times (1 day, 2, 4, 6, and 10 weeks) were evaluated. The experiments involved two fruit seasons within each harvest time (two replicates). After 7 days of incubation, fruits were dissected and the same variables as in experiment 1 were recorded. When a given fruit had less than 10 eggs, it was not included in the analysis of egg hatch percentage.

Data analysis

Experiments 1 and 3. Statistical analysis was performed using ANOVA. Depending on the experiment, the fixed factors were, citrus species (lemon, orange, or grapefruit), harvest season (summer or winter), or post-harvest storage time (1 day, 2, 4, 6, or 10 weeks). The dependent variables were number of successful oviposition events per fruit, number of eggs per clutch, egg hatch percentage, and number of pupae per kg of fruit. In all cases, the assumptions of normality and homoscedasticity were verified. Means were separated by multiple comparisons Tukey tests ($\alpha = 0.05$).

Experiment 2. To evaluate the oviposition preference for lemon or grapefruit, we used ANOVA with two fixed factors: citrus species (lemon or grapefruit) and test condition (choice or no-choice). Response variables were the number of successful oviposition events (clutches) and clutch size. To assess the impact of citrus species on egg location, we also applied ANOVA with fruit species and peel region as fixed factors and number of clutches and clutch size as response variables. To assess the impact of clutch location on egg hatchability, we followed the same procedure: ANOVA with citrus species and clutch position (in this case, in the essential oil's gland or between the glands) as fixed factors. The response variable was egg hatchability. Data for choice and no-choice experiments were pooled. In all cases, the assumptions of normality and homoscedasticity were verified.

When fruit peel attributes were recorded, we compared the different variables by means of an ANOVA (experiment 1) or Student's t test (experiment 2) using fruit species as fixed factor and the thickness of the flavedo and the albedo as well as the number of essential oil glands per cm^2 as response variables. In experiment 1, means were separated by multiple comparisons Tukey tests ($\alpha = 0.05$). All analyses were performed with InfoStat statistical software (Di Rienzo et al., 2012).

Results

Experiment 1. Effect of citrus species on oviposition behaviour and immature development

Fruit peel characteristics. The three citrus species presented particular physical and chemical attributes in their peels (Table 1). Whereas the thickness of the flavedo was similar for the three citrus species ($F_{2,22} = 1.54$, $P = 0.24$), the thickness of the albedo differed among species ($F_{2,22} = 39.94$, $P<0.001$); grapefruit had the thickest albedo, whereas that of sweet orange and lemon were similar to each other. Also the number of glands per cm^2 was different among species ($F_{2,22} = 63.65$, $P<0.0001$); the highest value occurred in sweet orange, whereas lemon and grapefruit presented similar values.

Chemical characterization of citrus ether extracts. The chemical composition of the ether extracts differed among citrus species (Table 2). The major chemical group presented in all extracts was monoterpene hydrocarbons. Within this group, the major compound was limonene, (95.6% in sweet orange, 82.7% in grapefruit, and 71.5% in lemon). The remaining compounds were either monoterpene hydrocarbons or from other chemical groups such as oxygenated monoterpenes, sesquiterpenes, and coumarins, and ranged from 0.01 to 9.4%. Lemon ether extract contained more β-pinene and γ-terpinene (6.1 and 9.4%, respectively) than grapefruit (0.2 and 0.1%). In sweet oranges, these compounds were present below 0.1%. The percentages of monoterpene hydrocarbons, sesquiterpene hydrocarbons, alcohols, aldehydes, esters, and coumarins in the various extracts were, respectively, 91.9-1.1-0.9-3.6-0.7-0.3 (lemon), 85.2-1.6-1.0-2.5-0.2-1.7 (grapefruit), and 98.0-0.1-0.6-1.0-0, 0 (sweet orange).

Oviposition behaviour and immature development. The number of successful oviposition events (clutches) was not affected by citrus species ($F_{2,37} = 0.88$, $P = 0.42$). Clutch size was marginally affected by citrus species ($F_{2,37} = 3.22$, $P = 0.051$). As a trend, lemon showed higher values than grapefruit (Table 3). Egg hatch rate was affected by citrus species ($F_{2,37} = 4.11$, $P = 0.025$); this value was higher for grapefruit than for lemon, and for sweet orange it was intermediate (Figure 1). In lemon and orange, all larvae were found dead 5 days after incubation. In grapefruit, after 5 days of incubation, we visualized the galleries made by the larvae on their way to the pulp. Pupae were recovered only from grapefruit with an average of 62.9 ± 12.1 pupae kg^{-1} of fruit (Table 4).

Experiment 2. Effect of fruit species on oviposition preference and effect of fruit peel characteristics on the location of eggs and immature development

Fruit peel characteristics. Flavedo thickness was not different between lemon and grapefruit ($T = 0.38$, $P = 0.72$). The albedo from grapefruit was significantly thicker than that from lemon ($T = 9.29$, $P<0.001$). The number of glands per cm^2 was also significantly higher in grapefruit than in lemon ($T = 4.67$, $P = 0.0016$).

Chemical characterization of ether extracts from lemon and grapefruit. Thirty-two compounds were detected by GC and GC-MS in the lemon extract, and only 22 in the grapefruit extract (Table 5). The main chemical groups present in both extracts were monoterpene hydrocarbons (85.6 and 90.8% in lemon and grapefruit extracts, respectively), sesquiterpenes (4.0 and 1.6%), alcohols (2.2 and 0.24%), aldehydes (3.4 and 0.35%), and coumarins (0.28 and 0.10%). Among hydrocarbon monoterpenes,

Table 1 Physical characteristics (mean ± SE) of citrus fruit peels from lemon cv. Eureka, sweet orange cv. Valencia, and grapefruit cv. Foster seedless (experiment 1)

	Thickness (mm)					
Citrus species	Flavedo	n	Albedo	n	No. oil glands cm^{-2}	n
Lemon	1.50 ± 0.01a	5	1.80 ± 0.01b	5	57.6 ± 2.8b	10
Sweet orange	1.90 ± 0.01a	10	2.70 ± 0.02b	10	101.8 ± 3.8a	10
Grapefruit	1.80 ± 0.01a	10	6.10 ± 0.05a	10	60.9 ± 2.5b	10

Means within a column followed by different letters differ significantly (one-way ANOVA followed by Tukey's test: $P<0.05$).

Table 2 Mean (± SE) relative percentages (area) of the chemical components of the ether extracts from the peel of lemon cv. Eureka, sweet orange cv. Valencia, and grapefruit cv. Foster seedless (experiment 1)

	RI[1]	Lemon	Sweet orange	Grapefruit
Component				
α-Thujene	935	0.25 ± 0.02	–	–
α-Pinene	942	1.20 ± 0.17	0.45 ± 0.16	0.22
Camphene	957	1.27 ± 0.17	0.23 ± 0.05	0.33 ± 0.06
β-Pinene	988	6.08 ± 0.73	0.04 ± 0.01	0.21 ± 0.05
Myrcene	1000	1.54 ± 0.17	1.53 ± 0.26	1.36 ± 0.12
Octanal	1010	0.02 ± 0.01	0.36 ± 0.07	0.58 ± 0.30
Pseudolimonene	1018	tr[2]	0.10 ± 0.01	–
o-Cimene	1032	0.18 ± 0.25	–	–
D-Limonene	1042	71.53 ± 1.37	95.57 ± 0.41	82.66 ± 0.98
(*E*)-β-Ocimene	1055	0.11 ± 0.00	0.02 ± 0.00	0.23 ± 0.05
γ-Terpinene	1068	9.38 ± 0.43	0.03 ± 0.00	0.10 ± 0.01
cis-Sabinene hydrate	1075	0.08 ± 0.02	0.01 ± 0.00	0.33 ± 0.44
Terpinolene	1097	0.32 ± 0.01	0.02 ± 0.00	0.07 ± 0.05
Linalool	1107	0.27 ± 0.05	0.54 ± 0.00	0.43 ± 0.28
Nonanal	1111	0.09 ± 0.01	0.06 ± 0.01	0.12 ± 0.08
Camphor	1158	0.04 ± 0.01	0.04 ± 0.00	0.12 ± 0.06
(*E*)-Isocitral	1190	0.38 ± 0.05	0.08 ± 0.01	0.43 ± 0.31
Decanal	1209	0.02 ± 0.01	0.28 ± 0.01	0.71 ± 0.30
Nerol	1229	0.32 ± 0.03	0.02 ± 0.01	0.14 ± 0.09
Neral	1240	1.25 ± 0.11	0.08 ± 0.00	0.17 ± 0.11
Geraniol	1252	0.23 ± 0.02	–	0.10 ± 0.08
Geranial	1267	1.82 ± 0.16	0.11 ± 0.01	0.36 ± 0.22
Undecanal	1299	0.03 ± 0.01	0.01 ± 0.00	0.07 ± 0.04
Neryl acetate	1357	0.44 ± 0.02	–	0.02
α-Copaene	1368	–	0.02 ± 0.00	0.27 ± 0.03
Geranyl acetate	1375	0.25 ± 0.01	–	0.16 ± 0.07
β-Elemenene	1381	0.01 ± 0.00	0.02 ± 0.01	0.23 ± 0.03
Dodecanal	1398	–	0.05 ± 0.01	0.06 ± 0.03
β-Caryophyllene	1409	0.33 ± 0.01	0.01 ± 0.00	0.65 ± 0.01
α-Humulene	1442	0.03 ± 0.01	–	0.11 ± 0.02
Bicyclo germacrene	1482	0.05 ± 0.02	–	0.07 ± 0.03
β-Bisabolene	1494	0.72 ± 0.03	0.01 ± 0.00	–
δ-Cadinene	1506	tr	0.03 ± 0.01	0.30 ± 0.01
Hexadecanoic acid	1865	0.02 ± 0.01	0.02 ± 0.00	0.10 ± 0.01
Citroptene	1875	0.24 ± 0.15	–	0.01
Bergamotene	1929	0.01 ± 0.00	–	0.09 ± 0.01
Ostole	1989	–	–	0.19 ± 0.01
Coumarin	2056	0.01	–	0.35 ± 0.47
Oxypseucedanin	2063	–	–	5.69 ± 1.82
Prangenin	2210	–	–	0.23 ± 0.01
Auraptene	2292	–	–	1.56
NI[3]	2304	0.05 ± 0.00	–	1.03 ± 0.76
Total		98.57	99.70	99.78
Chemical group				
Monoterpene hydrocarbons		91.86	97.98	85.16
Sesquiterpene hydrocarbons		1.14	0.09	1.63
Alcohols		0.90	0.57	0.99
Aldehydes		3.61	1.01	2.48
Esters		0.69	0	0.18
Coumarins		0.25	0	1.66

[1]RI, Retention index on a DB-1MS column relative to homologous series of *n*-alkanes.
[2]tr, trace (<0.01%).
[3]NI, not identified.

Table 3 Mean (± SE) number of *Anastrepha fraterculus* clutches and clutch size (no. eggs) on lemon cv. Eureka, sweet orange cv. Valencia, and grapefruit cv. Foster seedless (experiment 1)

Citrus species	n	No. clutches	Clutch size
Lemon	14	14.2 ± 2.9a	2.9 ± 0.2a
Sweet orange	8	12.4 ± 1.3a	2.8 ± 0.4ab
Grapefruit	18	16.6 ± 1.4a	2.3 ± 0.2b

Means within a column followed by different letters differ significantly (one-way ANOVA followed by Tukey's test: P<0.05).
n, number of fruit tested.

limonene was the main component in both extracts (88.5% in grapefruit, 64.3% in lemon). Other main components were γ-terpinene (11.0 and 0.23%) and β-pinene (5.9 and 0.34%). Lemon extract showed 1.1% of neral and 1.7% of geranial, whereas these compounds were traces in the extract from grapefruit.

Oviposition preference. The number of clutches per sample unit was affected by citrus species ($F_{1,20} = 7.15$, $P = 0.015$) but not by test condition ($F_{1,20} = 0.10$, $P = 0.75$). The interaction between the two factors was not significant ($F_{1,20} = 0.77$, $P = 0.39$). In choice assays, more clutches were laid on grapefruit than on lemon (Table 6). The number of eggs per clutch was neither affected by any of the analysed factors (citrus species: $F_{1,20} = 0.05$, $P = 0.82$; test condition: $F_{1,20} = 0.18$, $P = 0.68$), nor by their interaction ($F_{1,20} = 0.08$, $P = 0.79$) (Table 6).

Location of the clutch at different depths of citrus peel. The number of clutches per sample unit was affected by citrus species ($F_{1,54} = 7.8$, $P = 0.0072$) as well as its location in the citrus peel ($F_{2,54} = 6.36$, $P = 0.0033$). The interaction between these factors was not significant ($F_{1,20} = 0.13$, $P = 0.88$). Most clutches were recorded in the flavedo area of grapefruit, first in the space between the oil glands and then in the oil glands. The lowest value was recorded in the albedo of lemon (Table 7).

The number of eggs per clutch was influenced by its location in the citrus peel ($F_{2,43} = 8.4$, $P = 0.0008$), whereas citrus species did not affect this variable ($F_{1,43} = 0.229$, $P = 0.64$), and the interaction of these factors was not significant ($F_{1,43} = 0.13$, $P = 0.89$).

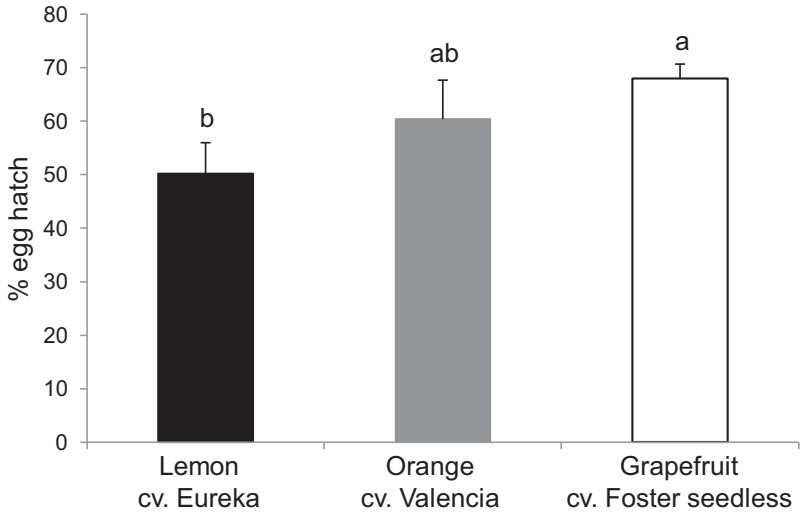

Figure 1 Mean (+ SE) egg hatchability (%) for *Anastrepha fraterculus* on three citrus species. Means capped with a different letter are significantly different (one-way ANOVA followed by Tukey's test: P<0.05).

Table 4 Physical characteristics (mean ± SE) of lemon cv. Eureka and grapefruit cv. Foster seedless peels (experiment 2)

		Thickness (mm)		
Citrus species	n	Flavedo	Albedo	No. oil glands cm^{-2}
Lemon	5	1.64 ± 0.04b	1.78 ± 0.09b	76.87 ± 5.95b
Grapefruit	5	1.58 ± 0.15b	3.06 ± 0.11a	114.73 ± 5.50a

Means within a column followed by different letters differ significantly (one-way ANOVA followed by Tukey's test: P<0.05).
n, number of fruit tested.

Table 5 Mean (± SE) relative percentages (area) of the chemical components of the ether extracts from the citrus peel of lemon cv. Eureka and grapefruit cv. Foster seedless (experiment 2)

	RI[1]	Lemon	Grapefruit
Component			
α-Tujene	935	0.14 ± 0.04	–
α-Pinene	942	0.69 ± 0.17	–
Sabinene	983	1.13 ± 0.23	0.28 ± 0.06
β-Pinene	988	5.94 ± 0.59	0.23 ± 0.31
Myrcene	1000	1.11 ± 0.21	1.30 ± 0.26
Octanal	1010	0.04 ± 0.01	0.23 ± 0.03
δ-3-Carene	1025	0.16 ± 0.04	–
o-Cimene	1032	0.66 ± 0.13	0.07
D-Limonene	1042	64.27 ± 4.58	88.46 ± 0.88
(*E*)-β-Ocimene	1055	0.10 ± 0.01	0.14 ± 0.03
γ-Terpinene	1068	10.96 ± 0.60	0.34 ± 0.49
cis-Sabinene hydrate	1075	0.13 ± 0.03	0.04 ± 0.03
NI[2]		0.05 ± 0.01	0.06
Terpinolene	1097	0.46 ± 0.09	0.03 ± 0.02
Linalool	1107	0.35 ± 0.08	0.24 ± 0.01
Nonanal	1111	0.11 ± 0.03	0.07 ± 0.01
Camfor	1158	0.18 ± 0.05	0.10 ± 0.02
NI		0.56 ± 0.12	0.12 ± 0.02
NI		0.08 ± 0.02	0.48 ± 0.08
Nerol	1229	0.86 ± 0.20	0.02
Neral	1240	1.13 ± 0.19	0.05 ± 0.00
Geraniol	1252	0.90 ± 0.20	0.04 ± 0.04
Geranial	1267	1.70 ± 0.29	0.08 ± 0.03
Perilla aldehyde	1269	0.04 ± 0.01	0.06
Neryl acetate	1357	1.53 ± 0.25	0.03 ± 0.01
NI	–	–	0.26 ± 0.06
Geranyl acetate	1375	0.44 ± 0.07	0.14 ± 0.04
β-Elemenene	1381	–	0.26 ± 0.07
cis-α-Bergamotene	1406	0.04 ± 0.01	0.08
β-Caryophyillene	1409	0.39 ± 0.08	1.05 ± 0.22
α-trans-Bergamotene	1425	1.17 ± 0.16	0.02
α-Humulene	1442	0.04 ± 0.01	0.12 ± 0.03
NI		0.02 ± 0.00	0.19 ± 0.05
Valencene	1478	0.49 ± 0.11	0.08 ± 0.02
Biciclo Germacrene	1482	0.10 ± 0.01	0.09 ± 0.02
β-Bisabolene	1488	0.15 ± 0.04	–
(*Z*)-γ-Bisabolene	1494	1.88 ± 0.35	–
BTH	1496	0.12 ± 0.06	0.35 ± 0.11
NI		–	0.36 ± 0.10
NI		0.07 ± 0.01	0.05 ± 0.06
NI		0.06 ± 0.02	0.09 ± 0.04
NI		–	1.84 ± 0.36
Citroptene	1875	0.28 ± 0.07	–
NI		0.13 ± 0.01	–
Ostole	1989	–	0.10 ± 0.06
NI		–	0.40 ± 0.06
Total		95.12	92.08
Chemical group			
Monoterpene hydrocarbons		85.58	90.25
Sesquiterpene hydrocarbons		4.02	1.57
Alcohols		2.24	0.24
Aldehydes		3.38	0.35
Coumarins		0.28	0.10

Table 5. Continued

[1]RI, Retention index on a DB-1MS column relative to homologous series of *n*-alkanes.
[2]NI, non-identified.

Anastrepha fraterculus females laid more eggs per clutch in the albedo than in the flavedo (Table 7).

Immatures development. Egg hatchability was affected by citrus species ($F_{1,10} = 31.36$) and clutch location ($F_{1,10} = 56.03$, both $P<0.001$) (Figure 2). In lemon, the percentage of egg hatch was significantly lower when the eggs were laid in the glands than when they were laid between the glands. In grapefruit, hatch rates were similar in and between the glands. At the time of recording this variable, all larvae were dead in lemon. In grapefruit, we observed the galleries made by the larvae on their way to the pulp. Pupae were recovered only in grapefruit (93.8 ± 19.7 pupae kg^{-1} of fruit).

Experiment 3. Effect of harvest season and post-harvest storage time on oviposition behaviour and immature development in lemon

Chemical characterization of ether extracts from the peel. The GC-MS analysis indicated differences in the relative amounts of the compounds present in the extracts of the lemon peel between the seasons in which fruit was harvested and the time it was stored after harvest (Table 8). In all cases, the main compound was limonene; for each harvest season, its percentage did not show significant variation as post-harvest storage time passed. The percentage of monoterpene hydrocarbons was not different between the harvest seasons and post-harvest storage times. In lemons harvested during winter, the percentage of hydrocarbons, sesquiterpenes, and oxygenated compounds decreased to half of their initial values after 10 weeks of post-harvest storage time (from 3.1 to 1.6%, and from 5.1 to 2.6%, respectively). For lemons harvested during summer, the percentage of

Table 6 Mean (± SE) number of clutches and clutch size (no. eggs) in lemon cv. Eureka and grapefruit cv. Foster seedless according to assay condition (experiment 2)

Citrus species	Assay condition	No. clutches	n	Clutch size	n
Lemon	Choice	4.29 ± 3.45b	7	1.88 ± 0.45b	6
	No-choice	8.80 ± 4.09ab	5	1.75 ± 0.43b	5
Grapefruit	Choice	17.71 ± 3.75a	7	1.86 ± 0.22b	7
	No-choice	15.60 ± 4.95ab	5	2.13 ± 0.37b	5

Means within a column followed by different letters differ significantly (two-way ANOVA followed by Tukey's test: $P<0.05$).

Table 7 Mean (± SE) number of clutches and clutch size (no. eggs) in lemon cv. Eureka and grapefruit cv. Foster seedless according to position in the peel (experiment 2)

Citrus species	Location	No. clutches	n	Clutch size	n
Lemon	Flavedo				
	Oil gland	2.89 ± 1.10bcd	9	1.26 ± 0.12b	7
	Interglandular	4.44 ± 1.68abc	9	2.15 ± 0.41ab	9
	Albedo	0.67 ± 0.24d	9	3.20 ± 0.80a	5
Grapefruit	Flavedo				
	Oil gland	6.09 ± 1.39ab	11	1.30 ± 0.10b	11
	Interglandular	7.55 ± 1.18a	11	2.01 ± 0.37ab	11
	Albedo	2.73 ± 1.12cd	11	2.83 ± 0.56b	6

Means within a column followed by different letters differ significantly (two-way ANOVA followed by Tukey's test: $P<0.05$).

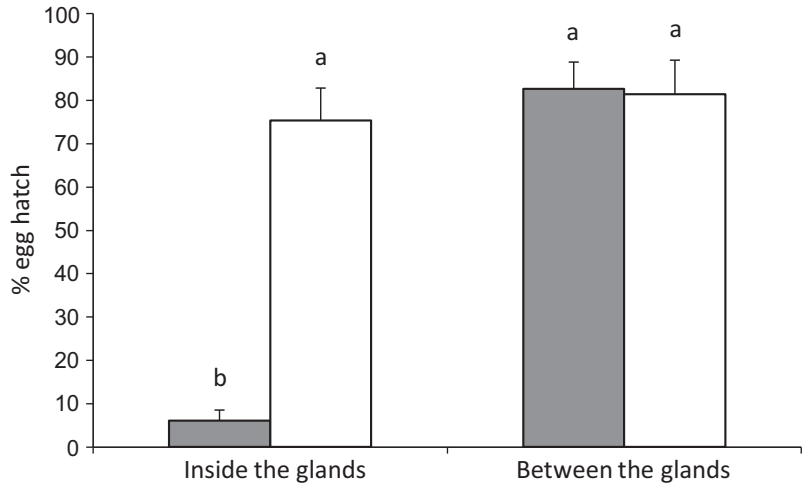

Figure 2 Mean (+ SE) egg hatchability (%) for *Anastrepha fraterculus* according to clutch location in the flavedo for lemon cv. Eureka (grey bars) and grapefruit cv. Foster seedless (white bars). Means capped with a different letter are significantly different (one-way ANOVA followed by Tukey's test: $P<0.05$).

sesquiterpenes doubled after 10 weeks (from 1.8 to 3.6%), whereas the percentage of oxygenates remained constant. The percentage of coumarins was affected for post-harvest storage time, decreasing from 0.25 to 0.09% in winter lemons (Table 8).

Harvest season and post-harvest storage time. The number of clutches per fruit was affected by harvest season ($F_{1,132} = 7.29$, $P = 0.0078$) and by post-harvest storage time ($F_{4,132} = 3.84$, $P = 0.0055$). The interaction of these two factors was not significant ($F_{4,132} = 0.91$, $P>0.05$). Lemons harvested in summer had more clutches than lemons harvested in winter (Table 9). Lemons stored for 4 weeks before being exposed to the females had more clutches than lemons stored for 6 weeks. Clutch size was not affected, neither by harvest season ($F_{1,132} = 0.89$,

Table 8 Chemical composition and mean (± SE) relative percentages (area) of the components in ether extracts of lemon cv. Eureka harvested in winter (W) or summer (S), and tested in the forced infestation trials, 1 day (W0, S0), 6 weeks (W6, S6), and 10 weeks (W10, S10) after sample harvest (experiment 3)

	RI[1]	W0	W6	W10	S0	S6	S10
Component							
α-Pinene	942	0.36 ± 0.01	0.72 ± 0.04	1.39 ± 0.23	1.52 ± 0.20	1.64 ± 0.19	1.19 ± 0.03
Sabinene	983	0.59 ± 0.00	0.99 ± 0.01	1.64 ± 0.23	2.12 ± 0.46	2.12 ± 0.17	1.79 ± 0.01
β-Pinene	988	3.12 ± 0.09	4.85 ± 0.81	7.72 ± 0.05	10.13 ± 0.58	11.43 ± 0.31	8.90 ± 0.02
Myrcene	1000	0.95 ± 0.01	1.34 ± 0.02	1.58 ± 0.35	1.39 ± 0.27	1.18 ± 0.10	1.37 ± 0.03
D-Limonene	1042	73.58 ± 4.19	73.78 ± 0.89	72.39 ± 3.33	65.97 ± 2.43	64.95 ± 4.12	63.92 ± 0.45
γ-Terpinene	1068	9.70 ± 0.08	8.59 ± 1.07	8.94 ± 0.48	10.94 ± 0.19	10.50 ± 1.25	11.65 ± 0.17
α-Terpineol	1190	0.58 ± 0.13	0.46 ± 0.01	0.30 ± 0.10	0.46 ± 0.09	0.43 ± 0.12	0.51 ± 0.01
Neral	1240	0.87 ± 0.17	0.82 ± 0.01	0.56 ± 0.20	0.54 ± 0.07	0.39 ± 0.07	0.63 ± 0.01
Geranial	1267	1.30 ± 0.28	1.06 ± 0.04	0.74 ± 0.28	0.81 ± 0.10	0.54 ± 0.11	0.81 ± 0.01
Neryl acetate	1357	0.71 ± 0.16	0.73 ± 0.13	0.38 ± 0.17	0.64 ± 0.07	0.80 ± 0.23	1.47 ± 0.04
β-Caryophyllene	1409	0.71 ± 0.16	0.53 ± 0.00	0.30 ± 0.14	0.37 ± 0.05	0.39 ± 0.18	0.62 ± 0.03
trans-α-Bergamotene	1425	0.90 ± 0.21	0.79 ± 0.01	0.46 ± 0.21	0.49 ± 0.04	0.55 ± 0.25	0.95 ± 0.02
β-Bisabolene	1494	1.31 ± 0.40	1.13 ± 0.00	0.66 ± 0.35	0.72 ± 0.05	0.92 ± 0.43	1.60 ± 0.08
Citroptene	1875	0.90 ± 1.10	0.23 ± 0.04	0.13 ± 0.06	0.13 ± 0.03	0.20 ± 0.11	0.09 ± 0.02
Total		95.58	96.02	97.19	96.23	96.04	95.50
Chemical group							
Monoterpene hydrocarbons		89.16	91.27	94.89	93.27	93.21	89.94
Sesquiterpene hydrocarbons		3.10	2.69	1.56	1.76	2.16	3.57
Alcohols		1.19	0.64	0.45	1.05	0.57	0.80
Aldehydes		2.82	2.39	1.66	1.90	1.50	2.05
Esters		1.07	1.12	0.52	0.92	1.08	1.91
Coumarins		1.35	0.35	0.15	0.25	0.26	0.09

[1]RI, Retention index on a DB-1MS column relative to homologous series of *n*-alkanes.

Table 9 Mean (± SE) number of clutches and clutch size (no. eggs) in lemon cv. Eureka harvested in summer or winter and with different post-harvest storage time (weeks) (experiment 3)

Harvest time	Post-harvest storage time	No. clutches	n	Clutch size	n
Summer	0	17.6 ± 3.9abc	14	2.1 ± 0.1b	14
	2	20.3 ± 3.3ab	15	2.2 ± 0.2b	15
	4	26.3 ± 3.1a	10	2.3 ± 0.2b	10
	6	11.9 ± 2.9cd	14	1.9 ± 0.2b	14
	10	17.1 ± 4.8abcd	7	2.1 ± 0.2b	7
Winter	0	7.5 ± 1.5d	16	1.8 ± 0.2b	16
	2	16.8 ± 1.9bcd	15	1.9 ± 0.2b	15
	4	16.9 ± 2.3bcd	16	1.9 ± 0.2b	16
	6	10.5 ± 3.1cd	17	3.1 ± 0.3a	17
	10	14.6 ± 2.9bcd	18	2.3 ± 0.3b	18

Means within a column followed by different letters differ significantly (two-way ANOVA followed by Tukey's test: $P<0.05$).

$P = 0.35$), nor by post-harvest storage time ($F_{4,132} = 2.08$, $P = 0.087$). The interaction of these factors was significant ($F_{4,132} = 4.54$, $P = 0.0018$) (Table 9).

Egg hatchability was neither affected by harvest season ($F_{1,102} = 0.38$, $P = 0.54$), nor by post-harvest storage time ($F_{4,102} = 0.91$, $P = 0.46$). The interaction between the two factors was significant ($F_{4,102} = 3.42$, $P = 0.011$). Lemon harvested in winter and stored for 6 weeks had the highest egg hatch percentage (Figure 3).

Discussion

We explored the behavioural and developmental effects of exposure to three citrus species on *A. frater-*

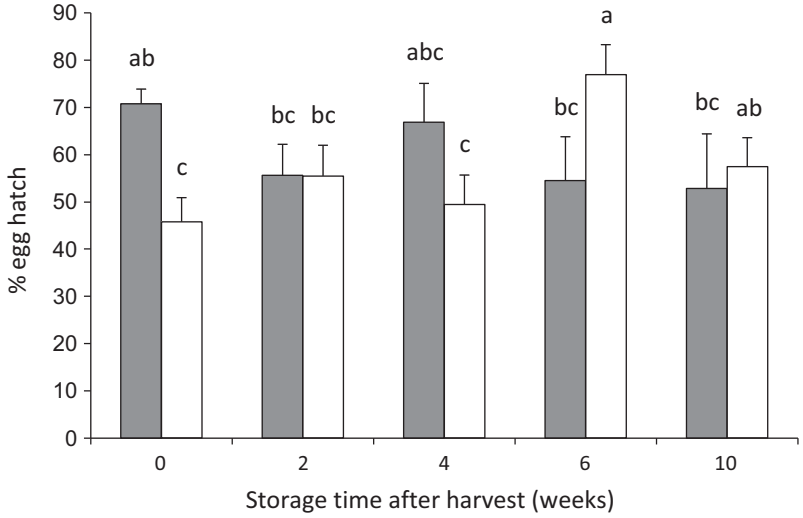

Figure 3 Mean (+ SE) egg hatchability (%) for *Anastrepha fraterculus* according to storage time after harvest for lemons harvested in summer (grey bars) and in winter (white bars). Means capped with a different letter are significantly different (one-way ANOVA followed by Tukey's test: $P<0.05$).

culus. We found evidence that citrus species conditions oviposition behaviour and affects survival of immature stages. Although females laid eggs in lemon, sweet orange, and grapefruit, the way in which this was done differed and development was not always completed. In orange and lemon, larvae were found dead close to the oviposition areas. When given a choice, females preferred grapefruit over lemon. Most eggs were laid in the flavedo but in the albedo the clutch size was higher. Lemon resistance was not affected by harvest season, nor by fruit storage time.

Anastrepha fraterculus laid eggs in lemon, orange, and grapefruit. The number of clutches per fruit was equal among species, but clutch size was higher in lemon and orange than in grapefruit. Egg hatchability was higher in grapefruit and pupae were obtained only in this fruit species. This indicates that even when oviposition occurs, development is not possible in all citrus species. Díaz-Fleischer & Aluja (2003) found that clutch size in *A. ludens* varied with host firmness and degree of ripeness, and they considered this a strategy to compensate for the high mortality of larvae in a bad host. Similar results were found by da Silva-Branco et al. (2000) in *C. capitata*: larval survival in citrus increased as clutch size increased. The high number of dead larvae found in lemon and orange suggested that toxic properties kill the larvae before they reach the pulp (Greany, 1989; Leyva et al., 1991; Birke et al., 2006). Differences in fruit species suitability are also reflected in infestation patterns in the field. In Argentina, there are records of recovery of *A. fraterculus* pupae from mandarin, *Citrus reticulata* Blanco, bitter orange, *Citrus aurantium* L. (Rootstock), grapefruit, and sweet orange (Ovruski et al., 2003; Schliserman & Ovruski, 2004; Segura et al., 2006; Oroño et al., 2008); unfortunately, not all records indicate the variety of fruit species analysed. Regarding lemon, there are no records of naturally occurring infestations (Augier et al., 2007).

When given a choice, *A. fraterculus* females preferred grapefruit over lemon. This was reflected by the higher number of clutches per unit area in grapefruit compared to lemon. However, this did not occur under no-choice conditions. Studies in Mexican populations of *A. fraterculus* demonstrated that this morphotype chooses between hosts of different quality, based on the number of visits made to a particular fruit species and the number of oviposition attempts (Aluja et al., 2003). When grapefruit cv. Ruby Red and orange cv. Valencia were offered, females laid eggs at a very low frequency, only in the laboratory, under no-choice conditions. Field studies conducted in Argentina also showed that oviposition behaviour is affected by fruit species (Oroño, 2010). In spite of this, the number of eggs per clutch was equal regardless of the host and the trial situation. This is contrary to what was found in our first experiment and previous studies in which females modulate the number of eggs per clutch depending on the quality (da Silva-Branco et al., 2000; Aluja et al., 2003; Díaz-Fleischer & Aluja, 2003) and the variety of the host (Papachristos & Papadopoulos, 2009).

We found that females placed their egg clutches differentially within the layers of the peel. Most eggs were located in the flavedo (almost 90% in grapefruit, 85% in lemon), and within this region, the space between the oil

glands was preferred; the other eggs were located in the albedo. Similar results were presented by Papachristos & Papadopoulos (2009). These authors evaluated the host status of sweet orange (three varieties), bitter orange, and lemon for *C. capitata* and found that the percentage of eggs laid in the flavedo and albedo depended on citrus variety. Aluja & Mangan (2008) suggested that size, colour, penetrability, and phenological stage of the fruit and the presence of host-marking pheromones determine the oviposition behaviour in females. The differences found in the number of clutches and their location, indicate that *A. fraterculus* females can modulate oviposition behaviour once the fruit has been accepted as substrate. Interestingly, within a given fruit, the largest clutch sizes were registered in the albedo, the most favourable region for embryo development. The presence of eggs in the albedo was recorded for *C. capitata* in sweet orange, bitter orange, and lemon (Papachristos & Papadopoulos, 2009), for *A. ludens* in grapefruit (Birke et al., 2006) and lemon (Mangan & Moreno, 2012), for *Anastrepha obliqua* (Macquart) in grapefruit (Mangan et al., 2011a), and for *Anastrepha serpentina* (Wiedemann) in grapefruit and sweet orange (Mangan et al., 2011b). The capacity of females to reach this area of the peel has been linked to the length of the ovipositor (Birke et al., 2006). *Anastrepha fraterculus* has an intermediate ovipositor length of 1.65–2.1 mm (Stone, 1942), in between that of *C. capitata* (0.9–1.3 mm; Delrio & Cocco, 2012) and *A. ludens* (3.4–4.7 mm; Stone, 1942).

Egg hatchability was affected by the location of the eggs in lemon but not in grapefruit. Eggs laid inside the glands hatched <15% in lemon and >80% in grapefruit. The results for lemon are in agreement with those of Greany et al. (1983), who found that egg hatchability of *A. suspensa* was significantly higher between glands than within, in lemon cvs Lisbon and Eureka, white and pink grapefruit, and Temple orange. The high egg hatchability found in grapefruit in our study was unexpected but may be explained by the high number of eggs laid in this species (2× more than in lemon, as inferred from the differences in the number of clutches). Díaz-Fleischer & Aluja (2003) proposed that the metabolic heat produced by a large number of larvae can create a microenvironment that favours the growth of bacteria and these could metabolize the toxic compounds present in oil glands. However, this needs confirmation.

The equal number of clutches on lemon and grapefruit in no-choice trials suggests that, under certain conditions, *A. fraterculus* females laid their eggs on a poor host or even in a non-host plant. Many phytophagous insects lay their eggs on plants on which the larvae do not reach the adult stage (Krainacker et al., 1987). Internal physiological changes due to shortage of the preferred host have been proposed as one of the main reasons. When an optimal host is hard to find, it becomes important to have some 'flexibility' to use a sub-optimal host. Laying eggs in poor host plants may also have advantages (Craig et al., 1989), for example, when competition for food is minimized and/or when the energetic cost of searching for the optimal host is high (Mayhew, 1997; Papaj, 2000). In extreme cases, when the female lays its eggs in an atypical plant, it exposes dozens or hundreds of them to a new host, increasing the possibility of exploring a new feeding environment. This phenomenon has been postulated as one possible basis for the population divergence observed within this species (Rull et al., 2013).

Unsuitability of lemon as a medium to complete development was neither affected by the harvest season nor by storage duration of the fruit after harvest. Although citrus fruits stop the ripening process once harvested, significant changes occur in the chemical composition of essential oils of the peel as well as its hardness (Bodenheimer, 1951). This may have an impact on the oviposition behaviour and development of some fruit fly species. Our third experiment indicated differences in the number of *A. fraterculus* clutches; more clutches were recorded for lemons harvested in summer, though complete development was not achieved.

The physical and chemical characteristics of the citrus peel differed among species and these differences may have affected the levels of resistance to infestation by *A. fraterculus*. Thickness of the flavedo did not differ among the citrus species evaluated, but the albedo was thicker in grapefruit. Albedo thickness may be directly correlated with the ability of development of eggs and larvae, as grapefruit was the only species from which pupae were obtained. Interestingly, clutch size was higher in albedo than in flavedo. For other species of the genus *Anastrepha*, there are records of high mortality in the albedo, caused by some chemical compounds present in this region. In *A. obliqua* mortality occurs both in the flavedo and albedo, whereas in *A. ludens* it occurs mostly in the albedo (Mangan et al., 2011b). Regarding the chemical attributes, the spectrogram area represented by the hydrocarbon monoterpenes was (above) 85% in grapefruit, 90% in lemon, and 98% in orange. In addition, grapefruit had a large variety of coumarins. Essential oils perform an important function as attractants, repellents, and toxins. Although attraction for oviposition could have been elicited by the large number of coumarins present in grapefruit, toxicity could have been generated by monoterpenes and aldehydes as shown in other fruit flies (Salvatore et al., 2004; Papachristos et al., 2009) and recently in *A. fraterculus* (Ruiz et al., 2014). The differences between

grapefruit and lemon in the areas occupied by monoterpene hydrocarbons may explain the different egg hatchability in lemon and grapefruit. We also found differences in oil composition of these species. The content of secondary metabolites in plants varies between locations and years, and is influenced by factors such as temperature, humidity, and soil composition (Isman et al., 2007). For example, the concentration of 1,8-cineole and α-pinene ranged from 7 to 55% and from 11 to 30%, respectively, in rosemary plants from different locations in Italia (Flamini et al., 2002). There are similar examples in basil (Pascual-Villalobos & Ballesta-Acosta, 2003) and myrtle (Flamini et al., 2004). During storage of lemon, the amounts of some compounds that are reported as highly toxic to other fruit fly species decreased, such as geranial, β-bisabolene, and citroptene (Salvatore et al., 2004; Papachristos et al., 2009). However, but this did not improve the status of this fruit as a host much, because even under the most favourable conditions, development was not completed.

Our results suggest that *A. fraterculus* females recognize the citrus species, modulate the number of clutches accordingly, and locate their clutches in the layers of the peel where embryonic development is favoured. All this proposes that female behaviour evolved to maximize reproductive success. Yet, females still lay eggs in non-favoured hosts suggesting some flexibility due to host availability. Such findings have implications for the study of insect-plant interactions and, more particularly, in determining the host status of lemon cv. Eureka. The inability to obtain pupae in lemon, even when oviposition and embryonic development occurred, supports the non-host status of this fruit. These results were obtained under laboratory conditions, which are expected to favour complete development. The lack of development in fruit that was stored for several weeks gives additional quarantine security, as it indicates that lemon does not become susceptible to infestation after harvest. Sweet orange was already reported as a non-host of the Mexican morphotype of *A. fraterculus*. Given that the Mexican and the Brazilian 1 morphotypes (Argentinean populations belong to Brazilian 1 morphotype) have the same ovipositor length (Hernández-Ortiz et al., 2004), we propose more comprehensive studies to define a more accurate host status of sweet orange for Argentine *A. fraterculus*. The complete larval mortality close to the egg shells in lemon and orange suggest that chemical resistance acts at the early stages of development and compounds present in the flavedo of the peel are the most likely responsible for this toxicity (Ruiz, 2013; Ruiz et al., 2014). We trust this information is of practical importance at the time of bilateral negotiations between fruit-producing areas and pest-free importing countries.

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