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ORIGINAL CONTRIBUTION

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Response of two parasitoid species (Hymenoptera: Braconidae, Figitidae) to tephritid host and host food substrate cues

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Abstract

The Neotropical-native figitid *Aganaspis pelleranoi* (Brèthes) and the Asian braconid *Diachasmimorpha longicaudata* (Ashmead) are two parasitoids of Tephritidae fruit flies with long and recent, respectively, evolutionary histories in the Neotropics. Both species experienced a recent range of overlap. In Argentina, *A. pelleranoi* is a potential species in biological control programs against the pestiferous tephritid species, *Anastrepha fraterculus* (Wiedemann) and *Ceratitis capitata* (Wiedemann), whereas *D. longicaudata* is already used in open-field releases against Medfly in central-western Argentina. To characterize the host-foraging strategies of *A. pelleranoi* and *D. longicaudata*, olfactometer experiments were conducted comparing responses to *C. capitata* and *A. fraterculus* larvae, in two kinds of food substrate: fruit and artificial larval medium. To control the possible influence of host larvae used for parasitoid rearing on olfactory response, two strains of both parasitoid species, reared on both tephrtid species, were studied. Volatiles directly emanating either from *A. fraterculus* or *C. capitata* larvae may be detected by both *A. pelleranoi* and *D. longicaudata*, although chemical stimuli originating from the combination of host larvae and the habitat of the host were preferred. However, olfactory cues associated with host larvae probably play a relevant role in host searching behaviour of *A. pelleranoi*, whereas for *D. longicaudata*, the host-habitat olfactory stimuli would be highly essential in short-range host location. The strain of the parasitoids did not affect host search ability on the two tephritid species evaluated. These evidences are relevant for mass production of both parasitoids and their impact following open-field augmentative releases.

KEYWORDS
Aganaspis pelleranoi, *Anastrepha fraterculus*, *Ceratitis capitata*, *Diachasmimorpha longicaudata*, olfactory cues, parasitoid behaviour

1 | INTRODUCTION

Success in a biological control program involving parasitoids depends among other factors on the host search efficiency (Lewis & Martin, 1990; Mills & Wajnberg, 2008). This is because suppressing pests relies on many factors that guide parasitoids to locate and attack suitable hosts in complex environments (Hilker & McNeil, 2008). Thus, during the host-location process, physical and chemical cues play an important role in driving parasitoid search behaviour (Godfray, 1994; Vinson, 1998). Although chemical signals are crucial for long- and short-range parasitoid orientation (Hilker & McNeil, 2008; Steidle & Van Loon, 2003; Vet & Dicke, 1992), from

a biological control perspective, chemical cues involved in tritrophic level interactions among parasitoids, hosts and host plants are of major importance in determining parasitoid effectiveness (Meiners & Peri, 2013). In this context, parasitoids may react to olfactory signals coming from both the host food plant and the host itself, often showing arrestment behaviour after contact with host-derived substances (Van Alphen & Jervis, 1996). Additionally, host-location processes may be influenced by previous experience, the rate of host encounter, food substrate type and conditions such as the presence of oviposition deterring pheromones, or changes in stimulus intensity directly issued by either the host species or the host plant (Bernstein & Driessen, 1996).

The literature attests to a great diversity of infochemicals mediating parasitoid foraging for tephritid hosts, such as those coming from uninfested fruit (Eitam, Holler, Sivinski, & Aluja, 2003; Segura, Viscarret, Ovruski, & Cladera, 2012; Stuhl, Sivinski, Teal, & Aluja, 2012), infested fruit (Aluja, Díaz-Fleischer et al., 2009; Canale, Geri, & Benelli, 2014; Eben, Benrey, Sivinski, & Aluja, 2000; Guimarães & Zucchi, 2004; Messing & Jang, 1992; Segura et al., 2016; Silva, Nascimento, Deus, Souza, & Oliveira, 2007), flower-nectar (Rohrig, Sivinski, Teal, Stuhl, & Aluja, 2008), fungi that grow on decaying fruit (Segura et al., 2012), rotting fruit and leaves of the host plant (Messing, Klungness, Jang, & Nishijima,1996), fruit fly host marking pheromone (Prokopy & Webster, 1978), sex pheromone (Benelli et al., 2014) and host larvae (Dias, Stuhl, & Sivinski, 2014; Stuhl, Sivinski, Teal, Paranhos, & Aluja, 2011).

Some fruit fly parasitoids can specialize in exploiting a particular host species. Such is the case of the braconid *Diachasma alloeum* (Muesebeck), which exploits larvae of several sibling species of Tephritidae in the *Rhagoletis pomonella* (Walsh) species group in temperate areas of North America (Forbes, Hood, & Feder, 2010). This braconid parasitoid tends to meet and mates near the plants where its tephritid hosts, are attracted by the host odour, and repelled by plants exploited by sibling species of *Rhagoletis* Loew, a series of features leading to cascading genetic differentiation within *D. alloeum* (Forbes, Powell, Stelinski, Smith, & Feder, 2009). Conversely, both tropical and subtropical fruit fly parasitoid species tend to exploit larvae of multivoltine tephritid species, which often use different host plants throughout the year (Schliserman, Aluja, Rull, & Ovruski, 2016; Sivinski, Piñero, & Aluja, 2000; Vargas, Leblanc, Harris, & Manoukis, 2012). In such a case, host driven specialization could be triggered by responses to signals emanating by the larva itself and probably, not mainly, by the host plant. However, in those cases where a behaviourally relevant substrate has been found, relatively few semiochemicals that attract (host-habitat cues), arrest (host-location cues) and stimulate oviposition behaviour of parasitoids of multivoltine tephritid species have been characterized (reviewed by Sivinski & Aluja, 2012; Dias et al., 2014). Among these chemical compounds, only one coming from tephritid larvae (Dias et al., 2014; Stuhl et al., 2011) and several from infested fruits (Benelli, Flamini, Fiore, Cioni, & Conti, 2013) have been identified.

Relatively little is known on the potential effect of direct cues from the host on attraction of Neotropical fruit fly parasitoids (Dias et al., 2014). In consequence, this study compares the Neotropical-native figitid *Aganaspis pelleranoi* (Brèthes) to the originally Asian braconid *Diachasmimorpha longicaudata* (Ashmead) in experiments dealing with response to both host tephritid flies (hosts) and host-habitat cues. These two parasitoid species belong to a guild of synovigenic, solitary, koinobiont, endoparasitoids that attack late-instar larvae of tephritid fruit flies (Ovruski, Aluja, Sivinski, & Wharton, 2000). Both are generalist fruit fly parasitoids (Aluja, Sivinski et al., 2009). However, these parasitoid species differ in geographic origin; *A. pelleranoi* is the subtropical and tropical rainforest of the Neotropical region (Ovruski et al., 2000), whereas the exotic *D. longicaudata* is native to the Indo-Pacific region. Another important difference between both parasitoid species lies in their host-foraging behaviours. *Aganaspis pelleranoi* females mostly search host larvae within fallen fruit (Ovruski, Schliserman, & Aluja, 2004). By contrast, *D. longicaudata* females forage host larvae both at the soil level and in the canopy, and oviposit by drilling through the pericarp from the exterior (Sivinski & Aluja, 2003). Such differences in foraging behaviour could result in the use of different cues during the process of host finding, where host fruit and larval cues may play different hierarchical roles. Furthermore, host searching behaviour differences would allow the combined use of both parasitoids in fruit fly biological control programmes, since *A. pelleranoi* females can attack larvae in large fruits by entering through the fissures produced in the fruit to fall or by holes produced by its jaws (Aluja, Sivinski et al., 2009), while *D. longicaudata* is limited in this kind of fruit (Sivinski & Aluja, 2003).

Currently, *D. longicaudata* is being mass reared on larvae of a *Ceratitis capitata* temperature-sensitive lethal (tsl) vienna-8 genetic sexing strain at the Bioplanta San Juan facility, Argentina (Suárez et al., 2014), and is being mass released against Medfly in fruit-producing irrigated-valleys of San Juan, central-western Argentina (Sánchez et al., 2016). Regarding *A. pelleranoi*, it has been laboratory-reared on both *C. capitata* and *Anastrepha fraterculus* (Gonçalves et al., 2013; Núñez-Campero, Aluja, Rull, & Ovruski, 2014), and it is a candidate species to be incorporated in augmentative biological control (Aluja, Ovruski, Guillén, Oroño, & Sivinski, 2009). Nevertheless, despite several records of *A. pelleranoi* in Latin American countries attacking *C. capitata* (Aluja, Ovruski et al., 2009; Aluja, Sivinski et al., 2009; Wharton, Ovruski, & Gilstrap, 1998), this native figitid would not be commonly associated with this exotic pest under natural conditions, but rather to *A. fraterculus* (Schliserman et al., 2016). So far, no information has been published on the effect of the parasitoid strain (larval rearing maternal effect) on adult offspring host preferences and host finding behaviour for this species.

The inclusion of both the Neotropical-native *A. pellenaroi* and the exotic *D. longicaudata* in this study allows examining responses to olfactory stimuli of parasitoid species with both long and short evolutionary histories in the Neotropics since both species belong to a recent range of overlap.

Therefore, the main goal here was to compare olfactory responses of different strains of both *D. longicaudata* and *A. pelleranoi*

to cues of *C. capitata* and *A. fraterculus* larvae provided with or deprived of a food substrate. In order to control potential maternal effects, two strains of *D. longicaudata* and two strains of *A. pelleranoi*, either laboratroy-reared on *C. capitata* or *A. fraterculus* larvae, were studied. Tests were designed on the basis of the following two predictions: (a) *A. pelleranoi* could be more likely than *D. longicaudata* to perceive volatiles directly emitted by the host larvae feeding inside the fruit, whereas *D. longicaudata* could be more oriented to exploit broadly disseminated cues of indirect evidence of host larvae; and (b) *A. pelleranoi* females, regardless of the strain, would be particularly more proficient in detecting odours directly produced by *A. fraterculus* larvae than those emanated from *C. capitata* larvae.

2 | MATERIALS AND METHODS

2.1 | Source and rearing of insects

Parasitoids and fruit flies were reared at the Laboratory of Ecoetological Research of Fruit Flies and their Natural Enemies (LIEMEN) of the Laboratory of Ecoetological Research of Fruit Flies and their Natural Enemies (PROIMI), San Miguel de Tucumán, Argentina. The *D. longicaudata* colony was originally established in 1999 with individuals imported from México (Ovruski, Colin, Soria, Oroño, & Schliserman, 2003). Initially, *D. longicaudata* was reared of *C. capitata* (Dl_{Cc} strain). In 2005, a second *D. longicaudata* colony was established of *A. fraterculus* (Dl_{Af} strain) (Van Nieuwenhove, Bezdjian, & Ovruski, 2012). In the case of *A. pelleranoi*, individuals were obtained in 2015 by harvesting peach and guava. The recovered adults of were used to make two colonies; one reared of *A. fraterculus* (=Ap_{Af} strain) and another of *C. capitata* (Ap_{Cc} strain). The *A. pelleranoi* cohorts used in the experiments were the 11th generation under artificial rearing, whereas the cohorts of the two *D. longicaudata* strains, Dl_{Af} and Dl_{Cc}, were the 120th and 180th generation, respectively.

The four parasitoid strains were held in cubical Plexiglas cages (30 × 30 × 30 cm) covered by organdy screen on two opposite sides, at a density of 300 parasitoid pairs per cage under 25 ± 1°C; 75 ± 5% RH and 12:12 (L:D) h photoperiod, until females reached appropriate age for the trials (7–11 days old). Each cage was provided with water and honey every other day. About 1,000 laboratory reared host larvae mixed with artificial diet were exposed to mated parasitoid females in sandwich-type oviposition devices similar to those described by Aluja, Ovruski et al. (2009). The same larval diet was used for rearing both *A. fraterculus* and *C. capitata*.

2.2 | Olfactometers

A glass Y-tube olfactometer was used in the first part of the study. The olfactometer (stem: 25 cm; arm length:10 cm; Y-arm angle: 45°; internal diameter: 1.5 cm) An air pump (ATMAN CX-1000®, 2.5 W, 50 hz, China) was used to produce an air flow, which was initially purified and humidified by passage through a 500-ml vacuum flask filled with a 350-ml distilled water and an activated charcoal solution. The speed of the airstream was set at 1.4 cm/s (=300 ml/min), measured at the exit of Y-tube, controlled by a needle valve and monitored by a flow metre. The clean airstream was then carried into two 200 ml glass vials via a 0.5 × 50-cm silicone tube (inner diameter × length), which in turn were finally connected to each arm of the tube Y through a 1.5 × 10-cm glass central tube (inner diameter × length). The end of both glass tubes was connected with a rubber tube. The distal part of the stem arm of the Y-tube had an opening where parasitoid females were introduced. Once a parasitoid entered into the tube, this opening was covered with a cloth stopper.

A four-arm olfactometer, similar to those described by Vet, Lenteren, Heymans, and Meelis (1983), with modifications, was used in the second part of the study. The apparatus consisted of a 200-ml glass air tight exposure chamber connected to four-arm, each one of 1 × 10-cm glass tube (inner diameter × length), conforming a star-shaped design. This central arena consisted of four glass semi-circles (arc 90°, 135 mm radius). Each arm was connected to a 400-ml glass vial through a 0.5 × 25 cm silicone tube (inner diameter × length). It also had two opposing 0.7 cm diameter round holes, one at the bottom and the other at the top. Each vial contained a different odour source. The air flow was generated by an air pump (PRECISION SR-7500®, 3.7 W, 50 hz, China) and passed through a filter of 350-ml distilled water and an activated charcoal before entering the airstream diffusion system. This device was a 3 × 5 cm sealed plastic container (inner diameter × height) with an entrance hole in the upper part and four equidistant outlet openings in each of the lateral sides in the central part. The container was attached to a plastic tripod 30 cm above the floor of the box in a central position equidistant from the four odour sources. This ensured an equitable distribution of airflow to the vials with odour source through 0.5 × 50-cm silicone tube (inner diameter × length). An airflow rate of approximately 300 ml/min was adjusted for each arm controlled by a flow metre.

Both olfatometers were put in the centre of a white box to avoid the effect of misleading visual stimuli. The pump and the flask with activated charcoal solution were placed outside the box and located on a separate table to avoid vibrations. Light came from 1000-lux daylight fluorescent tubes. All olfactometer assays were carried out at 25 ± 1°C and 75 ± 1% RH. The tests took place between 0900 a.m. and 0600 p.m.

2.3 | Response of parasitoids to extracts of the host larvae

Response of adult parasitoids of the four strains (Dl_{Cc}, Dl_{Af}, Ap_{Af} and Ap_{Cc}) to extracts of whole *C. capitata* and *A. fraterculus* larvae was tested. This combination of parasitoids from different strains was used to avoid a conditioned response by previous experience with the host on which the parasitoid was reared (Godfray, 1994).

One hundred *A. fraterculus* and *C. capitata* mid-third instar larvae were separately placed in 2 ml of hexane for 1 hr, to extract cuticle and exocrine gland substances of these hosts. This is because different parts of the host larval body, such as haemolymph, alimentary

canal, fat bodies, labial and mandibular glands, as well as larval frass, may be the source of kairomones that stimulate attractiveness and/or oviposition behaviours in larval parasitoid species (Arthur, 1981). Host larvae were macerated with the solvent using a porcelain pestle inside a laboratory porcelain mortar and allowed to stand for 15 min. Then, 1 ml of host larva extract, either *A. fraterculus* or *C. capitata*, was placed on 5-cm round filter paper piece (diameter), and tested against a 5-cm roundfilter paper piece soaked in 1 ml of the pure hexane (control). Each piece of filter paper was placed inside of each one glass vial of the olfactometer. These vials had a cover that allowed tight closure after placing the odour source. The host extract and the solvent were applied on the filter paper pieces 15 min before the first parasitoid female was released, in order to allow the odour to reach a constant release rate. Individual parasitoid females were released one at a time within the first cm of the central arm of the distal Y-tube with a plastic aspirator. For acclimatization, parasitoids remained 3 min in the tube before turning on the air pump. A female was considered as having made a choice when it reached the far end of one of the arms of the Y-tube and stayed for 15 s. However, whenever a parasitoid made no choice within 30 min, this was recorded as a no choice and discarded. Experiments consisted of 100 choices for each parasitoid strain. Each individual female was mated and naïve (7–11 days old) at the time of experiments, tested only once, and discarded afterwards. Filter paper pieces for control and odour treatments were replaced after every trial. To avoid biases, the positions of the stimulus and the control airstreams were changed randomly throughout the experiments. All parts of the olfactometer were cleaned with 95% alcohol, rinsed with distilled water and dry in an electrical stove at 60°C during 1 hr to prevent further contamination.

Three treatments were compared for each of the four parasitoid strains: (T1) = *C. capitata* larvae extract vs. solvent alone (hexane); (T2) = *A. fraterculus* larvae extract vs. solvent alone; and (T3) = *A. fraterculus* larvae extract vs. *C. capitata* larvae extract. The first two treatments were conducted to test whether host larva alone (without food/host fruit substrate) represented a stimulus attractive to female parasitoids. In the third treatment, the preference for one or the other host species was analysed and, in addition, whether the parasitoid strain influenced attraction to a particular host fly. A complementary multiple-choice test involving behavioural observations was made to determine the number of female parasitoid visits and ovipositor probes in oviposition devices soaked with *C. capitata* larvae extract, or *A. fraterculus* larvae extract, or solvent alone (hexane). The oviposition unit device consisted of a plastic dish (5 cm diameter, 0.3 cm high) containing either 2 ml of host larval extract or 2 ml of solvent alone (hexane) on a 5-cm roundfilter paper piece and covered with a piece of organdie cloth. Oviposition devices were positioned in the centre of a Plexiglas cage (42 × 40 × 40 cm) in circle (30 cm diameter), equidistant from each other, with positions randomized. Ten naïve, mated, 7- to 11-days-old females parasitoid, either *D. longicaudata* or *A. pelleranoi*, were released at the centre of the circle formed by the devices. Female parasitoids were observed once every 15 min throughout 3 hr and each observation lasted 30 s (Duan & Messing, 2000). A visit was recorded each time a female landed on the oviposition unit after release. An ovipositor probe was confirmed each time a female parasitoid inserted its ovipositor through the organdy screen of the oviposition device. Each treatment was replicated 22 times. For each replicate, new oviposition devices and parasitoid females were used. For data analysis, the percentages of female parasitoids probing according to the number of visits in each device were estimated.

2.4 | Response of parasitoids to volatiles produced by the substratum on which host larvae feed

In this experiment, the response of adults of each parasitoid strain to odours emitted either by fruit or larval diet with either *C. capitata* or *A. fraterculus* larvae, as well as clean fruit or clean larval diet (no host trace) and diet previously used by host tephritid larvae, was tested. Trials were performed using a four-arm olfactometer. The parasitoids were individually introduced into the centre of the chamber through a 3 × 11-cm glass tube (inner diameter × height). This tube could be moved up and down, allowing female parasitoids to remain confined in the centre of the camera for the setting time (1 min). Once the tube was slightly raised, the parasitoid was released to walk into the chamber, and the tube worked as an air extractor, which removed odours concentrated in the centre of the chamber.

The parasitoid movements between and within the four air fields were recorded from observations on orientation. The first and final parasitoid choices, as well as residence time (walking time + stopping time) in the corresponding evaluation field were assessed. The response time was set to a maximum of 30 min and started when the female parasitoid left the central part of the main chamber and crossed one of the four lines that delimited air fields (first choice). The final choice of the parasitoid (directional preference) was estimated when it approached to the base of one of the arms (narrow tube). Each female was mated and naïve (7–11 days old), tested only once and discarded to prevent associative learning. Experiments consisted of 100 choices, no choices being discarded. Six trials were carried out for each of the four parasitoid strains: (a) host larvae vs. clean peach fruit (no host trace); (b) host larvae vs. clean larval diet (no host trace); (c) infested diet vs. clean diet; (d) host larvae + diet vs. infested diet; (e) host larvae + diet vs. host larvae; and (f) host larvae + fruit (peach) vs. host larvae. Each trial consisted of four treatments (Table 1).

The first two trials were conducted to test whether larva, either *C. capitata* or *A. fraterculus*, would represent the main stimulus of attraction for parasitoids in the presence of fruit (peach) or artificial food substrate. Peach pulp was prepared from uninfested and unsprayed fruit collected from backyards. For this purpose, branches of peach trees, with 4–6 unripe fruits, were covered with an organdy mesh. Once the fruit ripened, they were harvested and taken to the laboratory, where the pulp was removed and used in tests. In both third and fourth trials, the question was whether the artificial rearing diet subsequent to larval development though without host was equally attractive to parasitoids as the diet plus host or clean diet (no host trace). Fifth and sixth trials were carried out to evaluate host's

TABLE 1 Description of the trials and treatments performed in the four-arm olfactometer

	Treatments/Odour sources[a,b]			
Trials	**Treatment #1**	**Treatment #2**	**Treatment #3**	**Treatment #4**
Host larvae vs. clean fruit	100 macerated *Af* larvae	Ripe peach pulp + skin (5 g)	100 macerated *Cc* larvae	Ripe peach pulp + skin (5 g)
Host larvae vs. clean diet	100 macerated *Af* larvae	Clean artificial larval diet (5 g)	100 macerated *Cc* larvae	Clean artificial larval diet (5 g)
Infested diet vs. clean diet	Artificial diet (5 g) previously used by *Af* (non-host)	Clean artificial larval diet (5 g)	Artificial diet (5 g) previously used by *Cc* (non-host)	Clean artificial larval diet (5 g)
Host larvae + diet vs. infested diet	100 macerated *Af* larvae + artificial larval diet (5 g)	Artificial diet (5 g) previously used by *Af*	100 macerated *Cc* larvae + artificial larval diet (5 g)	Artificial diet (5 g) previously used by *Cc*
Host larvae + diet vs. host larvae	100 macerated *Af* larvae	100 macerated *Af* larvae + artificial larval diet (5 g)	100 macerated *Cc* larvae	100 macerated *Cc* larvae + artificial larval diet (5 g)
Host larvae + fruit vs. host larvae	100 macerated *Af* larvae	100 macerated *Af* larvae + ripe peach pulp + skin (5 g)	100 macerated *Cc* larvae	100 macerated *Cc* larvae + ripe peach pulp + skin (5 g)

Notes. *Af*: *Anastrepha fraterculus*; *Cc*: *Ceratitis capitata*.
[a]Each treatment was a source of odour located in each arm of the olfactometer. [b]All maceration containing 0.2 ml of hexane.

attraction to parasitoids vs. the host food substrate, either larval rearing diet or fruit (pulp + skin), without host trace.

In each trial, the first two treatments were placed in opposite arms, and the same was carried out with the last two treatments. Each test material was put upon a piece of filter paper and located within the respective vial. The odour source material was used five times, and the chamber was rotated 90° after each use to remove directional bias. After every five tests, the glass vials and chamber were washed with distilled water and 95% alcohol to prevent contamination.

2.5 | Statistical analysis

Generalized linear models with a binomial distribution and logit link-function at $\alpha = 0.05$ were used (Crawley, 1993) to evaluate olfactory responses of both parasitoid species and strains. Fixed factors were as follows: (a) parasitoid species; (b) strain of the parasitoid; and (c) odour source or treatment. Interactions between fixed factors were also included. The frequency data recorded for both first and last olfactometer air field chosen were singly analysed for each parasitoid strain. The data of the time the parasitoid spent in a particular olfactometer area within the chamber (residence time) were expressed in percentages and also analysed for each parasitoid strain. Means were compared through the Sidak test at $\alpha = 0.05$.

The percentages of parasitoid females inserting ovipositors into oviposition devices were analysed through a general linear model at $\alpha = 0.05$. Parasitoid species, parasitoid strain and treatment were categorical variables. Percentage data were previously transformed using an arcsin square root function to meet parametric assumptions (Zar, 1999). However, the untransformed data are shown as means (±*SE*) in the figure. Mean comparisons were analysed with Tukey's honesty significant difference (HSD) test at $\alpha = 0.05$. All analyses were performed with the IBM SPSS Statistics for Windows software, version 22.0 (IBM Corp. Released 2013).

3 | RESULTS

3.1 | *Anastrepha fraterculus/Ceratitis capitata* larvae vs. control or host larvae

A preferential response of adults from both parasitoid species and strains to host tephritid larvae odours was found (Wald $\chi^2_{(1)} = 334.576$, $p < 0.0001$ for *C. capitata*; Wald $\chi^2_{(1)} = 274.026$, $p < 0.0001$ for *A. fraterculus*). This response was statistically similar for *D. longicaudata* and *A. pelleranoi*, regardless of the strain (Figure 1a–b). Interactions between categorical factors were not significant. Secondly, females of the four parasitoid strains did not discriminate between *C. capitata* and *A. fraterculus* larvae odours (Wald $\chi^2_{(1)} = 0.229$, $p = 0.632$). Attraction to a particular host was not influenced by the parasitoid strain (Wald $\chi^2_{(1)} = 0.014$, $p = 0.905$), and the interactions between categorical factors were not significant.

The percentages of females of both parasitoid species that initiated ovipositor-probing into devices containing host larvae extracts were markedly higher than those containing solvent alone ($F_{2,252} = 795.495$, $p < 0.0001$; Figure 2). Nevertheless, *A. pelleranoi* females inserted their ovipositor into such devices much more frequently than *D. longicaudata* females ($F_{1,252} = 85.159$, $p < 0.0001$; Figure 2). In addition, the interaction between the two factors, parasitoid species and treatment, was highly significant ($F_{2,252} = 19.725$, $p < 0.0001$). The parasitoid strain did not significantly influence the choice of females of parasitoid species for any host tephritid species ($F_{1,252} = 0.003$, $p = 0.958$; Figure 2).

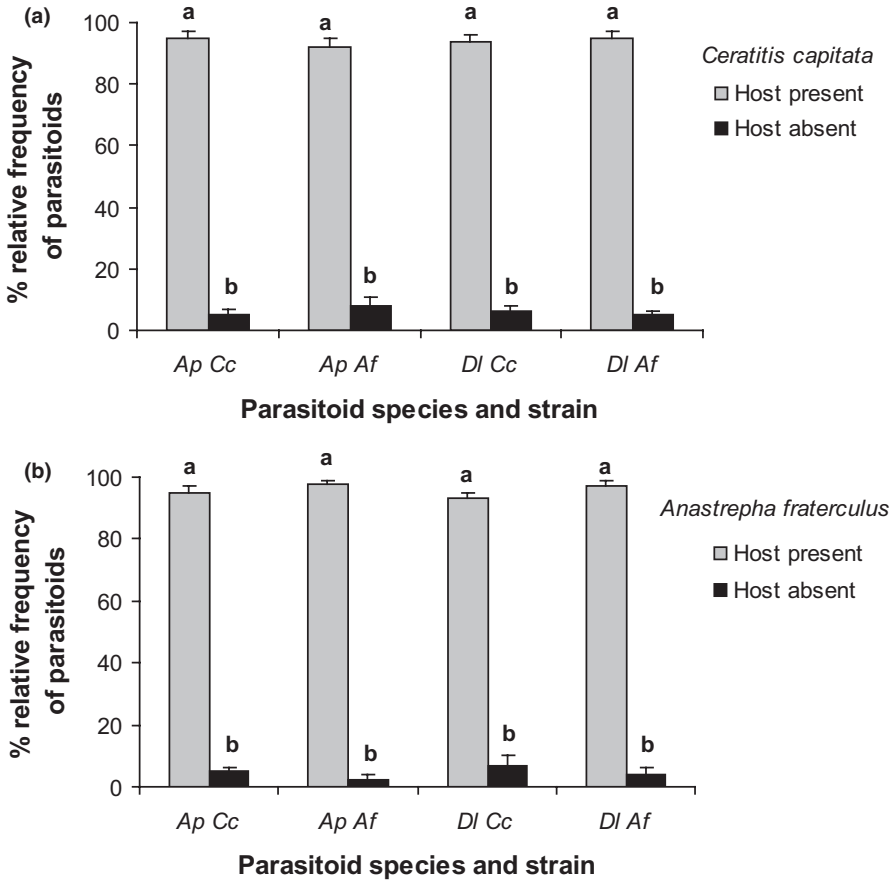

FIGURE 1 Olfactory responses of females of the four parasitoid strains to volatiles separately emanating from *Ceratitis capitata* larvae extracts (a) or *Anastrepha fraterculus* larvae extracts (b) vs. solvent (non-host, control). Bars followed by the same letter indicate no significant differences (Generalized Linear Models). Notations: Ap_{Af}: *A. pelleranoi* reared on *A. fraterculus* larvae; Ap_{Cc}: *Aganaspis pelleranoi* reared on *C. capitata* larvae; Dl_{Af}: *D. longicaudata* reared on *A. fraterculus* larvae; Dl_{Cc}: *Diachasmimorpha longicaudata* reared on *C. capitata* larvae

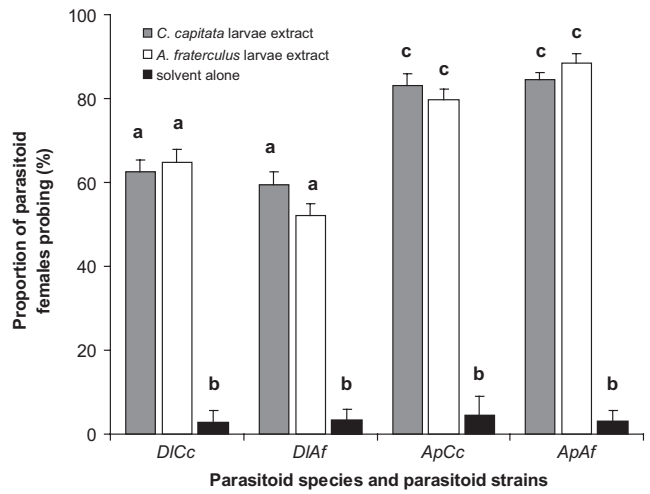

FIGURE 2 Ovipositor-probing responses of *Diachasmimorpha longicaudata* and *Aganaspis pelleranoi* to artificial oviposition devices containing *Anastrepha fraterculus* larvae extracts, *Ceratitis capitata* larvae extracts or solvent (control). Bars in each graph followed by the same letter indicate no significant differences in response levels (Tukey HSD test, $p = 0.05$). Notations: Ap_{Af}: *A. pelleranoi* reared on *A. fraterculus* larvae; Ap_{Cc}: *A. pelleranoi* reared on *C. capitata* larvae; Dl_{Af}: *D. longicaudata* reared on *A. fraterculus* larvae; Dl_{Cc}: *D. longicaudata* reared on *C. capitata* larvae

3.2 | Host larvae vs. clean fruit

Taking into account data recorded from first and final choice, the presence of females of the four parasitoid strains in the air fields permeated with fruit + host volatiles was 2.6 (±0.2) and 4.5 (±0.4) (mean ± *SE*) times greater, respectively, than that recorded in sectors soaked with host larvae volatiles alone (Table 2). Correspondingly, *D. longicaudata* and *A. pelleranoi* females spent more time in the two-quadrants permeated with fruit+host volatiles than in air fields soaked, with host volatiles alone (Table 2).

3.3 | Host larvae vs. clean diet

Similarly, females of the four parasitoid strains were also mainly attracted to volatile emanating from the artificial food substrate, either containing *C. capitata* or *A. fraterculus* larvae, compared with volatiles generated by host larvae alone. This was strikingly evidenced by data of first choice, final choice and residence time (Table 3). The air fields permeated with larval diet + host extracts were 3.2 (±0.5) and 5.6 (±0.3) (mean ± *SE*) more times visited by females of the four parasitoid strains in the first choice and the final choice, respectively, than those quadrants impregnated with host volatiles alone

TABLE 2 Summary of the olfactory response of females of four parasitoid strains to volatiles emitted by ripe peach pulp with either *Ceratitis capitata* larvae or *Anastrepha fraterculus* larvae ($N = 100$)

Treatments[b]	1st choice (N)[b]				Final choice (N)[b]				Residence time (%, mean ± SE)[b]			
	DI_{Cc}	DI_{Af}	Ap_{Cc}	Ap_{Af}	DI_{Cc}	DI_{Af}	Ap_{Cc}	Ap_{Af}	DI_{Cc}	DI_{Af}	Ap_{Cc}	Ap_{Af}
Cc with P	39 a	29 a	31 a	36 a	45 a	35 a	36 a	39 a	51.8 ± 4.6 a	25.2 ± 3.9 a	39.5 ± 3.4 a	45.9 ± 3.2 a
Cc alone	16 b	14 b	13 bc	11 b	9 b	8 b	10 b	7 b	9.6 ± 1.4 b	6.3 ± 1.2 b	6.6 ± 1.5 b	4.9 ± 1.3 b
Af with P	33 a	40 a	37 ac	41 a	37 a	46 a	42 a	45 a	31.3 ± 3.4 a	61.4 ± 5.1 c	45.8 ± 3.6 a	43.3 ± 5.2 a
Af alone	12 b	17 b	19 b	12 b	9 b	11 b	12 b	9 b	7.3 ± 1.6 b	7.1 ± 1.4 b	8.4 ± 1.4 b	5.9 ± 1.2 b
GLZs												
Wald χ^2(5)=	15.975	12.609	12.014	22.127	25.094	29.483	22.824	34.389	63.274	74.865	51.874	45.816
p=	0.001	0.006	0.007	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Notes. Values followed by the same letter in the column are not significantly different by the Sidak test at $p = 0.05$.

[a]Cc with P, *C. capitata* larvae and ripe peach pulp in solvent; Cc alone, *C. capitata* larvae in solvent; Af with P, *A. fraterculus* larvae and ripe peach pulp in solvent; Af alone, *A. fraterculus* larvae in solvent.

[b]Parasitoid strains: DI_{Cc}, *Diachasmimorpha longicaudata* reared on *C. capitata* larvae; DI_{Af}, *D. longicaudata* reared on *A. fraterculus* larvae; Ap_{Cc}, *Aganaspis pelleranoi* reared on *C. capitata* larvae; Ap_{Af}, *A. pelleranoi* reared on *A. fraterculus* larvae.

(Table 3). The average residence time for the four parasitoid strains in the air fields permeated with diet+host volatiles was 10.0 (±1.3) (mean ± *SE*) times higher than that recorded in the sectors soaked with host volatiles alone (Table 3).

3.4 | Infested diet vs. clean diet

Females of both parasitoid species were markedly attracted to volatiles emanating from larval diet previously used by the host compared with volatiles generated by clean diet (no host traces; Table 4). This was particularly evident in the final choice and residence time of *A. pelleranoi* (Table 4).

3.5 | Host larvae + diet vs. infested diet

There was no propensity towards any one particular olfactometer field by females of the four strains when both volatiles stemming from larval diet + host and from artificial diet previously used by the host were compared. The data for first choice, final choice and residence time in the four quadrants did not show significant differences between treatments (Table 5).

3.6 | Host larvae + diet or fruit vs. host larvae

Data from final choice and residence time showed that the level of *A. pelleranoi* females response to chemical cues generated by host larvae extract alone, either from *C. capitata* or *A. fraterculus*, was not significantly different from that caused by both clean artificial larval diet (Table 6) and clean fruit pulp + skin (no host traces; Table 7). In contrast, far more *D. longicaudata* females were attracted to quadrants soaked with volatiles from clean diet or clean fruit pulp+skin than those pervaded with volatiles generated by host larvae alone (Tables 6 and 7).

4 | DISCUSSION

Firstly, in agreement with the formulated prediction, revealed that females of both *A. pelleranoi* and *D. longicaudata* were attracted to odours emanating from larvae of both *A. fraterculus* and *C. capitata* in a Y-tube olfactometer, where the host larva was analysed without artificial larval diet or fruit. In addition, females of both parasitoid species showed no differences in their response patterns to olfactory cues emanating either from *C. capitata* or *A. fraterculus* larvae. These results would suggest that both the native *A. pelleranoi* and the exotic *D. longicaudata* may be able to detect volatile emissions from tephritid larvae during the host-location process, at least from a short distance. In turn, during egg-laying activity, chemical cues from larvae of both tephritid species would also have an influence on probing behaviour on both *A. pelleranoi* and *D. longicaudata* females. Previous studies by Duan and Messing (2000) revealed that *C. capitata* larvae outside the substrate on which they fed not only generated vibration cues but also chemical cues that stimulated

TABLE 3 Summary of the olfactory response of females of four parasitoid strains to volatiles emitted by artificial food diet substrate associated with either *Ceratitis capitata* larvae or *Anastrepha fraterculus* larvae (*N* = 100)

	1st choice (*N*)[b]				Final choice (*N*)[b]				Residence time (%, mean ± *SE*)[b]			
Treatments[b]	DI_{Cc}	DI_{Af}	Ap_{Cc}	Ap_{Af}	DI_{Cc}	DI_{Af}	Ap_{Cc}	Ap_{Af}	DI_{Cc}	DI_{Af}	Ap_{Cc}	Ap_{Af}
Cc with D	31 ac	30 a	43 a	38 a	40 a	36 a	47 a	40 a	46.6 ± 3.3 a	42.2 ± 4.8 a	49.2 ± 3.9 a	41.6 ± 3.5 a
Cc alone	19 bc	12 b	12 b	12 b	8 b	6 b	8 b	7 b	4.9 ± 1.3 b	3.1 ± 1.9 b	7.3 ± 1.6 b	4.7 ± 1.9 b
Af with D	35 a	42 a	35 a	43 a	42 a	49 a	39 a	45 a	41.9 ± 4.3 a	49.6 ± 4.8 c	40.2 ± 3.1 a	50.4 ± 3.6 c
Af alone	15 b	16 b	10 b	7 b	10 b	9 b	6 b	8 b	6.6 ± 1.4 b	5.2 ± 1.2 b	4.5 ± 1.3 b	3.2 ± 1.3 b
GLZs												
Wald $\chi^2_{(5)}$=	8.263	17.428	23.959	31.403	30.774	36.876	38.163	38.201	238.601	29.588	191.027	301.287
p=	0.041	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Notes. Values followed by the same letter in the column are not significantly different by the Sidak test at $p = 0.05$.
[a]*Cc* with D, *C. capitata* larvae and artificial larval diet in solvent; *Cc* alone, *C. capitata* larvae in solvent; *Af* with D, *A. fraterculus* larvae and artificial larval diet in solvent; *Af* alone, *A. fraterculus* larvae in solvent.
[b]Parasitoid strains: DI_{Cc}, *Diachasmimorpha longicaudata* reared on *C. capitata* larvae; DI_{Af}, *D. longicaudata* reared on *A. fraterculus* larvae; Ap_{Cc}, *Aganaspis pelleranoi* reared on *C. capitata* larvae; Ap_{Af}, *A. pelleranoi* reared on *A. fraterculus* larvae.

TABLE 4 Summary of the olfactory response of females of four parasitoid strains to volatiles emitted by artificial food diet previously used by *Ceratitis capitata* larvae or *Anastrepha fraterculus* larvae vs. clean larval diet (no host traces) (*N* = 100)

	1st choice (*N*)[b]				Final choice (*N*)[b,]				Residence time (%, mean ± *SE*)[b]			
Treatments[a]	DI_{Cc}	DI_{Af}	Ap_{Cc}	Ap_{Af}	DI_{Cc}	DI_{Af}	Ap_{Cc}	Ap_{Af}	DI_{Cc}	DI_{Af}	Ap_{Cc}	Ap_{Af}
D_{Cc}	33 a	41 a	27 ac	28 ac	37 a	45 a	32 a	35 a	30.1 ± 2.4 a	44.5 ± 3.3 a	22.9 ± 2.2 a	19.6 ± 2.3 a
CDa	12 b	13 b	21 bc	19 b	11 b	09 b	17 b	17 b	3.1 ± 1.0 b	4.9 ± 0.9 b	9.2 ± 1.4 c	8.1 ± 1.5 b
D_{Af}	38 a	31 a	41 a	36 a	39 a	35 a	36 a	30 a	39.5 ± 2.9 a	31.8 ± 2.6 a	40.9 ± 3.5 b	31.2 ± 3.2 a
CDb	17 b	15 b	11 b	21 bc	13 b	11 b	15 b	18 b	4.2 ± 0.8 a	5.7 ± 1.2 b	5.9 ± 1.0 c	10.3 ± 1.7 b
GLZs												
Wald $\chi^2_{(5)}$=	27.500	26.640	28.529	13.818	40.178	60.476	18.605	12.867	281.689	133.863	151.131	37.851
p=	0.001	0.001	0.001	0.003	0.001	0.001	0.001	0.005	0.001	0.001	0.001	0.001

Notes. Values followed by the same letter in the column are not significantly different by the Sidak test at $p = 0.05$.
[a]D_{Cc}, diet previously used by *C. capitata* larvae; D_{Af}, diet previously used by *A. fraterculus* larvae; CDa–b, clean larval diet. [b]Parasitoid strains: DI_{Cc}, *Diachasmimorpha longicaudata* reared on *C. capitata* larvae; DI_{Af}, *D. longicaudata* reared on *A. fraterculus* larvae; Ap_{Cc}, *Aganaspis pelleranoi* reared on *C. capitata* larvae; Ap_{Af}, *A. pelleranoi* reared on *A. fraterculus* larvae.

TABLE 5 Summary of the olfactory response of females of four parasitoid strains to volatiles emitted by artificial food diet substrate on which the *Ceratitis capitata* larvae or *Anastrepha fraterculus* larvae fed (*N* = 100)

	1st choice (*N*)[b]				Final choice (*N*)[b]				Residence time (%, mean ± *SE*)[b]			
Treatments[a]	Dl_{Cc}	Dl_{Af}	Ap_{Cc}	Ap_{Af}	Dl_{Cc}	Dl_{Af}	Ap_{Cc}	Ap_{Af}	Dl_{Cc}	Dl_{Af}	Ap_{Cc}	Ap_{Af}
D with *Cc*	32 a	28 a	26 a	23 a	33 a	28 a	30 a	24 a	33.0 ± 5.1 a	24.0 ± 3.7 a	24.9 ± 4.2 a	28.7 ± 3.7 a
D_{Cc} alone	19 a	24 a	25 a	20 a	20 a	21 a	22 a	19 a	21.9 ± 3.1 a	25.6 ± 3.1 a	29.1 ± 3.7 a	19.1 ± 3.6 a
D with *Af*	27 a	31 a	31 a	35 a	26 a	33 a	28 a	36 a	24.3 ± 4.1 a	29.4 ± 5.2 a	25.4 ± 4.0 a	35.5 ± 6.2 a
D_{Af} alone	22 a	17 a	18 a	22 a	21 a	18 a	20 a	21 a	20.8 ± 2.9 a	21.0 ± 2.9 b	20.6 ± 3.0 a	22.4 ± 3.8 a
GLZs												
Wald $\chi^2_{(3)}$=	3.030	4.083	5.321	5.081	2.986	4.290	2.846	5.864	4.893	2.285	2.041	6.710
p=	0.387	0.253	0.150	0.166	0.394	0.232	0.416	0.118	0.180	0.515	0.564	0.082

Notes. Values followed by the same letter in the column are not significantly different by the Sidak test at $p = 0.05$.
[a]D with *Cc*, artificial larval diet plus *C. capitata* larvae in solvent; D_{Cc} alone, diet used by *C. capitata* larvae in solvent (without host larvae); D with *Af*, diet plus *A. fraterculus* larvae in solvent; D_{Af} alone, diet used by *A. fraterculus* larvae in solvent (without host larvae). [b]Parasitoid strains: Dl_{Cc}, *Diachasmimorpha longicaudata* reared on *C. capitata* larvae; Dl_{Af}, *D. longicaudata* reared on *A. fraterculus* larvae; Ap_{Cc}, *Aganaspis pelleranoi* reared on *C. capitata* larvae; Ap_{Af}, *A. pelleranoi* reared on *A. fraterculus* larvae.

TABLE 6 Summary of the olfactory response of females of four parasitoid strains to volatiles emitted by clean larval diet (no host traces) vs. volatiles emanated from both *Anastrepha fraterculus* and *Ceratitis capitata* larvae extract (*N* = 100)

	1st choice (*N*)[b]				Final choice (*N*)[b]				Residence time (%, mean ± *SE*)[b]			
Treatments[a]	Dl_{Cc}	Dl_{Af}	Ap_{Cc}	Ap_{Af}	Dl_{Cc}	Dl_{Af}	Ap_{Cc}	Ap_{Af}	Dl_{Cc}	Dl_{Af}	Ap_{Cc}	Ap_{Af}
Cc alone	12 a	11 a	24 ab	12 a	13 a	10 a	21 a	18 a	5.2 ± 1.2 a	3.9 ± 0.8 a	15.1 ± 2.1 ab	16.7 ± 2.0 ab
CDa	45 b	36 b	37 a	30 bc	43 b	40 b	32 a	33 a	39.9 ± 2.8 b	31.2 ± 2.2 b	22.9 ± 2.9 c	24.6 ± 3.4 b
Af alone	10 a	13 a	13 b	19 ab	06 a	08 a	19 a	25 a	4.0 ± 1.1 a	7.4 ± 1.2 a	12.2 ± 1.9 b	14.3 ± 2.1 a
CDb	33 b	40 b	27 ab	39 c	38 b	42 b	28 a	24 a	30.9 ± 2.5 b	42.5 ± 2.8 c	17.8 ± 2.1 ab	16.5 ± 2.4 ab
GLZs												
Wald $\chi^2_{(3)}$=	49.817	50.219	17.027	25.241	68.812	64.198	5.885	5.569	234.899	291.552	151.353	10.187
p=	0.001	0.001	0.001	0.001	0.001	0.001	0.117	0.135	0.001	0.001	0.006	0.017

Notes. Values followed by the same letter in the column are not significantly different by the Sidak test at $p = 0.05$.
[a]*Cc* alone, *C. capitata* larvae in solvent; *Af* alone, *A. fraterculus* larvae in solvent; CDa–b, clean larval diet. [b]Parasitoid strains: Dl_{Cc}, *Diachasmimorpha longicaudata* reared on *C. capitata* larvae; Dl_{Af}, *D. longicaudata* reared on *A. fraterculus* larvae; Ap_{Cc}, *Aganaspis pelleranoi* reared on *C. capitata* larvae; Ap_{Af}, *A. pelleranoi* reared on *A. fraterculus* larvae.

TABLE 7 Summary of the olfactory response of females of four parasitoid strains to volatiles emitted by clean fruit (no host traces) vs. volatiles emanated from both *Anastrepha fraterculus* and *Ceratitis capitata* larvae extract (*N* = 100)

Treatments[a]	1st choice (N)[b]				Final choice (N)[b]				Residence time (%, mean ± SE)[b]			
	Dl_{Cc}	Dl_{Af}	Ap_{Cc}	Ap_{Af}	Dl_{Cc}	Dl_{Af}	Ap_{Cc}	Ap_{Af}	Dl_{Cc}	Dl_{Af}	Ap_{Cc}	Ap_{Af}
Cc alone	14 a	17 a	19 a	20 a	09 a	11 a	23 a	21 a	3.4 ± 0.7 a	5.7 ± 1.1 a	10.7 ± 1.5 b	12.6 ± 1.8 a
CDa	34 b	35 b	29 a	34 a	35 b	38 b	30 a	26 a	30.6 ± 2.4 b	28.2 ± 2.1 b	19.5 ± 2.3 a	18.9 ± 2.0 ab
Af alone	16 a	15 a	21 a	16 a	12 a	10 a	22 a	24 a	5.1 ± 1.0 a	6.4 ± 1.3 a	12.1 ± 1.7 ab	13.8 ± 2.0 a
CDb	36 b	33 b	31 a	30 a	44 b	41 b	25 a	29 a	41.1 ± 3.0 c	33.8 ± 2.5 b	16.8 ± 1.9 ab	23.7 ± 2.5 b
GLZs												
Wald χ^2(5)=	21.036	18.401	5.653	11.876	53.377	50.834	1.429	1.837	272.576	190.264	14.404	18.544
p=	0.001	0.001	0.130	0.008	0.001	0.001	0.587	0.607	0.001	0.001	0.002	0.001

Notes. Values followed by the same letter in the column are not significantly different by the Sidak test at $p = 0.05$.

[a]*Cc* alone, *C. capitata* larvae in solvent; *Af* alone, *A. fraterculus* larvae in solvent; CDa–b, clean fruit. [b]Parasitoid strains: Dl_{Cc}, *Diachasmimorpha longicaudata* reared on *C. capitata* larvae; Dl_{Af}, *D. longicaudata* reared on *A. fraterculus* larvae; Ap_{Cc}, *Aganaspis pelleranoi* reared on *C. capitata* larvae; Ap_{Af}, *A. pelleranoi* reared on *A. fraterculus* larvae.

oviposition in the opine parasitoids *Diachasmimorpha tryoni* Cameron and *D. longicaudata*. Nevertheless, a recent study by Stuhl et al. (2011) proved that fruigivorous tephritid larvae, such as *Anastrepha suspensa* (Loew), *Bactrocera dorsalis* Hendel, *Zeugodacus cucurbitae* Coquillett and *C. capitata*, release para-ethylacetophenone (PEA), a volatile that stimulates attraction of female *D. longicaudata*, and essentially incites both probing and oviposition behaviours.

Secondly, results derived from a four-arm olfactometer. The combination of chemical stimuli related to the host larvae mixed with fruit pulp or with artificial larval diet compared to those individually produced by both clean host food substrate (no host) and host larvae alone strongly influenced host finding behaviour in both fruit fly parasitoid species. Therefore, the short-range orientation of *A. pelleranoi* and *D. longicaudata* females to the host appears to be regulated by a blend of odours emanating from host larvae and host food substrate. This finding supports results published by Guimarães and Zucchi (2004) and Aluja, Díaz-Fleischer et al. (2009) on *A. pelleranoi*, and by Messing and Jang (1992), Eben et al. (2000), Silva et al. (2007), and Segura et al. (2012) on *D. longicaudata*. Furthermore, Stuhl et al. (2011) highlighted the diversity of chemical stimuli involved in *D. longicaudata* attraction to find the host and to oviposit in it. These authors recorded a significantly higher parasitoid emergence rate in a treatment involving a fruit (*Pyrus communis* L.) plus PEA (derived from host larvae) plus ethanol, this latter compound known to attract *D. longicaudata* females to decaying fruit (Greany, Tumlinson, Chambers, & Boush, 1977). Similarly, both *D. longicaudata* and *A. pelleranoi* females showed a remarkable capacity for recognizing and selecting volatiles emanating from used artificial diet following host larval development. These volatiles were broadly preferred over olfactory cues emitted from clean artificial diet (no host trace), but were equally attractive to parasitoid females when compared to a blend of volatiles stemming from artificial larval diet plus host larvae. Segura et al. (2012) also found that *D. longicaudata* females were highly attracted to larval diet previously used when compared to fresh larval diet. A possible reason explaining this result would be the one given by Stuhl et al. (2011) found that *D. longicaudata* females inserted their ovipositors into oviposition devices in the complete absence of larvae, but particularly if PEA was present.

Thirdly, based on residence time and final choice data foraging *A. pelleranoi* females were equally attracted to odours emitted from both host larvae and fresh host food substrates, either fruit pulp or artificial diet devoid of host, when both options were compared in the four-arm olfactometer. In contrast, odours generated from clean host food substrates (non-host) were the main attraction source for *D. longicaudata* females under choice conditions. This finding suggests that olfactory cues associated with host larvae probably play a relevant role in host searching behaviour of *A. pelleranoi*, whereas for *D. longicaudata*, the host-habitat olfactory stimuli would be highly essential in short-range host location. The latter is fully in line with results previously published by Eben et al. (2000), who pointed out that *D. longicaudata* is attracted to unidentified volatiles emitted by uninfected fruit, and by Silva et al. (2007) and Segura et al. (2012, 2016) who found a positive response of *D. longicaudata* females

to ripe and rotten fruit volatiles, even in the absence of larvae. In addition, parasitoids ovipositional activity records showed that cues from any host larvae elicited a noticeably higher percentage of probing responses in *A. pelleranoi* than in *D. longicaudata* females (83.6 ± 1.5 vs. 61.5 ± 1.4). Presumably, the distinctive host-foraging strategies between *A. pelleranoi* and *D. longicaudata* may have selected for response to different sets of host-related cues, which generate dissimilar responses to volatiles. A similar assumption was previously raised by Dias et al. (2014) who found that *D. longicaudata* actively responded to PEA, but this chemical cue did not influence oviposition in the Neotropical-native *Utetes anastrephae* (Viereck), another braconid larval parasitoid of tephritids. Although both braconids always seek host larvae on the fruit surface, different host-foraging techniques produced distinct perceptions of chemical host cues (Dias et al., 2014).

Fourthly, contrary to the prediction that chemical cues produced by *A. fraterculus* larvae would be more attractive to *A. pelleranoi*, females of this figitid showed no preference for any tephritid species in particular under a host-choice situation. It is likely that the generalist condition of *A. pelleranoi*, besides having a history of sympatry with *Anastrepha* Schiner, allows it to react to chemical cues shared between various host tephritid species. This is a distinctive feature of generalist parasitoids (Godfray, 1994). Against this background, a foraging generalist parasitoid must be versatile in its response to host-related odours because it may be confronted with the problem of high variability of chemical cues (Hilker & McNeil, 2008). Hence, learning to respond to host and plant odours might be a strategy of generalist parasitoids that allows them to locate a particular host under high environmental variability (Steidle & Van Loon, 2003). This is likely to be the case of *A. pelleranoi*, as it has already been shown for *D. longicaudata*; this generalist braconid parasitoid is capable of associative learning during host finding (Segura, Viscarret, Paladino, Ovruski, & Cladera, 2007), a fact which allows it to modulate its innate preference for host habitats depending on prior experience and the abundance of hosts (Segura et al., 2016). Therefore, future complementary studies focused on this issue should be conducted using *A. pelleranoi* in order to contrast with *D. longicaudata* and/or other generalist braconid parasitoids native to the Neotropics. Larvae of both host tephritid species were equally attractive to both parasitoid females, regardless of the parasitoid strain. Such data would support results provided by Silva et al. (2007). These authors pointed out that *D. longicaudata* females had no preference either for *C. capitata* or *A. fraterculus* larvae although hosts were inside rotting guavas when evaluated, and thus host choice may have been masked by decaying fruit odours. It is well known that habitat cues associated with host larval activity plus those produced by rotten fruit, particularly fruit colonized by fungi, are highly attractive to *D. longicaudata* females (Segura et al., 2012). In the present study, parasitoid attraction to host-related odours was evaluated deprived of any host food substrate.

To sum up, findings from this study add knowledge on chemically mediated foraging behaviours of *D. longicaudata* and *A. pelleranoi*, and highlight the potential use of both parasitoids species for augmentative release programs in Argentinean fruit-growing regions where *A. fraterculus* and *C. capitata* coexist. In Argentina, *D. longicaudata* is mass reared on larvae of *C. capitata* and mass released against this pest (Sánchez et al., 2016). However, in several Argentinean regions, the presence of *A. fraterculus* is also a main concern for growers. Regarding *A. pelleranoi*, this parasitoid could be successfully used for starting a mass rearing system in Argentina using *A. fraterculus* as a host (Núñez-Campero et al., 2014). Both parasitoid would allow mass-releases tailored to the climatic and ecological conditions of a particular fruit-growing regions in Argentina.

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AUTHOR CONTRIBUTION

All authors conceived and designed the experiments. Author 1 and author 6 conducted experiments. Author 1, author 2, author 3, author 5 and author 6 wrote the manuscript. Author 1, author 4 and author 6 analysed data and conducted statistical analyses. Author 1, author 2 and author 3 contributed material. Author 5 and author 6 secured funding. All authors read and approved the manuscript.

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