

## BIOLOGICAL CONTROL

### Parasitism Behavior of Three Species of Eucoilinae (Hymenoptera: Cynipoidea: Figitidae) Fruit Fly Parasitoids (Diptera) in Brazil

JORGE A. GUIMARÃES<sup>1</sup> AND ROBERTO A. ZUCCHI<sup>2</sup>

<sup>1</sup>EMBRAPA/CNPAT, Rua Sara Mesquita, 2270, C. postal 3761, 60511-110, Fortaleza, CE  
e-mail: jorge@cnpat.embrapa.br

<sup>2</sup>Depto. Entomologia, Fitopatologia e Zoologia Agrícola, ESALQ/USP, Av. Pádua Dias, 11, C. postal 9, 13418-900 Piracicaba, SP, e-mail: razucchi@esalq.usp.br

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Comportamento de Parasitismo de Três Espécies de Eucoilinae (Hymenoptera: Cynipoidea: Figitidae) Parasitóides de Moscas-das-Frutas (Diptera)

RESUMO - Os eucoilíneos são endoparasitóides larvais de dípteros ciclorrhafos. Várias espécies têm sido associadas a larvas de moscas frugívoras (Tephritidae, Lonchaeidae e Drosophilidae). Algumas espécies desses dípteros causam danos aos frutos cultivados. Para minimizar as perdas, tem-se utilizado o Manejo Integrado de Pragas (MIP), onde o controle biológico com himenópteros parasitóides tem papel fundamental. O conhecimento a respeito do comportamento de parasitismo pode auxiliar na maximização do uso de parasitóides em programas de controle biológico aplicado. Dessa forma, este trabalho teve como objetivos: (1) estudar a resposta olfativa de *Aganaspis pelleranoi* (Brèthes) e *Dicerataspis grenadensis* Ashmead aos voláteis emitidos de goiaba infestada com larvas de moscas-das-frutas em olfatômetro de quatro vias, com fluxo de ar e (2) verificar a seqüência comportamental de localização e avaliação do hospedeiro por *A. pelleranoi*, *D. grenadensis* e *Odontosema albinerve* Kieffer, em goiabas infestadas com larvas frugívoras em laboratório e em campo. Nos experimentos com olfatômetro de quatro vias, *A. pelleranoi* e *D. grenadensis* foram mais atraídos pelos voláteis dos frutos infestados por larvas frugívoras. Ao estudar a seqüência comportamental dos eucoilíneos, em polpa de goiaba madura infestada com larvas de moscas, verificou-se que *A. pelleranoi* e *O. albinerve*, ao chegarem nos frutos por meio dos voláteis, localizam as larvas hospedeiras nos frutos por meio de vibrotaxia e que *D. grenadensis* localiza as larvas hospedeiras nos frutos com o ovipositor. Foi estabelecida a especificidade de *D. grenadensis* às larvas da família Drosophilidae em goiaba podre.

PALAVRAS-CHAVE: *Odontosema albinerve*, *Dicerataspis grenadensis*, *Aganaspis pelleranoi*, olfatômetro, semioquímico

ABSTRACT - Eucoilinae are larval endoparasitoids of cyclorrhaphous dipterous. Several species have been associated with frugivorous larvae (Tephritidae, Lonchaeidae and Drosophilidae). Some species of these dipterous cause serious damage to fruit crops. In order to minimize their damage, Integrated Pest Management (IPM) using biological control with hymenopteran parasitoids has been used. Studies on parasitism behavior can maximize the use of parasitoids in biological control programs. Thus, this paper had as objectives (1) to study the olfactory response of *Aganaspis pelleranoi* (Brèthes) and *Dicerataspis grenadensis* Ashmead to the volatile emitted by guava infested with fruit fly larvae and (2) to verify the behavioral sequence of location and evaluation of hosts by *A. pelleranoi*, *D. grenadensis* and *Odontosema albinerve* Kieffer in laboratory and in field conditions. Experiments in a four-armed airflow olfactometer showed that *A. pelleranoi* and *D. grenadensis* were more attracted to volatiles of fruits infested with frugivorous larvae. The behavioral sequence of Eucoilinae, in ripe guava pulp infested by frugivorous larvae, showed that *A. pelleranoi* and *O. albinerve* located the host larvae by vibrotaxis and *D. grenadensis* found the host larvae with the ovipositor. These results established that *D. grenadensis* showed specificity to larvae of the family Drosophilidae in rotting guava.

KEY WORDS: *Odontosema albinerve*, *Dicerataspis grenadensis*, *Aganaspis pelleranoi*, olfactometer, semiochemical

The Eucilinae are the largest group of Cynipoidea, consisting of approximately 1,000 species in 82 genera distributed throughout the world. It is the most diversified subfamily of Figitidae in Brazil, with 55 species in 29 genera (Ronquist 1995, Diaz 1998). They are koinobiont endoparasitoids of cyclorrhaphous dipterous that parasitize preferentially the last larva instars, emerging from the fly pupa (Diaz 1998). Some Eucilinae species only parasitize the larvae of flies of the Tephritoidea superfamily, making them potential biological control agents for use in fruit fly integrated management programs (Ovruski *et al.* 2000). Recently seven Eucilinae species were detected associated with fruit-eating flies (*Anastrepha* spp., *Ceratitis capitata* Wied and *Lonchaeidae*) in 34 species of fruit trees belonging to 15 botanical families. The Myrtaceae fruit are the most attractive for these parasitoids, indicating a strong tritrophic relationship among these organisms (Guimarães *et al.* 1999, 2000, 2003).

The Eucilinae act at the end of the fruit succession process in the field. They parasitize the larvae that escape the opiines (Braconidae) that, because of the size of the ovipositor, only parasitize the larvae that are found on the fruit surface (Sivinski *et al.* 1999, 2001). The parasitism of the fly larvae in the fruits probably leads to inter and intraspecific competition that imply different search and host assessment strategies (Vet & Alphen 1985).

The process by which the microhymenopterous parasitoids find the hosts can be divided into three stages: (1) habitat localization, that generally involves long distance displacements, guided by the volatiles released from the plants; (2) localization of the host, that consists in the search for signals emitted directly by the host (feces, exuvia), vibrotaxis (search based on the movements produced by the larvae inside a determined substrate) and examination with the ovipositor (fruit fly parasitoids) to locate the larvae in the fruit and (3) host suitability (size, age, parasitism) (Weseloh 1981, Vet 1985, Vinson 1985, Tumlinson *et al.* 1993, Lewis & Sheehan 1997).

Little is known about the behavior and biology of the Eucilinae in the neotropical region and most of the studies have been about taxonomy and populational surveys (Diaz 1998, Guimarães 1999, Guimarães *et al.* 2003). Most of the behavior studies are restricted to the *Leptopilina* species, drosophilid parasitoids in Europe (Vet *et al.* 1983, Vet 1985, Vet & Alphen 1985, Vet & Bakker 1985, Vet & Opzeeland 1985). Recently, parasitism behavior has become more important because it was observed that natural enemies have great capacity for associative learning that may be used to maximize the use of these parasitoids in biological control programs (Tumlinson *et al.* 1993, Powell & Poppy 2001). Thus aiming to contribute with knowledge of the parasitism behavior of neotropical Eucilinae parasitoids of frugivorous larvae, the objectives of this study were: (1) study the olfactory response of *Aganaspis pelleranoi* (Brèthes) and *Diceraspis grenadensis* Ashmead to the semiochemicals released by guava infested with tephritid and drosophilid larvae in a four-armed airflow olfactometer, and (2) establish the behavioral sequence of host location and assessment by *A. pelleranoi*, *D. grenadensis* and *O. albinerve* in guava pulp infested with larvae of these flies in the laboratory.

## Material and Methods

**Olfactory Response to Volatiles in an Olfactometer.** The Eucilinae olfactory response to the volatiles released from the association of frugivorous larvae and host fruits was observed in a four-armed airflow olfactometer (Vet *et al.* 1983). The apparatus consisted of a star-shaped central arena with four exits (Fig. 1A-B). The central arena consisted of four acrylic semi-circles (arc 90°, 135 mm radius) placed on a 3-mm thick acrylic sheet. There was a 5-mm diameter hole at each end of the arena to attach the hose of the side tubes containing the test sample. In the center of the arena there was a 5 mm hole to attach the vacuum hose. The arena roof is a 3-mm thick acrylic sheet. Complete sealing of the system was ensured with the use of vaseline to join the base (central arena) to the roof. Each arm of the chamber was connected to a series of three glass tubes (50 ml). The tube closest the chamber was to capture the insect that responded positively to the tested odor, the sample to be studied was placed in the second tube and the distilled water was placed in the third tube to moisten the air and filter the impurities that penetrate the system (Fig. 1B). An air flow of 300 ml/min was used in each arm of the olfactometer, obtained by a vacuum pump attached to the lower hole located in the central part of the arena and regulated by four flowmeters installed in each arm of the apparatus (Vet *et al.* 1983).

Natural parasitoid populations were used obtained from fruit-eating fly pupae collected directly from guavas in the counties of Monte Alegre do Sul and Piracicaba, SP. The fruits were harvested randomly from the trees and/or the ground, placed on plastic trays containing about 2 cm vermicelli covered with organza and kept in a greenhouse for approximately 15 days. The vermicelli was then sieved to remove the pupae. These were counted and transferred to glass containers with a layer of wet vermicelli and covered with organza. The parasitoids (male and female) were identified at emergence and transferred to maintenance cages where they were supplied daily with water and honey at 30% as food supply. The cage was kept in the laboratory (T = 25 ± 3°C, RH = 75 ± 5% and light period = 14h) until the experiments were carried out. The female parasitoids had no direct contact with either the host larva or the fruit until the experiment was carried out. Each female was tested once in the olfactometer to prevent associative learning and consequently, tendacious results (Vet *et al.* 1983).

The test samples were formed by *Anastrepha* spp. or *C. capitata* (Tephritidae) larvae and by *Zaprionus indianus* Gupta (Drosophilidae) in guava fruit (ripe or rotting). The *Anastrepha* spp. larvae were obtained from ripe guavas collected in the field and the *C. capitata* larvae were reared on an artificial diet at CENA/USP. The *Z. indianus* larvae were collected from rotting guavas in the field. In the laboratory, the fly larvae were removed from the guavas by pincers and used in the experiments.

After setting up the olfactometer with the test samples and establishing the air flow (300 ml/min), the female parasitoid was placed in the central arena through the hole located in the lower part of the apparatus. For this, the hose was quickly disconnected and the female entered the arena. As soon as the insect moved about 5 mm away from the central hole, the

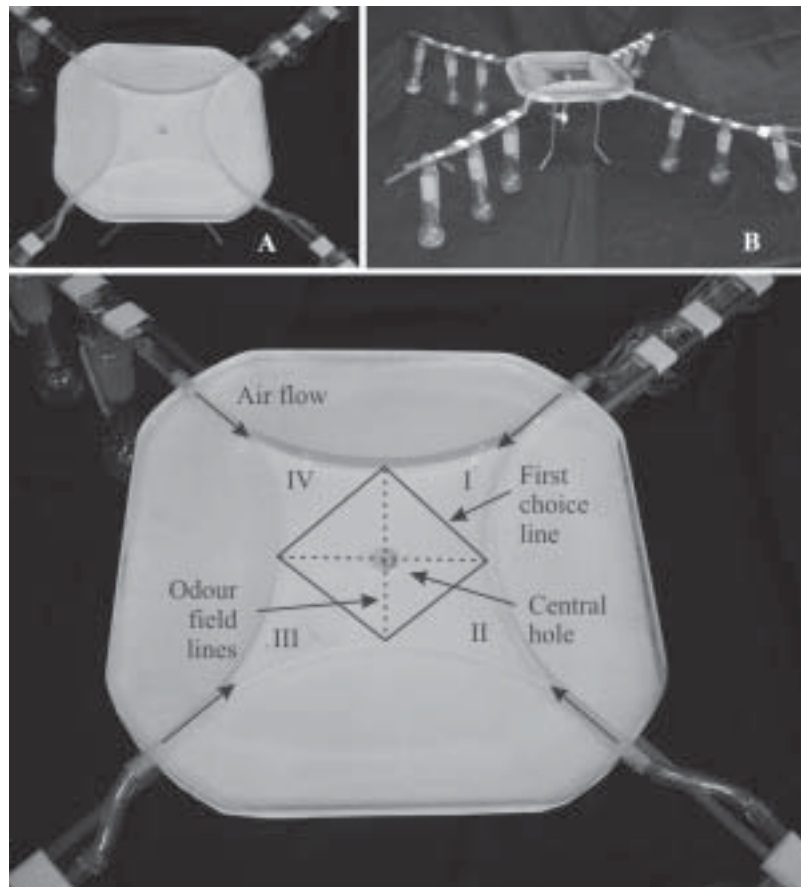


Figure 1. Four-armed airflow olfactometer (Vet *et al.* 1983).

hose was reconnected and the air flow restored. When the parasitoid crossed the arbitrary line of the 'first choice', the corresponding field was recorded and scoring began of the length of time the female stayed in each odor field (I, II, III or IV). Each female stayed in the arena for 10 min. If it entered one of the side arms, leaving the central arena before the end of the set period, the final choice was made and the remaining observation time was added to the chosen sector. After each treatment, the olfactometer and the side tubes were washed with neutral detergent and disinfected with 90% alcohol (Vet *et al.* 1983, Alphen *et al.* 1991).

Five treatments were carried out for the study of the behavioral response to the volatiles in the olfactometer:

1. Olfactory response of 44 *A. pelleranoi* females ( $3 \pm 1$  day old) to ripe guava pulp infested with frugivorous larvae (*Anastrepha* spp at 3<sup>rd</sup> instar) and to non-infested ripe guava.
2. Olfactory response of 60 *A. pelleranoi* females ( $3 \pm 1$  day old) to ripe guava pulp infested with native tephritids (*Anastrepha* spp.) and exotic larvae (*C. capitata*).
3. Olfactory response of 42 *A. pelleranoi* larvae ( $8 \pm 1$  day old) to ripe guava pulp infested with *C. capitata* larvae from different instars (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> instars).
4. Olfactory response of 40 *D. grenadensis* females ( $4 \pm 1$  day old) to volatiles from ripe guava infested with tephritids (*Anastrepha* spp.) and rotting guava infested with drosophilid larvae (*Z. indianus*). Different substrates were used because in nature the drosophilid larvae usually develop on rotting

fruit while the tephritid larvae are more commonly found in ripe fruit, or fruits close to this stage.

5. Olfactory response of 50 *D. grenadensis* ( $4 \pm 1$  day old) to rotting guava pulp infested with drosophilid larvae and non-infested rotting guava (same ripeness stage).

The treatments were developed under laboratory conditions ( $T = 25 \pm 3^{\circ}\text{C}$ ,  $\text{RH} = 75 \pm 5\%$  and light period = 14h). *O. albinerve* was not studied in the olfactometer because of the small number of samples obtained. The Friedman test was used to calculate the difference between the percent of time spent in each of the volatile fields (I, II III and IV) (Vet 1983, Vet *et al.* 1991). The statistical analyses were performed by the 'BioEstat' programme 1.0 version for Windows.

#### Behavior Sequence for Host Localization and Assessment.

The study of the behavior sequence of *A. pelleranoi*, *D. grenadensis* and *O. albinerve* on guava infested with frugivorous larvae was divided into two stages: (1) qualitative study: establishment of a behavior pattern – vibrotaxis or search with the ovipositor (Vet & Alphen 1985) and (2) quantitative study: quantification of the movements performed during the host search and assessment sequence (Vet & Bakker 1985).

The larvae of the flies were placed in petri dishes (3.5 cm diameter) on a thin layer of ripe guava pulp (substrate) where the female parasitoids were released individually. The experiments were carried out in the laboratory ( $T = 25 \pm 3^{\circ}\text{C}$ ,  $\text{RH} = 75 \pm 5\%$  and light period = 14h). The females remained in

the maintenance cages in a separate room to prevent contact with the guava volatiles. After placing the parasitoid on the petri dish, there was a 15-min wait to the start of the experiment, to allow the parasitoid to adapt to the new surroundings, then each female was assessed for 10 min. The behavior was recorded with the help of a stereoscopic microscope adapted to a video camera. After the tests, the video tape was analyzed and the time taken for each activity was marked using a digital chronometer. The time values were added and presented as a mean of each activity. Motionless period, frequency and duration of the host search movements, substrate probing, host probing and the frequency with which the parasitoid cleaned itself during the host search were observed (Vet & Bakker 1985). Twenty *A. pelleranoi* and *D. grenadensis* and five *O. albinerve* females were studied.

The parasitism behavior of Eucoilinae species was observed directly in guava orchards in the counties of Monte Alegre do Sul and Piracicaba, SP.

## Results and Discussion

### Olfactory Response to Volatiles in Olfactometer.

*A. pelleranoi*: Treatment 1: The females were more attracted by the ripe guava substrate infested with frugivorous larvae than by the volatiles emitted by ripe non-infested fruits (same stage of ripeness) (Table 1). Therefore, under these conditions, *A. pelleranoi* could recognize the semiochemicals emitted by the fruits attacked by fruit fly larvae. This fact is very important because *A. pelleranoi* specifically attacks tephritid larvae in a great variety of fruits (Ovuruski *et al.* 2000, Ovuruski & Aluja 2002). Thus the females of this parasitoid need to recognize the caïromones of the host larvae developing in the fruits to prevent dispersion of the target species (Vet 1985, Waage 1979).

Treatment 2: The females did not show preference in the choice between the fruits infested with *Anastrepha* spp. larvae from those infested with *C. capitata* larvae (Table 2). *A. pelleranoi* is probably able to recognize the volatiles of different larva species in the fruits but does not show a preference because the larvae of both tephritid are potential hosts of this parasitoid.

Treatment 3: The females of the parasitoids did not distinguish between the volatiles from guavas infested with 3<sup>rd</sup>-instar larvae and those emitted by guavas infested with young larvae (Table 3). In this case, it should be pointed out

Table 1. Response of 44 *A. pelleranoi* females ( $3 \pm 1$  day old) to the odors of ripe guava infested or not with frugivorous larvae (Diptera).

Treatments	Mean percentage of stay of the females in each quarter of the olfactometer
Water	12.9 b
Water	4.1 b
Ripe guava	19.5 b
Ripe guava with frugivorous larvae	63.3 a

Values followed by the same letter do not differ by the Friedman test at 5% probability.

Table 2. Response of 60 *A. pelleranoi* females ( $3 \pm 1$  day old) to the odors of ripe guava infested with *Anastrepha* spp. larvae and ripe guava infested with *C. capitata* larvae.

Treatments	Mean percentage of stay of females in each quarter of the olfactometer
Water	13.5 b
Water	13.0 b
Ripe guava with <i>C. capitata</i> larvae	31.6 a
Ripe guava with <i>Anastrepha</i> spp. larvae	41.6 a

Values followed by the same letter do not differ by the Friedman test at 5% probability.

Table 3. Response of 42 *A. pelleranoi* females ( $8 \pm 3$  day old) to the odors of ripe guava infested with *C. capitata* larvae of different instars.

Treatments	Mean percentage of stay of the females in each quarter of the olfactometer
Water	10.1 b
Water	12.9 b
Ripe guava with <i>C. capitata</i> larvae (3 <sup>rd</sup> instar)	33.4 a
Ripe guava with <i>C. capitata</i> larvae (1 <sup>st</sup> and 2 <sup>nd</sup> instars)	43.4 a

Values followed by the same letter do not differ by the Friedman test at 5% probability.

that the females studied were about eight days old, and thus it would be necessary to assess the influence of age on the ovarian charge and the effects on the decision limiters and choice of hosts for these parasitoids.

*D. grenadensis*. Treatment 4: *D. grenadensis* females were more attracted by the rotting guava substrate infested with *Z. indianus* larvae (Drosophilidae) than by the volatiles emitted from ripe fruits infested with *Anastrepha* spp. (Table 4). Thus the preference of *D. grenadensis* was detected for the volatiles released from the drosophilid + rotting guava complex.

Treatment 5: The females were more attracted by the rotting guava substrate infested by drosophilid larvae (plant + host complex) than by the volatiles emitted from non-infested rotting fruits (Table 5). This is probably because this species parasitizes drosophilid larvae, naturally associated with rotting fruits on the ground (Wharton *et al.* 1998). The rotting fruit volatiles have high concentrations of alcohols, due to the fermentation produced by fungi and bacteria (Vet 1983, Dicke *et al.* 1984, Vet & Opzeeland 1985). Adult drosophilids are bacteria and yeast vectors that, in contact with the substrate, increase the speed of fruit decomposition and consequently release alcohols as by-products of this fermentation process (Vet 1983, Dicke *et al.* 1984, Vet & Opzeeland 1985).

### Behavioral Sequence of Host Localization and Assessment.

*A. pelleranoi*. Initially the females presented a motionless period (mean 30s). At this stage, the antennae remained raised,

Table 4. Response of 40 *D. grenadensis* females ( $4 \pm 1$  day old) to the odors of ripe guava infested with Tephritid larvae and to the volatiles released by rotting guava infested with drosophilid larvae.

Treatments	Mean percentage of stay of the females in each quarter of the olfactometer
Water	12.9 b
Water	4.8 b
Ripe guava with tephritid larvae	18.8 b
Rotting guava with drosophilid larvae	63.3 a

Values followed by the same letter do not differ by the Friedman test at 5% probability.

making discrete vertical movements without touching the substrate. Shortly afterwards, the females walked actively on the fruit, moving their antennae vertically, quickly touching the clave segments on the fruit surface. The antennae moved rhythmically and alternately (Figs. 2A and 3). This period of antennal searching during which the female explored the fruit surface was observed for up to four consecutive min. When they located the fly larvae, the females generally remained immobile (mean 25s) (Figs. 2B and 3) and then inserted the ovipositor rapidly into the fruit (mean 5s) in a typical “probe” (Figs. 2C and 3). If the host is suitable for parasitism, the female positions itself at an angle of approximately 45° to the

Table 5. Response of 50 *D. grenadensis* females ( $4 \pm 1$  day old) to the odors released from rotting guava infested or not with drosophilid larvae.

Treatments	Mean percentage of stay of the females in each quarter of the olfactometer
Water	23.4 b
Water	9.7 b
Rotting guava	18.4 b
Rotting guava with drosophilid larvae	48.4 a

Values followed by the same letter do not differ by the Friedman test at 5% probability.

substrate and inserts the ovipositor in the host larva (mean 30s). After parasitism, the females began a cleaning process, using the tibia spurs on the forelegs to clean the antennae and the hind legs to clean the gaster, the ovipositor and the wings. The species that behave in this manner to locate the larvae inside the fruit only use the ovipositor for the “probe” and for the oviposition. Therefore, the behavior observed for the *A. pelleranoi* females consists mainly of the search using the antennae (vibrotaxis). This fact had already been observed in *A. pelleranoi* by Ovruski (1994) but the species of the *Ganaspis* genus also present this behavior (Vet & Bakker 1985).

In two field observations, five *A. pelleranoi* females were counted parasiting larvae in rotting fruits on the ground that

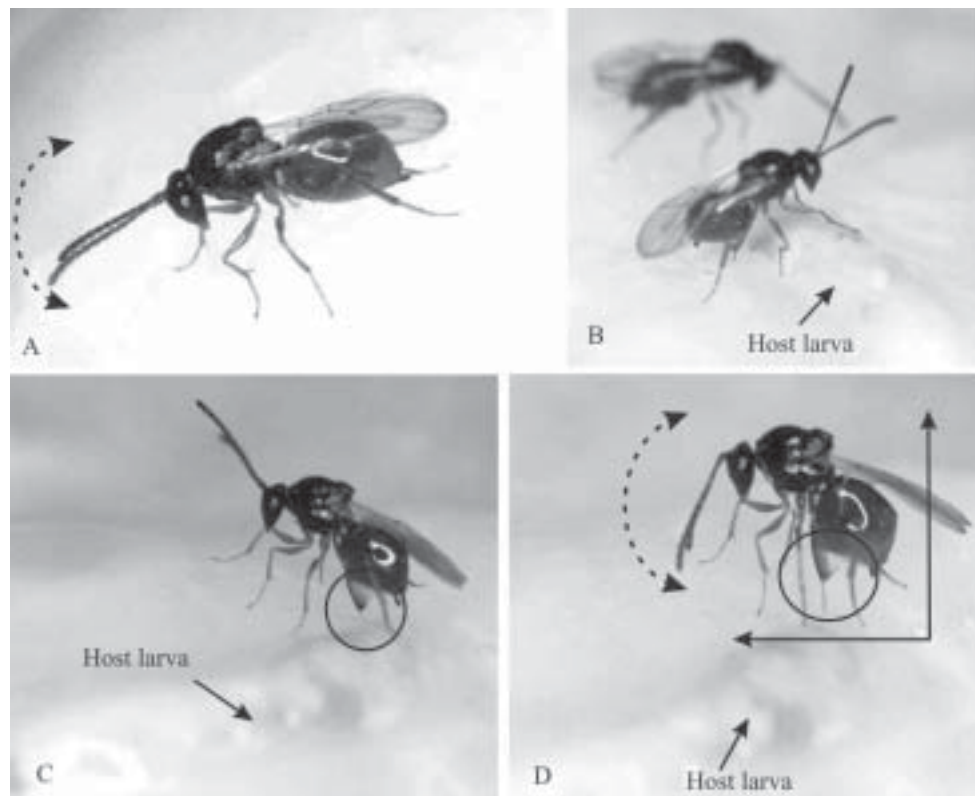


Figure 2. *A. pelleranoi* parasitoid sequential behavior. A. Antennal searching; B. Short stop; C. Quick introduction of the ovipositor “probe”; D. Parasitism (dotted lines indicate the antennal search).

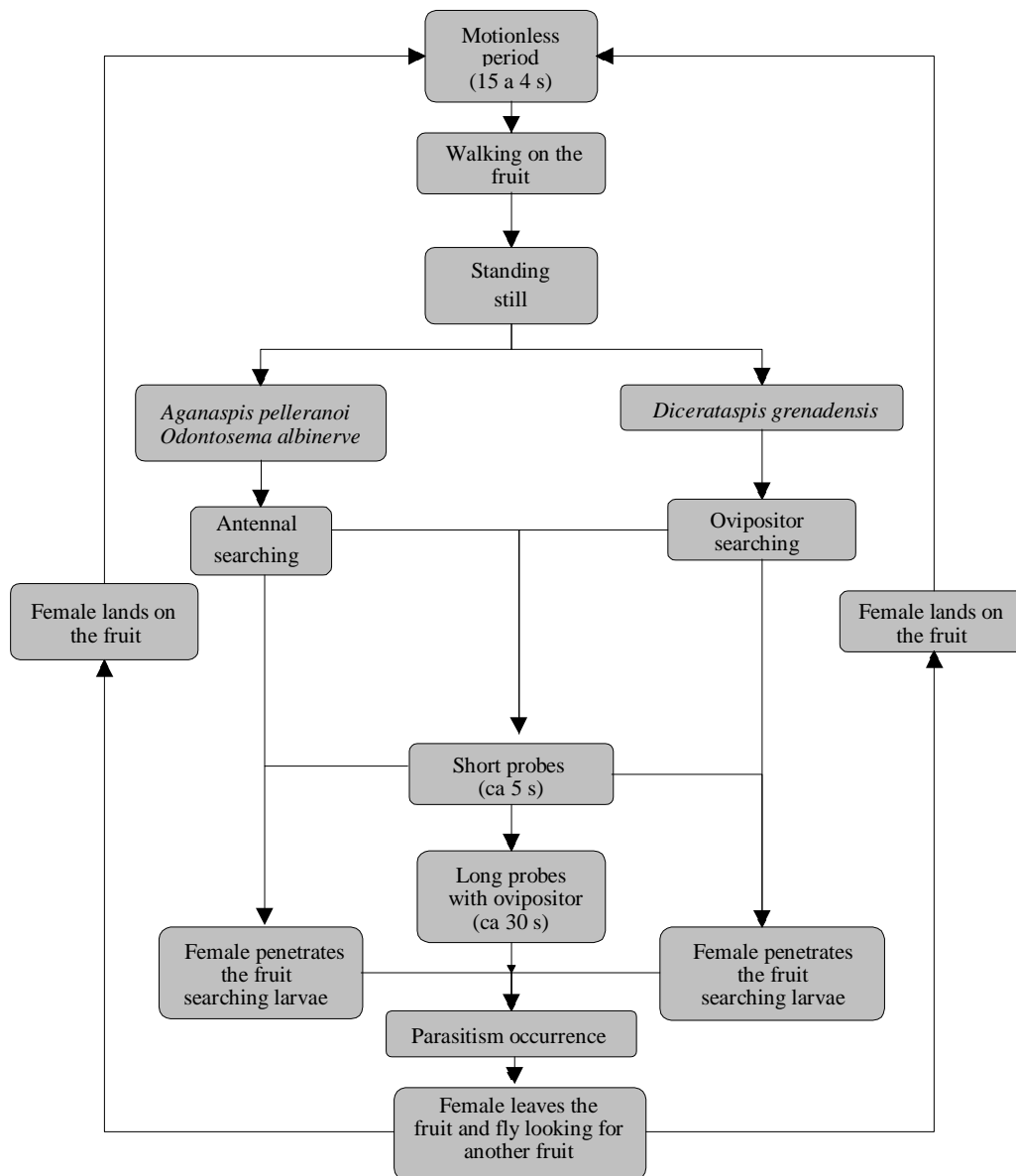


Figure 3. *A. pelleranoi*, *O. albinerve* and *D. grenadensis* parasitoid sequential behavior.

they penetrated through holes made by the fly larvae or through the cracks in the fruit resulting from the fall or from predatory insect activity (wasps and staphilinid beetles). Also in the field, on two occasions, three females were observed parasiting larvae in healthy fruit (without external injury) on the tree. In these observations it was possible to detect the sequence described previously (in the laboratory) that ended with the female inserting the ovipositor in the fruit.

*O. albinerve*. There was a period of motionless (mean 45s) where the females remained immobile, making gentle vertical antenna movements without touching the substrate. Next, the females walked on the fruit making vertical antenna movements, quickly hitting the fruit surface. Only the last segments of the antenna touched the substrate. This antennal searching was observed for periods of up to three consecutive minutes, and shortly afterwards, the females

generally remained immobile (mean 30s) and then quickly inserted the ovipositor in the fruit surface (mean 3s) in a typical "probe". After parasitism, the female cleaned its antennae with the mandibles and forelegs (tibial spurs) and cleaned its gaster, ovipositor and wings with the hind legs. The duration of the movements was obtained by the mean of the observation of five specimens. These parasitoids only used the ovipositor for "probe" and for oviposition, remaining retracted in the gaster at all other times (Figs. 2 and 3).

The larval parasitoids that used vibrotaxis tended to present a typical behavior of walking followed by periods of immobility. This immobility was necessary to perceive the noises produced by the larva activity inside the fruit. As soon as these noises were detected, they quickly inserted the ovipositor in the fruit, until they reached the larva, "probing" it to verify the suitability for the development of

the parasitoid (Vet & Bakker 1985).

The behavior of *O. albinerve* and *A. pelleranoi* was fairly similar and consisted mainly of searching with the antennae (vibrotaxis). Furthermore, *O. albinerve* females could also penetrate the voluminous fruits searching for fruit fly larvae. This kind of behavior was observed four times, when guavas infested with fly larvae were offered in the rearing cage. The *O. albinerve* females immediately penetrated the fruit, through the orifices made artificially by pincers or through the orifices made by the fly larvae and remained there for periods of up to 30 min. According to Fontal-Cazalla *et al.* (2002), *A. pelleranoi* and *O. albinerve* are fairly distant species phylogenetically. Thus these similarities in host search and assessment behavior are probably due to behavior convergence. This fact is quite common among parasitoids that use these same hosts (Vet & Alphen 1985).

*D. grenadensis*. There was a short motionless period (mean 15s) where the females remained immobile, making ovipositor cleaning movements with the hind legs. Afterwards, the females walked on the fruit "probing" the substrate several times with the ovipositor, even during the walk (Fig. 3). The ovipositor remained exposed and turned to face the front throughout the search period. The ovipositor touched the substrate for no more than one second. Approximately 60% of the time used by the females during the host search was spent in continuous substrate "probing" with the ovipositor (Fig. 3). When a larva was found, the females inserted the ovipositor in the substrate (mean of 10s) to assess the suitability of the host ("probe"). To perform parasitism, the females positioned themselves over the location where the larvae were, remaining at an angle of approximately 45° to the substrate and inserted the ovipositor (mean of 30s). The host search process was frequently interrupted for short stops (mean 15s) to clean the ovipositor and the wings. About 11% of the time was spent in cleaning.

The behavior pattern of the *D. grenadensis* parasitism was based mainly on the search with the ovipositor. This type of behavior is quite widespread among the drosophilid parasitoids (Vet & Alphen 1985). The behavior observed for *D. grenadensis* is similar to that of the species of the *Leptopilina* genus, that parasite drosophilid larvae associated with decomposing organic matter (Vet & Alphen 1985). These species were studied in detail by Lenteren *et al.* (1998) who observed the presence of many sensiles at the tip of the ovipositor, associated with host search and localization.

*D. grenadensis* presents a different parasitism behavior pattern than that observed for *A. pelleranoi* and *O. albinerve*. This may be related to the use of phylogenetically distant hosts (tephritids and drosophilids), with different biological and behavioral parameters, that probably selected different host search strategies in these parasitoids during the co-evolutionary process (Vet & Alphen 1985). The association of *D. grenadensis* with the hosts (tephritids or drosophilids) was doubtful (Wharton *et al.* 1998). Thus the observation of the host search and assessment sequential behavior of the olfactory response in the four-armed olfactometer enabled the establishment of the fact that *D. grenadensis* presents specificity to the volatiles released from the drosophilids + rotting guava complex.

In six field observations, eight penetrated inside fruits

searching for host larvae, as observed for *A. pelleranoi* and *O. albinerve*. Thus, this capacity seems to be widely spread among all the Eucoilinae parasitoids of larvae that develop inside fruits. This information is very important, because it shows that the Eucoilinae can complement opiines parasitism and thus could be used together in biological control programs.

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