

Defensive behaviors of the new mealybug citrus pest, *Delottococcus aberiae* (Hemiptera: Pseudococcidae), against three generalist parasitoids

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Abstract

Delottococcus aberiae De Lotto (Hemiptera: Pseudococcidae) is an invasive mealybug that has become a citrus pest in Europe. This mealybug species causes serious damage because it deforms the fruits. Here, we studied the defensive behavior of *D. aberiae* when it was attacked by three parasitoid species: *Acerophagus angustifrons* (Gahan), *Anagyrus* sp. near *pseudococi* (Girault), and *Leptomastix algerica* Trjapitzin (Hymenoptera: Encyrtidae). *Anagyrus* sp. near *pseudococi* and *L. algerica* detected and accepted nymphs and adult females of *D. aberiae*, whereas *A. angustifrons* only accepted adults. We recorded four defensive responses of *D. aberiae* to parasitoid attacks: abdominal flipping, swiveling around the inserted stylet, withdrawing the stylet and walking away, and, occasionally, they secreted ostiolar fluids. Despite these defensive behaviors, the mealybug did not escape parasitism from any of the tested parasitoids, even though *A. angustifrons* needed more than 15 min to parasitize. We also analyzed the nutritional value of the honeydew excreted by *D. aberiae* for *A. angustifrons* and *A.* sp. near *pseudococi*. Females and males of these parasitoids lived more than 28 d when fed sucrose, but they lived fewer than 3 d when fed *D. aberiae* honeydew. Therefore, *D. aberiae* excretes honeydew of poor quality for parasitoids. The consequences of these biological traits of *D. aberiae* for its biological control are discussed.

Key words: *Acerophagus*, *Anagyrus*, *Leptomastix*, biological control, honeydew

Delottococcus aberiae (De Lotto) (Hemiptera: Pseudococcidae) is a polyphagous mealybug native to sub-Saharan Africa (Miller and Giliomee 2011, Beltrà et al. 2015b), which was detected for the first time in 2009, as a citrus pest in the Mediterranean basin (Beltrà et al. 2013). After a comprehensive but unsuccessful eradication program, this mealybug established as a serious pest in the region of Les Valls (Valencia, Eastern Spain), which is located in the core of the main citrus producing area of the Mediterranean basin (Beltrà et al. 2013, Tena et al. 2014). *D. aberiae* increases its populations during spring and nymphs and adults settle and feed on the surface of young fruitlets under the calyces (Martínez-Blay et al. 2017). This feeding habit causes reduction and distortion of growing fruit (Pérez-Rodríguez 2017).

Encyrtid parasitoids and predatory coccinellids are among the most successful families of natural enemies used in biological control of mealybugs (Bartlett 1978, Moore 1988). Numerous biological control programs have been carried out and, as result of these efforts, most pseudococcids are under excellent biological control (Moore 1988, Franco et al. 2004). Some genera of the Encyrtidae

such as *Acerophagus* or *Leptomastix* have been used successfully in the biological control of other mealybug species, including *Phenacoccus peruvianus* Granara de Willink, *Phenacoccus herreni* Cox & Williams, and *Paracoccus marginatus* Williams and Granara de Willink (van Driesche et al 1987, Muniappan et al. 2006, Beltrà et al. 2015a). Parasitoids of the Anagyrini tribe, which contains the genus *Anagyrus*, have been cited as primary parasitoids of *Planococcus* mealybugs as *P. citri* (Risso) and *P. ficus* Signoret (Noyes and Hayat 1994, Franco et al. 2004). The success of mealybug parasitoids depends, in part, on their capacity to overcome their hosts' defenses (Bartlett 1978, Gross 1993, Blumberg 1997, Blumberg and van Driesche 2001, Daane et al. 2007, Campos-Rivela 2008, Tena et al. 2012, Sime and Daane 2014, Beltrà et al. 2015a, Bugila et al. 2015). In the case of *D. aberiae*, we have recently demonstrated that it is able to encapsulate the eggs of three generalist parasitoids of mealybugs: *Acerophagus angustifrons* (Gahan), *Anagyrus* sp. near *pseudococi* (Girault), and *Leptomastix algerica* Trjapitzin (Hymenoptera: Encyrtidae) (Tena et al. 2017). However, the behavioral defenses of this new citrus pest against parasitoids

have not been described. These parasitoids were selected because they are native or naturalized species in Spain, are considered generalist parasitoids of pseudococcids (Martínez-Ferrer 2003, Campos-Rivela 2008, Universal Chalcidoidea Database 2016), and are able to parasitize *D. aberiae* (Tena et al. 2017).

Despite being vulnerable to parasitoids, mealybugs have developed diverse defensive strategies that can hinder biological control (Blumberg 1997, Gullan and Kosztarab 1997, Gutierrez et al. 2008). They hide from their natural enemies by adopting cryptic behavior (Vet and Dicke 1992, Gross 1993, Foldi 1997, Gutierrez et al. 2008). When these defense mechanisms fail, mealybugs carry out evasive movements similar to those performed by aphids (Hemiptera: Aphididae) and soft scales (Hemiptera: Coccidae) (De Farias and Hopper 1999, Wyckhuys et al. 2008, Tena et al. 2012). Some of these movements, such as wriggling, swiveling around the stylet, getting up, walking away, and secreting defensive exudates, have been recorded to thwart parasitoid oviposition (Bynum 1937, Boavida et al. 1995, Bokonon-Ganta et al. 1995, Cadée and van Alphen 1997, Bugila et al. 2014, Beltrà et al. 2015a). The elucidation of such interactions is crucial to understanding the potential of these parasitoids and to select parasitoids for biological control.

Mealybugs excrete large amount of honeydew that can be used by their parasitoid as a carbohydrate source. Generally, honeydew has been considered a poorer carbohydrate source for adult parasitoids when compared with nectar (Wäckers et al. 2008, Tena et al. 2016). However, honeydew is available in citrus and the honeydew of at least two mealybug species significantly increases the longevity and fecundity of different parasitoids (Avidov et al. 1970, Tena et al. 2013). In the case of *D. aberiae*, it is unknown whether its honeydew is suitable for parasitoids. In order to design future biological control programs, it is important to determine the sugar sources available and its quality for parasitoids (Tena et al. 2016).

The aims of this study were: 1) to compare the host selection behavior of the parasitoids *A. angustifrons*, *A. sp. near pseudococci*, and *L. algerica* in relation to the mealybug *D. aberiae*; 2) to describe the defensive behavior of *D. aberiae* against these parasitoids, and 3) to analyze the suitability of *D. aberiae* honeydew as a carbohydrate source for these parasitoids. These results will increase our knowledge of the defensive mechanisms of this new citrus pest and whether additional carbohydrates are necessary to efficiently rear parasitoids in the insectary or conserve their populations in the field.

Materials and Methods

Mealybug Rearing and Parasitoid Sources

The colony of *D. aberiae* was established in the laboratory of Entomology at Instituto Valenciano de Investigaciones Agrarias (IVIA) using specimens collected in a citrus orchard located in Quartell (Les Valls, Valencia, Spain) in 2013. Mealybugs were reared on organically ripen lemons inside cardboard boxes (30 × 22 × 25 cm) in which egg cartons were placed in the bottom. Approximately 65% of the surface of each lemon was covered with red paraffin around the mid-section to retard its desiccation. The red paraffin was prepared with a mixture of 1 kg of paraffin pearls (Parafina USP Perlas, Guinama S.L., Alboraya, Spain) and 1 g of red pigment (Sudan III, Panreac Química S.A., Castellar del Vallés, Spain) following the same procedure used for the rearing of California red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) (Vanaclocha et al. 2012). Every 2 wk, four or five fresh lemons were introduced within each box and the dried ones were

removed. The mealybug colony was maintained in darkness in a climatic chamber at 25 ± 1°C and 70 ± 5% RH.

The parasitoids *A. angustifrons*, *A. sp. near pseudococci*, and *L. algerica* were obtained from Koppert Biological Systems S.L. (Águilas, Murcia, Spain). For the behavioral assay, the bottles with the pupae were opened and introduced in wooden and crystal rearing boxes (51 × 51 × 41 cm) with holes covered with mesh in the wall. Honey drops on the walls and an Eppendorf tube filled with water and a piece of cotton were provided. Rearing boxes were kept in the laboratory at room temperature until parasitoids were used. Emerged parasitoids were collected daily at 15:00, sexed and individualized in a 3.0 × 0.8 cm in diameter glass and sealed with cotton. Parasitoids could mate during the 24 h that both sexes were together in the rearing boxes. A drop of honey was provided on the wall. Female parasitoids were used 2–4 d later in the behavioral assay.

For the survival assay, parasitoid pupae were introduced in a 3.0 × 0.8 cm diameter glass vials sealed with a piece of muslin mesh. At emergence, parasitoids were sexed and diets were provided and replaced every 2 d as explained subsequently. Parasitoids were kept in a climatic chamber at 25 ± 1°C, 70 ± 5% RH, and a photoperiod of 14:10 (L:D) h in both assays.

Parasitoid Oviposition Behavior and Host Defense Responses

The oviposition behavior of *A. angustifrons*, *A. sp. near pseudococci*, and *L. algerica* were evaluated by direct observations, following methods similar to those described by Desneux et al. (2009). We also observed *D. aberiae* defensive behaviors and the effectiveness of these behaviors in preventing parasitism. Finally, we assessed the relationship between *D. aberiae* defensive behaviors and oviposition success of the three parasitoids.

The substrate for observations was the surface of a lemon under a binocular microscope illuminated with cold light. We placed one individual of *D. aberiae* on lemon surface using a fine brush and allowed it to establish for 24 h. The individual of *D. aberiae* was either a third instar or a preovipositing adult female, which are the preferred instars for these parasitoids (Tena et al. 2017). Then, a glass vial with a parasitoid female was opened and placed near the host mealybug on the lemon surface. Observations began when the vial came into contact with the lemon and ended when: 1) oviposition finished (parasitism); 2) the female contacted and rejected the host (rejection); or 3) the female did not contact the host after 15 min (indifference). The frequency and time spent by the parasitoid and the host in each behavioral event were recorded by voice recording with the program Audacity®, the Free, Cross-Platform Sound Editor (<http://www.audacityteam.org/>). The time spent to find the host was also recorded.

We recorded parasitoid behaviors following Beltrà et al. (2015a): 1) antenna drumming—drumming the host body with the antennae; 2) ovipositor tap—assessing the host by tapping the body with the ovipositor; 3) oviposition drill that included probing the host's body with the ovipositor and abdominal movements with the ovipositor inside the host's body; and 4) remove ovipositor—removing ovipositor.

Mealybug defensive action patterns were classified following Boavida et al. (1995) and Bugila et al. (2014): 1) abdominal flipping—refers to repeated up and down movements of the hind half of the mealybug; 2) moving—swiveling around the inserted stylet; 3) reflex bleeding—refers to the secretion of ostiolar fluid; 4) walking away—the mealybug may also escape from the aggressor. We

considered that a mealybug defended itself from an oviposition attempt when it carried out at least one of the above behaviors.

We also recorded the host detection and acceptance rates. We considered that a host was detected and accepted when the parasitoid drummed the mealybug with its antenna, and inserted its ovipositor in an attempt to deposit an egg, respectively. To confirm the oviposition, the parasitoid was removed and the mealybug was carefully transferred to a tender leaf of citrus clementine (*Citrus sinensis* cv. clemenules) after the observation period. The leaf was introduced over a layer of bacteriological agar (20 g/liter) with the petiole inserted in the agar within a 5.3-cm diameter Petri dish with a 3-cm diameter hole covered by a muslin mesh to permit ventilation (Tena et al. 2017). The arenas were placed undisturbed in a climatic chamber ($25 \pm 1^\circ\text{C}$, $70 \pm 5\%$ RH, and a photoperiod of 14:10 (L:D) h. Ten days later, we confirmed whether or not the mealybugs were parasitized (i.e., mummified or contained encapsulated parasitoid eggs) (Tena et al. 2017). Observations were replicated between 12 and 24 times depending on the parasitoid species and host instar.

Effect of *D. aberiae* Honeydew on Parasitoid Longevity

To assess the quality of the honeydew excreted by *D. aberiae* as a sugar source for *A. sp.* near *pseudococci* and *A. angustifrons*, we compared their longevity when they fed on 2M (mol/liter) sucrose (catalog number S0389, Sigma-Aldrich, United Kingdom), honeydew, and water. Female and male parasitoids were tested in this assay. Water was supplied for all treatments by spraying the vials through the muslin mesh. Honeydew and sucrose (three droplets of 5 μl) were provided ad libitum on 0.5 cm^2 piece of Parafilm (Tena et al. 2013). Honeydew was collected over a period of 24 h by placing pieces of Parafilm below a colony of mealybugs settled on lemons fruits in the climatic chambers. The presence of honeydew on the pieces of Parafilm was checked under a binocular and then kept at -20°C within a Petri dish until further use (Hogervorst et al. 2007, Tena et al. 2013). Vials with parasitoids were checked daily from 8:00 to 10:00 to determine the number of surviving parasitoids. Accidental deaths (wasps stuck in honey) were not used in the data analyses. Between 15 and 40 parasitoids were used per treatment.

Statistical Analysis

We applied generalized linear modeling techniques assuming Poisson and binomial error variance to construct models using the below-mentioned dependent variables and parasitoid species as the explanatory variable. Initially, we assumed a Poisson error variance for the count variable of 'defensive responses per host' and a binomial error variance for the proportional variable of host detection and acceptance. When an over- or underdispersion was detected, we re-evaluated the significance of the explanatory variables using an *F*-test after rescaling the statistical model by a Pearson's chi-square divided by the residual degrees of freedom (Crawley 2007). The data are presented as the means of untransformed proportion and count data (in preference to less intuitive statistics such as the back-transformed means of logit-transformed data). For the variable 'oviposition time,' we assumed normal distribution and an ANOVA was carried out with parasitoid as explanatory variable. Moreover, Tukey's posthoc tests were performed. The effect of the feeding treatments on the longevity of adult parasitoids was represented by Kaplan-Meier survivorship curves and analyzed by log-rank tests. All the analyses were performed by means of statistical software

R (<http://www.R-project.org>) and the packages 'OISurv' for the survival analysis and 'MULTCOMP' for the Tukey's posthoc tests.

Results

Parasitoid Oviposition Behavior and Host Defense Responses

Host Detection and Acceptance

Detection rates of third instar nymphs of *D. aberiae* by the three parasitoids ranged between 0.23 and 0.94 (Table 1). The rate of host detection in *A. sp.* near *pseudococci* was significantly higher than in *A. angustifrons* and *L. algerica* ($\chi^2 = 49.3$, $\text{df} = 2$, 50 , $P < 0.0001$). In the case of *D. aberiae* adult females, detection rates ranged between 0.67 and 0.88, but no significant differences were found among the three parasitoid species ($\chi^2 = 58.14$, $\text{df} = 2$, 52 , $P = 0.22$).

Anagyrus sp. near *pseudococci* and *L. algerica* accepted a significantly higher proportion of third instar nymphs than *A. angustifrons* ($\chi^2 = 53.65$, $\text{df} = 2$, 50 , $P = 0.0002$) (Table 1). In this last parasitoid species, only one female of *A. angustifrons* parasitized a third instar nymph. In the case of *D. aberiae* adult females, no significant differences were found among the three parasitoid species ($\chi^2 = 62.7$, $\text{df} = 2$, 52 , $P = 0.07$).

Oviposition Behavior

Anagyrus sp. near *pseudococci* and *L. algerica* needed about ~ 90 s to parasitize third instar nymphs of *D. aberiae* and there were no significant differences between the two species ($F_{1,18} = 0.40$, $P = 0.53$). These parasitoids spent ~ 65 s to parasitize adults of *D. aberiae* and, again, there were no significant differences between the two species ($F_{1,23} = 2.45$, $P = 0.13$). They spent most of this time drilling and laying the eggs (Fig. 1). In the few cases in which *A. angustifrons* accepted adult mealybugs of *D. aberiae* as hosts, females needed more than 15 min (1048–1489 s) to parasitize adult mealybugs. Similarly, they spent most of this time drilling and laying the eggs (84.26–98.46% of the total time spent).

Host Defence

Overall, 92% (23 out of 25) and 88.6% (31 out of 35) of the nymphs and adults of *D. aberiae* defended themselves from parasitoid attacks, respectively. Third instar *D. aberiae* nymphs showed a mean of 5.79 ± 1.1 defensive responses against *A. sp.* near *pseudococci* and 3.0 ± 0.69 against *L. algerica* ($F_{1,23} = 4.04$, $P = 0.056$). *D. aberiae* adults showed a mean of 5.8 ± 2.27 defensive responses against *A. angustifrons*, 6.29 ± 1.12 against *A. sp.* near *pseudococci* and 4.56 ± 1.48 against *L. algerica* ($F_{2,32} = 0.43$, $P = 0.65$) (Fig. 2). The most common defensive response of *D. aberiae* during the

Table 1. Proportion of *D. aberiae* (\pm SE) detected and accepted by the parasitoids *A. angustifrons*, *Anagyrus sp.* near *pseudococci*, and *L. algerica* when encountering third instar nymphs (N3) and preovipositing adult females of the mealybug (F1)

Host instar	Parasitoid species	Detection	Acceptance
N3	<i>A. angustifrons</i>	0.23 \pm 0.12 ^b	0.08 \pm 0.08 ^b
	<i>A. sp.</i> near <i>pseudococci</i>	0.94 \pm 0.06 ^a	0.78 \pm 0.10 ^a
	<i>L. algerica</i>	0.55 \pm 0.11 ^b	0.55 \pm 0.11 ^a
F1	<i>A. angustifrons</i>	0.67 \pm 0.14 ^a	0.42 \pm 0.15 ^b
	<i>A. sp.</i> near <i>pseudococci</i>	0.88 \pm 0.08 ^a	0.82 \pm 0.10 ^a
	<i>L. algerica</i>	0.67 \pm 0.10 ^a	0.67 \pm 0.10 ^{ab}

Different letters within a host instar indicates significant differences among parasitoid species ($P < 0.05$).

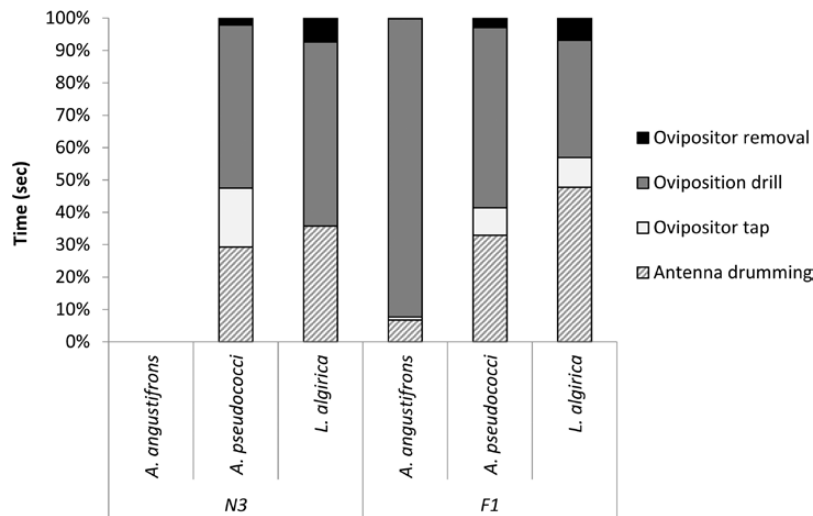


Fig. 1. Mean time spent (\pm SE) by *A. angustifrons*, *Anagyrus* sp. near *pseudococci*, and *L. algerica* when they parasitized third instar nymphs (N3) and preovipositing adult females (F1) of the mealybug *D. aberiae*.

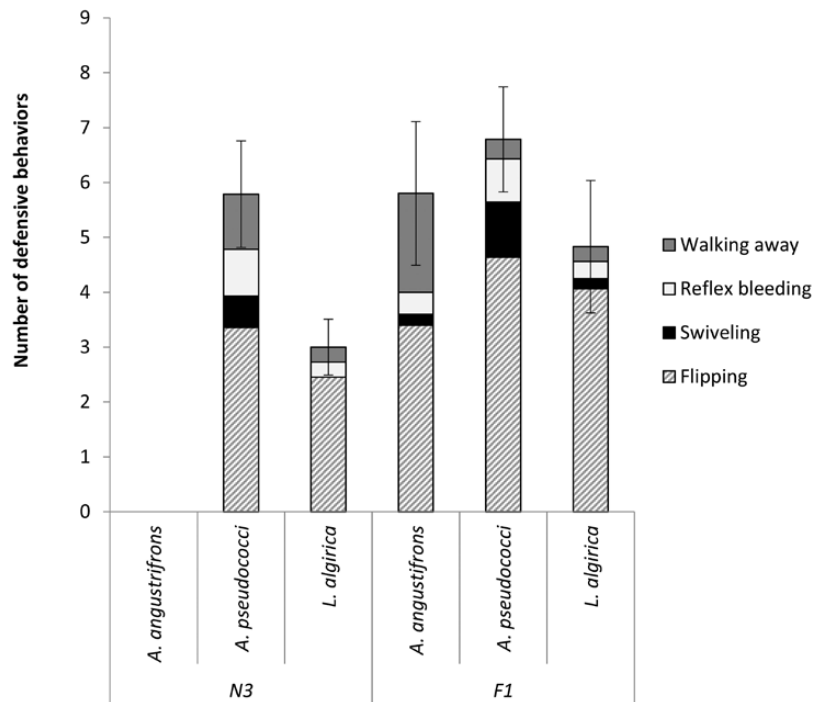


Fig. 2. Mean number of defensive responses (\pm SE) of third instar nymphs (N3) and preovipositing adult females (F1) of the mealybug *D. aberiae* when attacked by the parasitoid species *A. angustifrons*, *Anagyrus* sp. near *pseudococci*, and *L. algerica*.

oviposition process was abdominal flipping (Fig. 2). Mealybugs also defended themselves by swiveling around the inserted stylet and by withdrawing the stylet and walking away (Fig. 2). Finally, some individuals also secreted ostiolar fluids during the parasitoid oviposition. However, these defensive responses did not succeed and only three mealybugs out of the 54 escaped parasitism (one defended against *A. angustifrons* and two against *A. sp. near pseudococci*).

Effect of *D. aberiae* Honeydew on Parasitoid Longevity

The diet provided to the parasitoids significantly influenced the life span of both parasitoids (*A. angustifrons* females: [Log-rank test] $\chi^2 = 43.45$, $df = 2$, $P < 0.001$, *A. angustifrons* males: $\chi^2 = 47.78$,

$df = 2$, $P < 0.001$, *A. sp. near pseudococci* females: $\chi^2 = 89.38$, $df = 2$, $P < 0.001$, *A. sp. near pseudococci* males: $\chi^2 = 62.02$, $df = 2$, $P < 0.001$) (Fig. 3). Females and males lived longer when provided with sucrose than with honeydew or water. *D. aberiae* honeydew did not increase the longevity of *A. angustifrons* females and males and only marginally increased the longevity of *A. sp. near pseudococci* females and males (between 1 and 2 d) when compared with water (Table 2).

Discussion

The three generalist parasitoids, *A. angustifrons*, *A. sp. near pseudococci*, and *L. algerica*, detected and accepted the new citrus pest *D. aberiae* but both behaviors varied among parasitoids and depended also on the mealybug instar. Among the three parasitoid

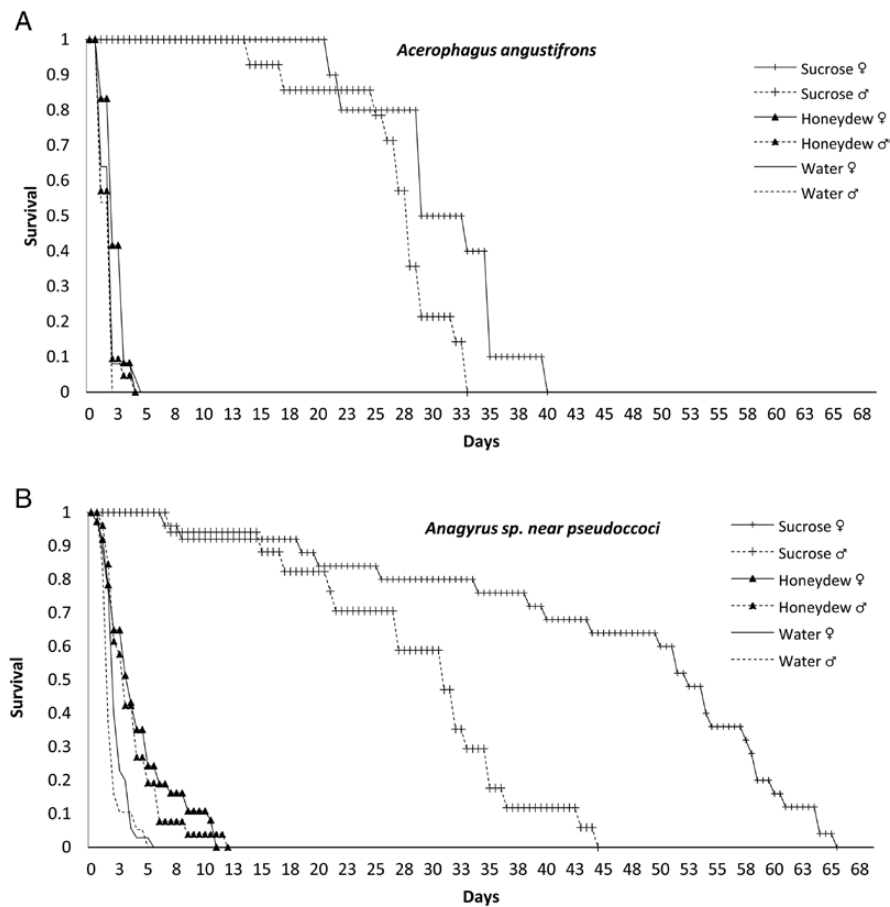


Fig. 3. Survival of females and males of the parasitoids *A. angustifrons* and *Anagyrus sp. near pseudococci* fed on sucrose 2M (mol/liter), honeydew excreted by *D. aberiae*, or water.

Table 2. Mean longevity in days (\pm SE) of females and males of the parasitoids *A. angustifrons* and *Anagyrus sp. near pseudococci* when fed on sucrose 2M, honeydew excreted by *D. aberiae*, and water

Species	Sex	Diet		
		Sucrose	Honeydew	Water
<i>A. angustifrons</i>	Female	30.8 \pm 1.9 ^a	2.3 \pm 0.18 ^b	1.8 \pm 0.18 ^b
	Male	26.9 \pm 1.45 ^a	1.7 \pm 0.17 ^b	1.5 \pm 0.14 ^b
<i>A. sp. near pseudococci</i>	Female	46.0 \pm 3.52 ^a	4.2 \pm 0.48 ^b	2.3 \pm 0.16 ^c
	Male	28.7 \pm 2.39 ^a	3.7 \pm 0.49 ^b	1.9 \pm 0.23 ^c

Different letters within the same row denote significant differences between diets ($P < 0.05$).

species, *A. sp. near pseudococci* detected and accepted *D. aberiae* at higher rates, especially the third instar. This generalist parasitoid tends to accept mealybugs independently of their geographical origin, phylogenetic relationships, and defensive behaviors (Bugila et al. 2015). *L. algerica* also tended to accept *D. aberiae* nymphs but it did not detect them easily. Unfortunately, the eggs of both parasitoids are encapsulated by *D. aberiae* (Tena et al. 2017). On the other hand, *A. angustifrons* females did neither detect nor accept *D. aberiae* nymphs and only accepted 40% of the adults. This parasitoid has been observed on mealybugs from genus *Dysmicoccus* (Trjapitzin 2008) and it might not be adapted to parasitize pseudococcids from other genera. Although its long oviposition time and defensive responses of *D. aberiae* were not an impediment to successfully parasitize the adults, *A. angustifrons* does seem be able to

detect *D. aberiae* and, moreover, its eggs are also encapsulated by *D. aberiae* (Tena et al. 2017).

D. aberiae responded to parasitoid attacks with four non-excluding active defensive behaviors: wriggling, swiveling around the inserted stylet, withdrawing the stylet and running away, and secreting defensive fluids. These defensive behaviors had previously been described in other mealybug species (Boavida et al. 1995, Bokonon-Ganta et al. 1995, Cadée and van Alphen 1997, Bugila et al. 2014, Beltrà et al. 2015a). However, these defensive responses did not allow *D. aberiae* to escape parasitism from any parasitoid. Even *A. angustifrons* females, which needed more than 15 min to parasitize, were not disturbed by *D. aberiae* active defenses. Moreover, and contrary to previous studies with other mealybug species (Cadée and van Alphen 1997, Beltrà et al. 2015a), we did not find differences

between mealybug instars. With all this said, the active defensive behavior of *D. aberiae* described in this paper cannot explain the low degrees of parasitism found in Spanish citrus since the detection of the mealybug in 2009 (Tena et al. 2014). Furthermore, our results support the hypothesis that most active host defenses might have not evolved as a response to parasitoid selective pressure but to other biological functions that also provide some protection against parasitoids (Gross 1993, Bugila et al. 2014). If there were specific defensive behaviors, we would expect differences among the responses to the three parasitoids.

Honeydew excreted by *D. aberiae* is a poor carbohydrate source for the studied parasitoids. Parasitoids fed honeydew extended their life for just 1 d more than unfed parasitoids and their lifespan was much shorter than those fed sucrose 2M (between 25 and 35 d long). The low nutritional suitability of honeydew may be based on plant-derived compounds (primary and secondary metabolites) as well as compounds synthesized by honeydew-producers (Wäckers et al. 2008). It is important to highlight that another mealybug *P. citri* excretes honeydew of high value for parasitoids and ants in citrus (Pekas et al. 2011, Tena et al. 2013, Tena et al. 2016). Therefore, either *D. aberiae* or the rearing conditions are responsible for the poor honeydew. In our study, we have tested honeydew excreted by *D. aberiae* reared on detached fruits that could be of poorer quality for the mealybug than growing fruits in the field. In this scenario, it is reasonable to believe that honeydew composition may be affected. Our field observations, however, also show that ants do not attend *D. aberiae* colonies as frequently as they attend *P. citri* (A.T., personal observations), suggesting that *D. aberiae* excretes a poor quality honeydew also in the field. This is because ants tend hemipterans that excrete honeydew of high quality for parasitoids (Tena et al. 2016). Further research in the field is necessary to corroborate this result because sugar sources are crucial for the primary parasitoids of mealybugs, which are generally synovigenic. For example, *A. sp.* near *pseudococci* is synovigenic, emerge without mature eggs, and females lay few eggs per day (~5–7 per day) but for an extended period of time (~40 d) (Tingle and Copland, 1989).

Finally, we did not observe host-feeding in any parasitoid species. However, we cannot exclude the possibility of host-feeding by *A. sp.* nr. *pseudococci* and *L. algerica* in younger host instars, such as first and second instars, as our observations were carried out only on third instar nymphs and prereproductive adult females. For example, Karamaouna and Copland (2000) and Bokononganta et al. (1995) observed females of *Leptomastix epona* (Walker) and *Anagyrus mangicola* (Noyes) (Hymenoptera: Encyrtidae) feeding on young instars of mealybugs in which they do not oviposit. If this is the case, host-feeding by *A. sp.* near *pseudococci* might cause the mortality of *D. aberiae* nymphs in Spanish citrus, where the parasitoid develops on *P. citri* (Martínez-Ferrer 2003, Campos-Rivela 2008), which coexist with *D. aberiae*. The importance of nonreproducing effects of parasitoids such as host feeding and mutilation or death of host probed and rejected by parasitoids are an underappreciated component of biological control services provided by parasitoids (Abram et al. 2016, Cebolla et al. 2017). The impact of host-feeding and other nonreproducing effects of *A. sp.* near *pseudococci* on *D. aberiae* should be studied under field and laboratory conditions to unravel the effect of this abundant parasitoid in Europe.

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sp. near *pseudococci*, and *L. algerica* individuals. This research was partially funded by an INIA project (Project No. RTA2014-00067) and the Conselleria d'Agricultura, Pesca i Alimentació de la Generalitat Valenciana.

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