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ETHOLOGY

Evaluating the quality of the Mexican fruit-fly, *Anastrepha ludens*, as host for the parasitoid *Melittobia digitata*

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Abstract

We investigated the presence of *Melittobia digitata* (Hymenoptera: Eulophidae) and its associations with its natural hosts in Veracruz, Mexico. The parasitoid was confirmed to be present and found to attack prepupae of *Trypoxylon* and *Sceliphron* (Hymenoptera: Crabronidae and Sphecidae, respectively) mud daubers as well as pupae of the Mexican fruit fly *Anastrepha ludens* (Diptera: Tephritidae). Since *A. ludens* is a very important pest of citrus and mango in Mexico and Central America, we evaluated, in laboratory experiments, the quality of *A. ludens* as host of *M. digitata* comparing the development of the parasitoid in another unrelated fly species (*Sarcophaga* [*Neobellieria*] *bullata*) (Diptera: Sarcophagidae) and in mud dauber wasps (*Trypoxylon* sp. and *Sceliphron* sp.). The Mexican fruit fly appears to be a good-quality host for *M. digitata*, and the results are discussed in the context of their possible interest as a natural enemy of *A. ludens.*

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Introduction

Melittobia is a cosmopolitan genus of parasitoid wasps with 12 species (Matthews & González, 2008; Matthews *et al*., 2009; Tanner *et al*., 2011). Wasps of this genus are small (1.0-1.5 mm) ectoparasitic arrhenotokous idiobionts that develop gregariously on their hosts (Matthews *et al*., 2009). *Melittobia* wasps are sexually dimorphic, and females are polymorphic (short winged and long winged) (Cônsoli & Vinson, 2002a,b; González & Matthews, 2005, 2008; Matthews *et al*., 2009). Long-winged females, also called macropterous females, are the dispersal stage, while some non-dispersing short-winged females, also called brachypterous females, may be produced in some large hosts (Cônsoli & Vinson, 2004).

In North America seven *Melittobia* species have been reported attacking a wide range of hosts but they are mainly associated with mud dauber wasp species (Matthews *et al*., 2009), while in Mexico only two of them, belonging to different species-groups are known: *Melittobia australica* Girault and *M. digitata* Dahms (González *et al*., 2008, 2010; Tanner *et al*., 2011). The first species was found attacking wasps (Hymenoptera) such as *Euodynerus hidalgo vierecki* (Cameron) (Vespidae), *Chrysis wasbaueri* Bohart (Chrysididae) and *Sceliphron* spp. (Sphecidae), while the latter has been found attacking *Trypoxylon mexicanum* (Saussure) (Crabronidae), but was unexpectedly recorded from pupae of the pestiferous tephritid fly *Anastrepha ludens* Loew (Tephritidae) also known as the Mexican fruit fly (Copeland *et al*., 2008; González *et al*., 2008, 2010). *Anastrepha ludens* is a very important pest of citrus and mango in Mexico and Central America (Hernández-Ortiz, 1992; Aluja, 1994; Aluja *et al*., 1996, 2009; Birke *et al*., 2006). In the USA it is considered a pest of quarantine importance, with management/eradication programs in place in Texas and California (Mangan *et al*., 1997; Thomas, 2003; Thomas, *et al*. 2008;

Aluja & Mangan, 2008). Biological control of tephritid fruit flies, including *A. ludens*, largely relies on larval/egg-prepupal braconid parasitoids (Purcell, 1998; Ovruski *et al*., 2000), but under restricted conditions, a pupal parasitoid could be used in combination with larval parasitoids, because pests escaping larval parasitism may be attacked during the pupal stage, thus reducing the overall pest population (López *et al.*, 1999).

The use of pupal parasitoids can be recommended especially when tephritid fruit flies infest large fruits, such as citrus and mangoes, because it has been shown that there is a negative correlation between fruit size and percentage of parasitism by braconid parasitoids (Sivinski, 1991; López *et al*., 1999; Cancino *et al*., 2009). Excluding *M. digitat*a, only a few pupal parasitoids associated with tephritid fruit flies have been reported in Mexico, including *Coptera haywardi* (Oglobin) (Diapriidae), *Pachycrepoideus vindemmiae* (Rondani) (Pteromalidae), and *Eurytoma sivinskii* Gates and Grisell (Eurytomidae) (Mena-Correa *et al*., 2010 and references within). *Melittobia digitata* is a species only known from North America; in the continental USA it has been reported attacking various Diptera and Hymenoptera at several locations (Dahms, 1984; La Salle, 1993; Matthews *et al*., 2009). It is one of the best studied species in the genus and is also widely used in science curricula (Matthews *et al.*, 2009).

The goals of this study were to i) investigate how common *M. digitata* is as a parasitoid of mud dauber wasps in the area of Veracruz, Mexico; ii) inspect field collections of *A. ludens* pupae stored at the Instituto de Ecología, A.C. (INECOL) to confirm the presence of *M. digitata* as a natural parasitoid of the Mexican fruit fly; iii) evaluate the quality of *A. ludens* as a host of *M. digitata* and to compare its development with another totally unrelated host fly species [*Sarcophaga* (*Neobellieria*) *bullata* Parker] (Sarcophagidae) which is commercially used to rear *M. digitata* wasps (Matthews *et al*., 1996), as well as mud dauber wasps within the Crabronidae and Sphecidae (*Trypoxylon* and *Sceliphron* species, respectively, both Hymenoptera). In addition to the importance of these basic ecological studies, this information could be relevant when trying to identify new biological control agents and their potential risks to the local entomofauna (Simberloff & Stiling, 1996).

Materials and Methods

Field-survey in Veracruz, Mexico

Several sites were visited along the roads of Xalapa and the surrounding areas of north central Veracruz, Mexico. Locations visited were all north of the Cordoba-Veracruz road, east of the Perote – Xalapa – Coatepec axis, up to Papantla de Olarte and along the coast from La Guadalupe to Veracruz. Surveys were carried out in May and June, 2008. Their main purpose was to collect mud dauber wasps (*i.e*., *Sceliphron*, *Trypoxylon*) to look for unparasitized host larvae and/or pre-pupae as well as to corroborate the presence of *Melittobia* wasps in natural situations in the field. During the two months before the May-June trips, trap nests made of hollow bamboo sticks (hole sizes ranging from 5 to 10 mm diameter; 150 mm long) were also placed in various sites in Xalapa and its vicinity. The collected mud dauber wasp nests and the bamboo trap nests were transferred in the Applied Entomology Unit laboratories at Instituto de Ecología, A.C. (INECOL) (Xalapa, Veracruz, Mexico) and dissected to look for *Melittobia* wasps.

Insect collections kept at INECOL, Veracruz, Mexico

About 100 Mexican fruit fly pupae samples collected from 1997 mainly in Xalapa, but also in Los Tuxlas, Veracruz, Soconusco, Chiapas, and an unknown locality in Michoacán, and gathered over about 10 years in the INECOL laboratories were inspected for the presence of *Melittobia* spp. Mexican fruit fly specimens were stored singly in vials in alcohol (70%). The insects found in storage were originally collected from field infested *A. ludens* pupae brought to the Fruit Flies laboratory at INECOL.

Insects used in bioassays

Parasitoids

Melittobia digitata experimental cultures were established with female wasps obtained from stock cultures at INECOL from field-collected *A. ludens* pupae. The *M. digitata* culture was kept in an incubator at 24±2°C and 70% RH in total darkness. Only macropterous (long wing) females less than 5 days old and inexperienced from oviposition were used in experiments. Because of their age, and because they were taken directly from cultures before use in the bioassays, parasitoids were assumed to be mated.

Hosts

Anastrepha ludens. Mexican fruit fly pupae <24 hours old were obtained from the rearing rooms at the Applied Entomology Unit laboratories (INECOL) in Xalapa, Veracruz, (for experiments in Mexico), and the USDA-APHIS-PPQ-CPHST laboratories, in Mission, Texas (for experiments in the United States). Upon receiving the pupae, they were immediately introduced individually with a female of *M. digitata*, in a vial, to be parasitized.

Trypoxylon sp. and *Sceliphron* sp. mud dauber prepupae, obtained from field collections (in Veracruz, Mexico), were kept in a refrigerator (<12°C) to maintain the prepupae in diapause (to be eventually used as hosts). Before use in these bioassays, each cocoon was gently broken at the subtruncate cap (Cross *et al*., 1975) opening a small orifice to verify that the prepupa was healthy and not parasitized.

Sarcophaga (*Neobelleria*) *bullata*. A factitious host, *S. bullata* pupae are often used in commercial production of *M. digitata* (Matthews *et al*., 2009) and were obtained from a commercial source (Carolina Biological Supply, Burlington, NC, USA).

Host-quality bioassays

Two hundred fresh pupae of *A. ludens* (<4 day-old; weight: 0.021±0.004 g (mean±SE) were individually placed in 2-dram glass vials. Subsequently, *M. digitata* females were transferred individually from the INECOL cultures into the vials containing one *A. ludens* pupa each. *Sceliphron* sp. prepupae (n=15; weight: 0.199±0.013 g) and *Trypoxylon* sp. prepupae (n=15; weight: 0.300±0.033 g) were also placed in 2 dram vials (1 prepupa/vial) into which one mated *M. digitata* female was also introduced. Even though a larger geographical area in Veracruz, Mexico was covered in an effort to collect mud dauber wasp nests, we were only able to collect a limited number of prepupae of *Trypoxylon* sp. and *Sceliphron* sp. from the field. That is the reason why we used only 15 hosts of each of these species in this investigation. All vials were plugged with raw cotton to preclude parasitoid females from escaping and were placed inside an incubator kept at a temperature of 24±2°C and 60% RH.

Development of *M. digitata* was studied for each of the three types of hosts. Emerging parasitoids were killed by freezing (-18°C) on the day of their emergence and preserved in alcohol (70%). For each host species, the fitness traits used to evaluate host-quality for *M. digitata* were (i) relative clutch size (number of emerged wasps/host weight), (ii) developmental time (recorded daily for both macropteorus ♀♀ and ♂♂), and (iii) sex ratio (% of emerged males). The relative clutch size was considered in order to make comparisons among hosts of different weight. The adult body size was not taken into account in these experiments because in several species of *Melittobia*, including *M. digitata*, females generally tend to adjust clutch size but not body size when developing on different hosts (González & Matthews, 2002; González *et al*., 2004a,b; Matthews *et al*., 2009).

For comparative purposes, the development of the same strain of *M. digitata* was also studied in *S.* (*Neobellieria*) *bullata* and in *A. ludens* in laboratories at Texas A&M University, measuring the same fitness traits as described above. One hundred pupae of *A. ludens* (<4 days old; weight: 0.021±0.001 g) were each placed inside 2-dram glass vials with a *M. digitata* female. The same was done with pupae of *S. bullata* (n=100; <5 days old; 0.133±0.015 g). These cultures were maintained under alternating light and dark cycles of 12 h each at 25±2°C and 70% RH in the rearing chambers of the Entomology Research laboratory of the Department of Entomology at Texas A&M University.

Statistical analysis

The effects of host type on relative clutch size, developmental time, and sex ratio were compared, within each locality, with the Kruskal-Wallis ANOVA followed by the Dunn's test for post-hoc comparisons (Siegel & Castellan, 1988; Zar, 1999). All statistical analyses were processed using STATISTICA7 software (StatSoft, 2001).

Results

Field-survey in Veracruz, Mexico

Live *M. digitata* wasps were only found and retrieved from three localities: (1) Emiliano Zapata, Veracruz (N 19° 26.7' W 96° 46.9', 890 m, VI-2008) inside a *Trypoxylon* wasp nest; (2) Colorado, Santa Monica, Veracruz, Finca San Marco (N 20° 4.9' W 96° 55.8', 49 m, VI-2008) inside a cocoon of *Sceliphron* sp. that was inside a cell of a *Trypoxylon* sp. nest; and (3a) INECOL, Col. El Haya, Xalapa, Veracruz, VI, 2009, bamboo trap nest used by *Trypoxylon* sp.; (3b) INECOL, Col. El Haya, Xalapa, Veracruz, 29-VI, 2009, *Trypoxylon* sp. nest; (3c) INECOL, Col. El Haya, Xalapa, Veracruz, 29-VI, 2009, *Trypoxylon* sp. nest.

Insect collections kept at INECOL, Veracruz, Mexico

A total of 7 vials in storage were found containing a variable number of old *A. ludens* pupae and pupal remains (in some cases hosts were broken in pieces and we were unable to know exactly how many pupae were originally placed in the vials) in the Fruit Flies laboratory at INECOL. They were originally collected between January 1997 and March 2005, and together with these old pupae numerous dead specimens of *M. digitata* were also found: [vial 1 (1997): 20 ♀♀, Michoacán; vial 2 (2003): 5♂♂; >300 ♀♀, Los Tuxlas, Veracruz; vial 3 (2003): 15 ♀♀, Los Tuxtlas, Veracruz; vial 4 (2005): 25 ♀♀, Soconusco, Chiapas; vial 5 (2005): 50 ♀♀, Xalapa, Veracruz; vial 6 (2005): 2 ♂♂, 10+ ♀♀, Los Tuxlas, Veracruz; vial 7 (2005): 6 ♀♀, Xalapa, Veracruz)].

Host-quality bioassays

Mexico

Almost all the exposed hosts yielded *M. digitata* adults; from *A. ludens*, from *Trypoxylon* sp., and from *Sceliphron* sp., 99.5, 100 and 100% of the hosts produced parasitoids, respectively. In terms of relative clutch size, significantly more wasps emerged from *A. ludens* (2.37±0.06 mg of host weight) compared with *Trypoxylon* sp. (1.66±0.07 mg of host weight) and *Sceliphron* sp. (1.51±0.07 mg of host weight) [H (2, n=229)=29.28, P<0.001] (Figure 1A). The developmental time (days) of macropterous *M. digitata* females was not significantly different between hosts [H (2, n=70)=1.26, P=0.5317] (Figure 2A), and values were very similar (*A. ludens*=23.58±0.22; *Trypoxylon* sp.=23.33±0.32; *Sceliphron* sp.=23.00 ±0.49). However males required significantly more time when developing on *A. ludens* (19.50±0.13) compared with *Trypoxylon* sp. (17.40±0.16) or with *Sceliphron* sp. (17.33±0.13) [H (2, n=68)=51.86, P<0.001] (Figure 2A). A significant effect of the host species was found

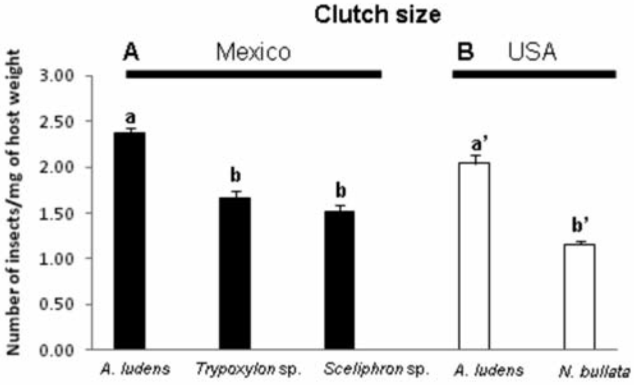

Figure 1. Values (mean±SE) of clutch size of *M. digitata* when developing on different host types at two localities. A) Mexico=black bars; B) USA=white bars. Different letters above bars indicate statistical significant differences within the same locality (Kruskal-Wallis ANOVA, Dunn's test, P<0.05).

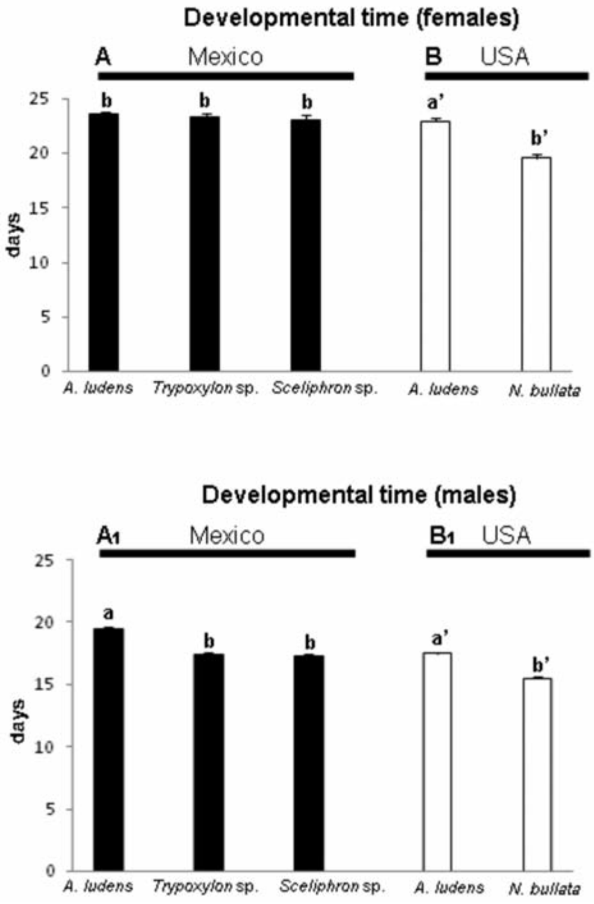

Figure 2. Values (mean±SE) of developmental time of *M. digitata* macropterous females (A) and males (B) when developing on different host types at two localities (Mexico=black bars; USA=white bars). Different letters above bars indicate statistical significant differences within the same locality (Kruskal-Wallis ANOVA, Dunn's test, P<0.05).

on sex ratio of *M. digitata* [H (2,n=229)=14.81, P<0.001] (Figure 3A). In fact, the proportion of males was higher on *A. ludens* (3.26±0.15%) compared with *Sceliphron* sp. (1.71±0.09%) whereas an intermediate value was found for *Trypoxylon* sp. (2.61±0.08%). Brachypterous females were found when *M. digitata* exploited both mud dauber wasp species but not on the Mexican fruit fly (Table 1).

USA

Melittobia digitata adults emerged from all the exposed *A. ludens* hosts whereas 84.0% of the *N. bullata* hosts yielded parasitoids. In terms of relative cutch size, significantly more *M. digitata* wasps emerged from *A. ludens* (2.04±0.09) *vs*. *N. bullata* (1.16±0.03) [H(1, n=184)=46.21, P<0.001] (Figure 1B). The developmental time (days) of macropterous *M. digitata* females was significantly higher on *A. ludens* (22.95±0.21) compared with *N. bullata* (19.55±0.31) [H (1, n=62)=36.09, P<0.001] (Figure 2B). Similarly, the developmental time of males was significantly higher on *A. ludens* (17.46±0.10) compared with *N. bullata* (15.50±0.15) [H (1, n=55)=36.61, P<0.001] (Figure 2B). Sex ratio was significantly higher when *M. digitata* developed on *A. ludens* (4.03±0.23%) compared to when it developed on *S. bullata* (2.28±0.12%) [H (1, n=184)=44.58, P<0.001] (Figure 3B). Brachypterous females were found when *M. digitata* developed on *N. bullata* but not when *A. ludens* was used as host (Table 1).

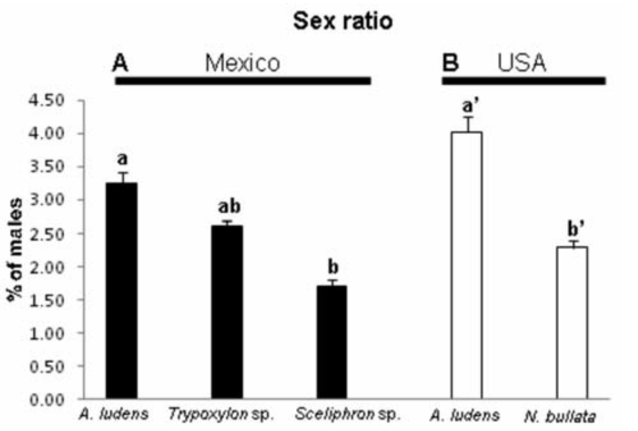

Figure 3. Values (percentage±SE) of sex ratio of *M. digitata* when developing on different host types at two localities. A) Mexico=black bars; B) USA=white bars. Different letters above bars indicate statistical significant differences within the same locality (Kruskal-Wallis ANOVA, Dunn's test, P<0.05).

Discussion

Our data confirmed that *M. digitata* is present in Mexico as a natural parasitoid of mud dauber wasps of the genus *Trypoxylon* and *Sceliphron*. Furthermore we also confirmed the natural host-parasitoid association *M. digitata* with *A. ludens* from at least 1997 (Copeland *et al*., 2008; González *et al*., 2008). Considering that pupae of the Mexican fruit fly have been found parasitized by *M. digitata* in at least three locations in Mexico (Los Tuxtlas, Veracruz; Soconusco, Chiapas; and Michoacán), it seems unlikely that parasitism could be coincidental even if such possibility cannot be completely excluded (González *et al*., 2008; 2010). It is thus interesting in trying to understand why this host-parasitoid association had been found only recently. A possible explanation could be due to the habit of fruit fly larvae of pupating in the soil at depths of ~5 cm from the surface (Hodgson *et al*., 1998), making it difficult to clearly assess the parasitoid complex associated with *A. ludens* pupae, especially when levels of parasitism are low.

Under laboratory conditions, *A. ludens* appears to be a good-quality host for *M. digitata* when comparing offspring fitness traits from different host species. In fact, our data on relative clutch size revealed that *M. digitata* produced significantly more offspring per mg of host resources when developing on *A. ludens* than on mud dauber wasps (*Trypoxylon* sp. and *Sceliphron* sp.) or *S. bullata*. Furthermore, in terms of developmental time, there are also no fitness costs for macropterous females associated with *A. ludens* compared with the other natural hosts tested in this study. However developmental time of males was significantly higher when developing on *A. ludens* and the proportion of males was significantly higher when *M. digitata* developed on *A. ludens,* compared with *Sceliphron* sp. and *S. bullata*, even though the sex ratio is still highly female biased (>95% females are produced). Our results suggest that *A. ludens* pupae are hosts of good suitability for rearing *M. digitata* but further data that take into account nutritional differences among host species could be useful in order to have a more complete view about the quality of *A. ludens* as host for *M. digitata*.

The individuals of *M. digitata* collected from Mexico were able to produce the brachypterous female morph only with some of the tested hosts, but none were produced on *A. ludens* pupae and rarely did a *Melittobia* female produce brachypterous females when *S. bullata* pupae are used as hosts. The mechanism controlling production of both female morphs in *Melittobia* wasps is poorly known, but it seems to be related with the host size (Cônsoli & Vinson, 2004; Matthews *et al*., 2009). In order to consume all the host resources of large size hosts like *Trypoxylon* sp. and *Sceliphron* sp. the ovipositing macropterous female produce brachypterous females, which do not disperse, but lay eggs on the host from which they have emerged: as a result, two distinct adult clutches develop from the same host (Matthews *et al*., 2009). In small

Table 1. Offspring (mean±SE) produced by *Melittobia digitata* from Mexico, on various hosts tested at two different localities (INECOL, Xalapa, Veracruz Mexico and Texas A&M University, College Station, Texas, USA).

Country	Host	Males	Brachypterous females	Macropterous females	Total
México	*Anastrepha ludens* n=200	1.53±0.06	0	49.25±1.27	50.78±1.30
USA	*Anastrepha ludens* n=100	1.65±0.09	0	40.3±1.78	41.95±1.86
México	*Trypoxylon* sp. n=15	12.66±0.64	5.73±0.60	481.8±19.83	500.2±20.76
México	*Sceliphron* sp. n=15	5.13±0.39	1.86±0.36	295.33±13.70	302.33±14.07
USA	*Sarcophaga (Neobellieria) bullata* n=100	3.25±0.11	0.36±0.07	151.36±4.37	154.98±4.41

hosts as *A. ludens*, the production of brachypterous females is not observed most likely because the ovipositing female can produce a clutch size big enough to consume all the host resources.

Our study suggested a need to evaluate *M. digitata* as a natural enemy of *A. ludens*. In fact *Anastrepha* species in citrus have a low parasitism rate due to the large fruit size and a scarcity of pupal parasitoids to reduce pest population density (López *et al*., 1999). Furthermore, *M. digitata* can be mass produced inexpensively in high quantities, and are already available commercially for use in teaching biological concepts (Matthews *et al*., 2009). Experiments are required especially for testing the capacity of *M. digitata* to reach and parasitize buried *A. ludens* pupae in the soil as well as to analyze host choice preferences comparing *A. ludens* to other potential hosts since non target effects could be a critical factor given the generalist habit of this parasitoid species. Taking into account that *M. digitata* uses indirect host-associated kairomones to locate mud dauber hosts (Cusumano *et al*., 2010; González *et al*., 2011) it would be interesting to test if such a group of semiochemicals also play a role when the parasitoid forages for *A. ludens* hosts.

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