Experimentalis et Applicata

The ovipositing female of *Ooencyrtus telenomicida* relies on physiological mechanisms to mediate intrinsic competition with *Trissolcus basalis*

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Abstract

Ongoing studies by our group showed that the outcome of the intrinsic competition between two solitary egg parasitoids, *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) and *Ooencyrtus telenomicida* (Vassiliev) (Hymenoptera: Encyrtidae), is dominated by *O. telenomicida*. In this article we investigated the role played by the ovipositing *O. telenomicida* female in the suppression of a *T. basalis* competitor. Laboratory experiments were conducted by allowing an *O. telenomicida* female to puncture the eggs of *Nezara viridula* (L.) (Heteroptera: Pentatomidae) with her ovipositor (= no oviposition) or to parasitize them. The results show that *O. telenomicida* relies on some physiological mechanisms to mediate its interspecific intrinsic competition with *T. basalis*. In fact, the emergence of *T. basalis* was strongly reduced in host eggs that were parasitized either before or after being punctured by *O. telenomicida* at fixed time intervals (5, 15, 30, or 45 h). The low percentage of emergence of *T. basalis* (ranging from approximately 4–20%) was a consequence of the delay and growth rate reduction of larval development. Furthermore, the percentage of eclosion of *N. viridula* nymphs was negatively affected by the *O. telenomicida* female's punctures (96% from healthy host eggs, 4% from punctured host eggs). Host eggs punctured or oviposited in by *O. telenomicida* showed alterations in the ooplasm including some melanized-like areas near the hole made with the ovipositor; such alterations indicate that the adult parasitoid releases substances that affect the host eggs survival. These results suggest that the *O. telenomicida* female influences both the physiological interspecific parasitoid-parasitoid interaction, as well as the host-parasitoid interaction, providing, for the first time in egg parasitoids, evidence that physiological suppression of some competitive egg parasitoids is mediated by the ovipositing female.

Introduction

Multiple parasitoids can coexist on a single host species (Hawkins, 1994). Coexisting species can often compete in the field especially when the host density is scarce. Competition between coexisting parasitoid species can be divided into extrinsic and intrinsic competition (Zwolfer, 1971; De Moraes et al., 1999). Extrinsic competition refers to the general interactions occurring between adults searching for hosts, whereas intrinsic competition refers to the inter-

actions occurring between larvae developing within the same host (multiparasitism sensu Mackauer, 1990). In the case of intrinsic competition, supernumerary larvae have to compete quickly to avoid losing some of the limited resources resulting in insufficient food intake for both competitors (Godfray, 1994). In western Sicily, a 3-year collection (2008–2010) of sentinel and naturally laid egg masses of the southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae), revealed that two solitary parasitoids, *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) and *Ooencyrtus telenomicida* (Vassiliev) (Hymenoptera: Encyrtidae), can exploit the same egg mass in about 25% of the total collected samples (Peri et al., 2011). These parasitoid species differ in their host location

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and larval competitive abilities and a trade-off between extrinsic and intrinsic competition may be important for species coexistence (Cusumano et al., 2011). In fact, *T. basalis* is able to exploit several chemical cues to locate *N. viridula* eggs including volatile and contact cues emitted from adult bugs and synomones induced by feeding and oviposition activities of the host (Colazza et al., 1999, 2004; Peri et al., 2006). In contrast, *O. telenomicida* eavesdrops the volatile sex pheromone which is emitted by *N. viridula* virgin males (Peri et al., 2011). However, when multiparasitism takes place, the inferior host location efficiency of *O. telenomicida* is counterbalanced by its superior larval survival. In fact, in laboratory experiments, *O. telenomicida* outcompetes *T. basalis* regardless whether ovipositions occur simultaneously or, in sequential exploitation, which species oviposits first (Cusumano et al., 2011).

The two main mechanisms involved in elimination of supernumerary parasitoids are physical attack and/or physiological suppression. The elimination of competitors by physical attack has been extensively observed in solitary parasitoids (Salt, 1961; Vinson, 1972; Chow & Mackauer, 1984, 1986; Mackauer, 1990; Quicke, 1997). In fact, their first instars are usually provided with large mandibles. On the contrary, larvae of gregarious parasitoids are generally equipped with smaller mandibles; consequently, solitary parasitoids may have an advantage when competing with gregarious ones (Laing & Corrigan, 1987; Kfir & van Hamburg, 1988) even if exceptions have also been reported (Boivin & van Baaren, 2000; Pexton & Mayhew, 2001). A similar situation often occurs when the time interval between ovipositions is such that one of the competitors has become a second instar and the other is a first instar. In fact, the falcate mandibles are lost when larvae molt into a second instar resulting in organs only adapted for feeding and, consequently, this larval instar is usually not able to injure the first instar (Chow & Mackauer, 1986; van Baaren et al., 1995). Interestingly, in the ectoparasitoid *Pachycrepoideus vindemmiae* Rondani, during intraspecific interactions, the older larvae can kill the younger ones (Goubault et al., 2003). Wounds inflicted as a consequence of fights became melanized and are readily seen on dead individuals (Fisher, 1961; Salt, 1961; Harvey & Partridge, 1987; Mackauer, 1990).

Physiological suppression between parasitoid species involves multiple mechanisms, including toxic factors, anoxia induction, and nutrient removal (Spencer, 1926; Fisher, 1963; Hagvar, 1988; Vinson & Hegazi, 1998; Uka et al., 2006; Bai et al., 2011). Often dead parasitoid eggs or larvae lacking any marks of a physical attack constitute the only available evidence of physiological suppression so that researches are forced to assume that a suppressive factor must be involved. Furthermore, first instars with large mandibles can employ other mechanisms than physical attack to eliminate rivals, such as physiological suppression (Strand, 1986; Mackauer, 1990).

In our system the mechanism adopted by *O. telenomicida* to outcompete *T. basalis* is still unexplored. However, morphological observations suggested that physical attack may not be the main outcompeting mechanism involved. In fact, the first instar of *O. telenomicida* has minute mandibles compared to the large sickle-shaped mandibles present in the first instar of *T. basalis*. Furthermore, *T. basalis* has a ring of 10 thoracic hairs that allow the larva to move in the host ooplasm 'seeking' for a competitor (Volkoff & Colazza, 1992). In contrast, the *O. telenomicida* larva is attached to the host chorion through its respiratory stalk and this mobility constraint gives it a physical disadvantage. Although it has been reported in endoparasitoids that the ovipositing female can physically injure the competitor's offspring through ovicide or infanticide (Netting & Hunter, 2000; Collier et al., 2007; Tena et al., 2008), there is a lack of studies focusing on the possible role of the ovipositing female in mediating the interspecific intrinsic competition through physiological suppression especially in oophagous insects (Mackauer, 1990). During oviposition, a wasp female could take over an already parasitized host and make it suitable for the development of her own offspring by injecting toxic substances for the competing parasitoid (Mackauer, 1990). Also, a wasp female can inject factors that influence the metabolism of their hosts preparing them for the survival of her progeny (Vinson, 1977, 1990; Stoltz & Vinson, 1979; Vinson & Iwantsch, 1980; Beckege, 1985; Dushay & Beckage, 1993). In *O. telenomicida* possible evidence of a physiological host-parasitoid interaction is suggested by the alteration of the ooplasm of *N. viridula* eggs. In fact, as earlier noted by Catalan & Verdu (2005), oviposition of *O. telenomicida* into *N. viridula* hosts is associated with a melanized-like area, visible through the host's translucent chorion, which is localized around the parasitoid egg stalk protruding from the host egg. Such ooplasm alteration does not simply result from a mechanical damage caused by the parasitoid female with the ovipositor, as it does not occur on stink bug eggs that were mechanically damaged with the tip of a tungsten probe simulating parasitoid punctures (Koppel et al., 2011). However, it is unclear whether the ooplasm alteration is due to the action of the ovipositing female, her egg, or to a substance released by the interaction between the parasitoid and the injured host egg. Therefore, investigating the role of *O. telenomicida* in mediating the competition with *T. basalis* eggs or larvae within the *N. viridula* egg appeared to be an interesting question.

The oviposition behavior of *O. telenomicida* is a complex sequence that includes drumming, drilling, and

concurrent host feeding (sensu Jervis & Kidd, 1986). During the host feeding, the female punctures the host with her ovipositor and feeds on the small droplet of ooplasm emerging from the wound repeating the drilling-host feeding sequence from 12 to 36 times before she lays the egg (A Cusumano, unpubl.). The purpose of this study was to test the ability of *O. telenomicida* females through punctures and host feeding to mediate the parasitoid-parasitoid (*T. basalis–O. telenomicida*) and host-parasitoid (*N. viridula–O. telenomicida*) interaction. In the first experiment we investigated whether or not the wasp female can affect the percentage of emergence of *T. basalis* according to the order of the parasitoid species exposures, and the time interval between exposures. Then we investigated in detail the stage at which the development of *T. basalis* is impaired by *O. telenomicida* female behavior. Finally, we investigated whether or not the melanization-like process that occurs when *N. viridula* eggs are parasitized by *O. telenomicida* is due to the action of the wasp female during the puncture and host feeding behavior.

Materials and methods

Insect rearing

The *N. viridula* colony, established from material collected from cultivated and uncultivated fields around Palermo, Italy (38°03′57″N, 13°28′10″E), was reared in wooden cages (50 × 30 × 35 cm), provided with mesh-covered holes (5·cm diameter), in a climatic chamber (24 ± 1 °C, 70 ± 5% r.h., and L16:D8 photoperiod), and fed with a diet of raw sunflower seeds, cabbages, cauliflowers, and beans. Food was changed every 2–3 days, and separate cages were used for nymphs and adults. Paper towels were hung inside each adult cage as ovipositional substrate. Egg masses were collected daily and used to maintain the colony which was also regularly augmented with bugs collected in fields around Palermo (38°03′57″ N, 13°28′10″E).

The *T. basalis* and the *O. telenomicida* colonies were originally collected from wasps emerging from sentinel and/or naturally laid *N. viridula* egg masses in tomato crops located around Palermo (37°44′23″N–13°08′27″E). Adult parasitoids were reared, separately, in 16-ml glass tubes, fed honey water solution, and kept in an incubator (L16:D8 photoperiod, 24 ± 2 °C, and 80 ± 5% r.h.). Daily collected *N. viridula* egg masses were exposed to parasitoids for 48 h, then they were removed and stored in another tube for incubation. After emergence, male and female parasitoids were kept together to allow for mating. In all experiments, female wasps of both species were mated, naïve concerning host oviposition, and 4–5 days old. This time window has been selected to use

wasps of both species at the same age and ready to lay eggs, as *T. basalis* is pro-ovigenic and *O. telenomicida* is synovigenic. About 24 h before the assays, female wasps were isolated in small vials (1.5 × 5 cm) with a drop of honey water solution, and kept in the bioassays room to acclimate.

Order of parasitoid species exposure and the time interval between exposures

The objective of the experiment was to investigate whether or not the sequence of the species exposure and the time interval between exposures play a role in the emergence (%) of *T. basalis*. A single egg of *N. viridula*, detached from a 24 h-old egg mass, was fastened onto a piece of Parafilm™ (Pechiney Plastic Packaging, Chicago, IL, USA) inside a plastic 4-cm Petri dish and exposed to both parasitoid species to obtain the following two treatments: (1) 'Ot then Tb': egg punctured by *O. telenomicida*, i.e., the female was removed after 10 drilling-host feeding bouts without egg deposition, and then parasitized by *T. basalis* at fixed intervals; (2) 'Tb then Ot': egg parasitized by *T. basalis* and subsequently punctured by *O. telenomicida* at fixed intervals. We decided to remove the *O. telenomicida* female after 10 drilling-host feeding bouts to interrupt the oviposition process just before the egg laying step (A Cusumano, unpubl.). The time intervals between exposures were 5, 15, 30, or 45 h, starting from the removal of the first female. As a control, eggs parasitized by *T. basalis* (non-punctured host eggs) were used. After the exposures, *N. viridula* eggs were stored in a climatic chamber (24 ± 2 °C and 80 ± 5% r.h.) until emergence of *T. basalis*. For each of four time intervals and for each treatment, 25 replicates were performed.

Developmental stage of *Trissolcus basalis* impaired by *Ooencyrtus telenomicida* female

The purpose of the experiment was to investigate the effects of *O. telenomicida* oviposition behavior on *T. basalis* immature development. Two eggs of *N. viridula*, detached from a 24-h-old egg mass, were fastened onto a piece of Parafilm™ inside a plastic 4-cm Petri dish and exposed to a single *T. basalis* female. After both eggs were parasitized, *T. basalis* was removed, and the eggs were exposed to a single female of *O. telenomicida*, which was removed soon after she punctured and performed 10 drilling-host feeding bouts in one of the two eggs. After the treatments, the eggs (one parasitized by *T. basalis*, and the other parasitized by *T. basalis* and punctured by *O. telenomicida*) were stored in an incubator (24 ± 2 °C, 80 ± 5% r.h.) until dissections were carried out to investigate the comparative development of *T. basalis* larvae. Eggs were dissected at 20, 45, or 70 h after *T. basalis* parasitization. These intervals are

representative of the *T. basalis* immature development from hatching in *N. viridula* eggs until the mature larva consumes the host yolk (Volkoff & Colazza, 1992). Dissections were performed with entomological needles and forceps by gently removing the operculum and flushing out all the egg content in a drop of Ringer solution. Observations were carried out under a stereomicroscope (Olympus SZX12 with a WHS15X/16 ocular and a De Plapo 1.2 XPF lense; Olympus, Tokyo, Japan) equipped with a calibrated cross-hair micrometer. For each of the development times, 10 hosts for both treatments were dissected and the distance between the tip of the cephalothoracic region and the end of the caudal region was taken as a measure of larval body length.

Host-parasitoid interactions within a host egg

The purpose of this experiment was to investigate the effects of *O. telenomicida* oviposition behavior on the development of *N. viridula* eggs. A single egg of *N. viridula*, detached from a 24-h-old egg mass, was fastened onto a piece of Parafilm™ inside a plastic 4-cm Petri dish, and exposed to an *O. telenomicida* female to obtain: a punctured host, i.e., a host in which *O. telenomicida* has performed 10 drilling-host feeding bouts (a dot of acrylic paint was put near the puncture to mark the hole), and a parasitized host, i.e., a host in which *O. telenomicida* has laid an egg. Preliminary experiments have shown that the acrylic paint did not induce any negative effect on the host development as well as on the eclosion of *N. viridula* eggs (A Cusumano, unpubl.). As control, healthy hosts were used, i.e., host eggs not exposed to *O. telenomicida*. The experiment was replicated 35× for all host types. All eggs were stored in a climatic chamber (24 ± 2 °C, 80 ± 5% r.h.). Twenty-five eggs were maintained until eclosion, 10 eggs were regularly observed to determine the presence/absence of a melanization-like area. Parasitized hosts in which such an area was found, were dissected in Ringer's physiological saline under a stereomicroscope as described in the previous experiment to document the relationship between the melanization and the developing egg of the parasitoid.

Image treatment and data analysis

Optical micrographs were taken using a Canon EOS Digital Rebel Xt (Canon, Tokyo, Japan) on an Olympus microscope to document: (1) the development of *T. basalis* in both punctured and non-punctured hosts, (2) the occurrence of melanization-like areas on host types that were punctured or parasitized by *O. telenomicida*, and (3) the relationship between melanization-like area and the developing egg of *O. telenomicida*. Emergence (%) of *T. basalis* was analyzed by χ^2 tests, whereas body lengths of *T. basalis* larvae were compared with a Mann–Whitney U-test. All data were analyzed using STATISTICA 7 software (StatSoft, 2001).

Results

Order of parasitoid species exposure and the time interval between exposures

The percentage of emergence of *T. basalis* was dependent on the puncture made by *O. telenomicida* (χ^2 = 155.40, d.f. = 8, P<0.001). In particular, parasitoid emergence was significantly different between punctured and non-punctured hosts, regardless of the order of species exposure and the time interval between exploitations (Tb then Ot, 5 h: χ^2 = 19.40, 15 h: χ^2 = 23.08, 30 h: χ^2 = 17.68, 45 h: χ^2 = 21.20; Ot then Tb, 5 h: χ^2 = 19.40, 15 h: χ^2 = 21.20, 30 h: χ^2 = 17.68, 45 h: χ^2 = 16.04; all d.f. = 1, P<0.001) (Figure 1). The percentage of emergence of *T. basalis* was not significantly different, regardless whether the puncture of *O. telenomicida* occurred before or after parasitization by *T. basalis* (χ^2 = 0.97, d.f. = 3, P = 0.81). In all bioassays it was found that, when *T. basalis* developed in host eggs punctured by *O. telenomicida*, only few *T. basalis* emerged [lowest percentage: Tb then Ot, 4 ± 3.2% (15 h between exposures); highest percentage: Ot then Tb, 20 ± 8.0% (45 h between exposures)]; most *T. basalis* adults died. On the contrary, when *T. basalis* developed in non-punctured host eggs most of the parasitoids emerged (96 ± 3.9%) (Figure 1).

Developmental stage of *Trissolcus basalis* impaired by *Ooencyrtus telenomicida* female

Larvae of *T. basalis* were found in all the dissected host eggs. Larvae obtained from dissections at 20 h had a similar body length, regardless whether they have developed in hosts punctured by *O. telenomicida* or not (Z = −0.466, P = 0.64) (Figure 2A). Larvae obtained by dissections at 45 h were significantly smaller when they developed in punctured hosts than in non-punctured hosts (45 h: Z = 3.797, P<0.001) (Figure 2A). In fact, after 45 h the body length of larvae that developed in punctured hosts was 0.30 ± 0.022 mm (mean ± SEM), whereas the body length of those larvae that developed in non-punctured hosts was 0.60 ± 0.003 mm (Figure 2B). Larvae obtained from dissections at 70 h were found to have reached different instars: larvae from non-punctured hosts had molted to the second instar (body length = 1.15 ± 0.029 mm), whereas larvae from punctured hosts were still first instar (body length = 0.37 ± 0.050 mm) (Figure 2C). In non-punctured hosts, the *T. basalis* larvae accumulated abundant yellow material in their guts. In contrast, all the *T. basalis* larvae developing in punctured hosts displayed melanization of their total body.
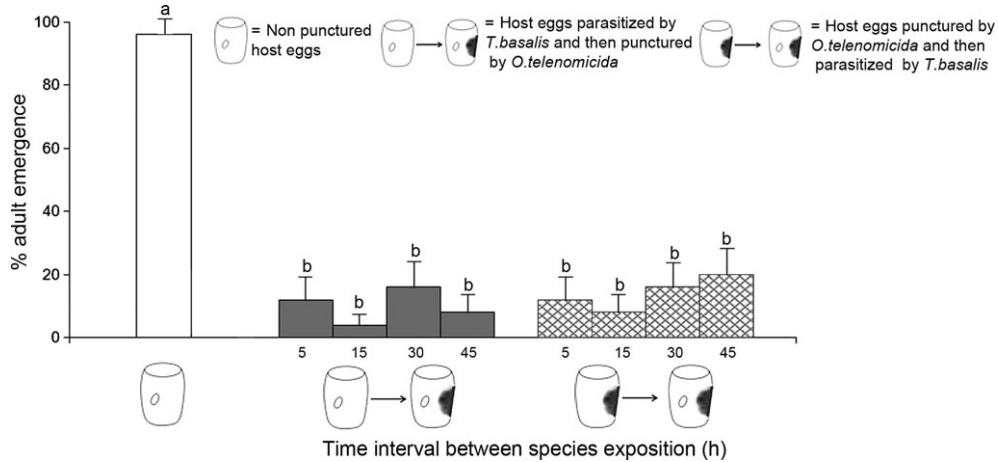
Figure 1 Mean (+ SEM) emergence rate (%) of *Trissolcus basalis* from host eggs exposed to both *T. basalis* and *Ooencyrtus telenomicida* in a different order and at different time intervals. Host eggs were first parasitized by *T. basalis* and then punctured by *O. telenomicida* (gray bars), or the other way around (cross bars). Time intervals were 5, 15, 30, or 45 h. The emergence of *T. basalis* from non-punctured host eggs is represented by the white bar. Means capped by different letters are significantly different (χ^2 test: P<0.05).
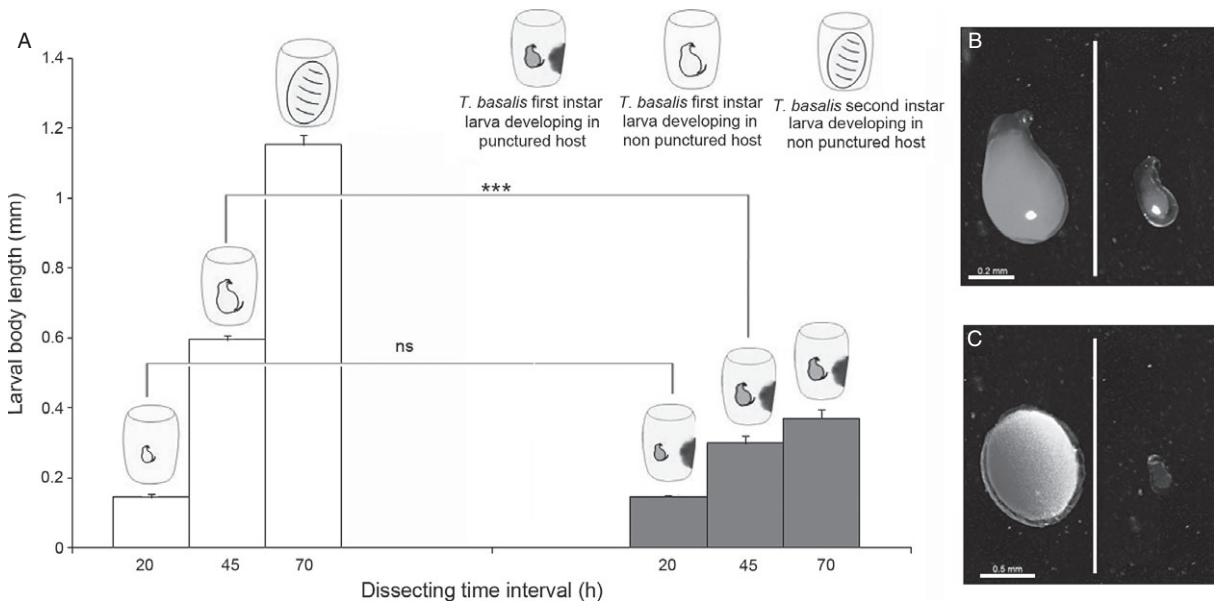

Figure 2 (A) Mean (± SEM) body length (mm) of *Trissolcus basalis* larvae obtained by dissecting at fixed times host eggs parasitized by *T. basalis* (non-punctured; white bars), or parasitized by *T. basalis* and punctured by *Ooencyrtus telenomicida* (punctured; dark bars). Eggs were dissected at 20, 45, or 70 h after *T. basalis* parasitization. Body lengths of larvae obtained at the 20 and 45 h dissection time intervals were analyzed by Mann-Whitney U test; ns, not significant, ***P<0.001. Data at 70 h were not analyzed because in punctured host eggs, first instar *T. basalis* never molted. (B) Optical micrographs of *T. basalis* larvae developing in non-punctured (left) or in punctured (right) host eggs by *O. telenomicida* female after 45 h development, and (C) after 70 h.

Host-parasitoid interactions within a host egg

The parasitoid puncture affected egg emergence of *N. viridula* nymphs, because stink bug nymphs were recorded from 96 ± 8.5% of healthy, and from 4 ± 2.0% of punctured hosts. No emergence of stink bug nymphs was obtained from parasitized hosts. Punctured hosts showed a melanized-like area around the puncture made by the adult female through the host chorion (Figure 3A). Similarly, hosts parasitized by *O. telenomicida* displayed a melanized-like area surrounding the parasitoid respiratory
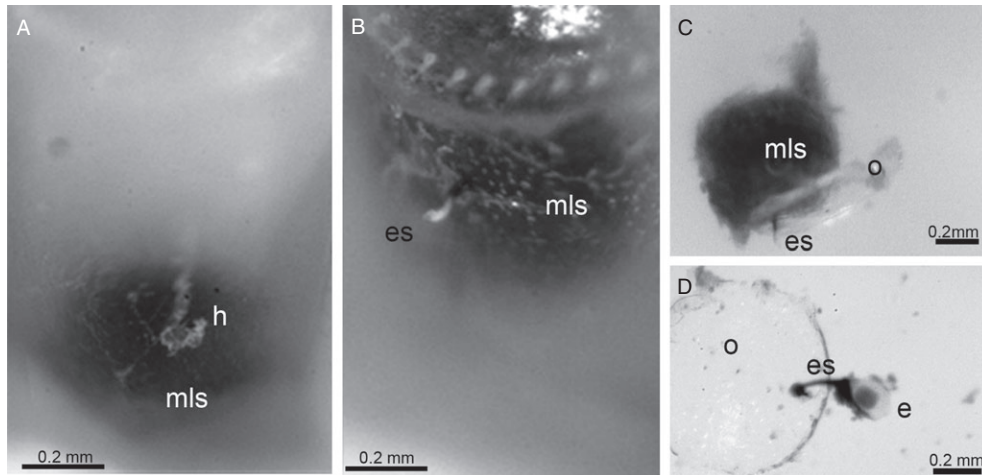
Figure 3 (A) Melanized-like area in *Nezara viridula* eggs attacked by *Ooencyrtus telenomicida* female localized around the hole in a punctured host egg, and (B) around the egg stalk in a parasitized host egg. (C) After 24 h the melanized-like substance envelops the parasitoid egg, which (D) appears translucent once the melanized-like substance is removed ad hoc. e, *O. telenomicida* egg; es, egg stalk; h, hole; mls, melanized-like substance; o, operculum.

stalk protruding from the host; part of the egg stalk closer to the chorion was also found melanized (Figure 3B). The developing egg of *O. telenomicida* was found completely immersed inside a melanized-like substance that was different in consistency and color from the host yolk (Figure 3C and D).

Discussion

The results presented in this study provide evidence that the ovipositing *O. telenomicida* relies on a physiological mechanism to mediate interspecific intrinsic competition. In fact, regardless of whether host eggs were first parasitized by *T. basalis* and then punctured by *O. telenomicida*, or vice versa, the percentage of *T. basalis* that emerges is strongly reduced. On the basis of these findings it is possible to exclude any mechanical damage leading to ovicide or infanticide that the *O. telenomicida* female may cause to its competitor while inserting several times the ovipositor into the host egg. When *O. telenomicida* punctures *N. viridula* eggs containing a *T. basalis* egg, the *T. basalis* eggs always hatch, and in few cases adults emerge. However, *T. basalis* larvae display a reduced growth rate and exhibit development delays compared to the larvae that developed in non-punctured host eggs. In fact, the results of host egg dissected 70 h after *T. basalis* parasitization revealed that non-punctured host eggs contain *T. basalis* second instars, whereas punctured hosts contain first instars. These results strongly support the possibility that competition between *T. basalis* and *O. telenomicida* is affected by factor(s) injected by *O. telenomicida* female prior to the egg

deposition, which likely makes the ooplasm unsuitable for *T. basalis* larval development. To the best of our knowledge, this is the first evidence in egg parasitoids that the ovipositing female can mediate physiological suppression. The superior intrinsic ability of *Ooencyrtus* species competing with *Trissolcus* or *Telenomus* species have been already highlighted, but in neither of these species has the mechanisms of suppression been elucidated (Laraichi, 1978; Conde & Rabinovich, 1979; Lee, 1979; Sjaarda, 1989).

The physiological suppression mechanisms that may be mediated by the ovipositing female have been shown or hypothesized in a few larval parasitoids. For example, there is the case of the intrinsic competitive interaction between aphid parasitoids of the genera *Ephedrus* and *Aphidius* (Hagvar, 1988; Mackauer, 1990). Hagvar (1988) reported that the eggs of *Aphidius matricariae* Haliday failed to hatch even when an egg of the dominant competitor, *Ephedrus cerasicola* Stary, had not yet hatched suggesting that the suppression was either due to factors injected by the ovipositing female of *E. cerasicola* or released from her egg. Further, Mackauer (1990) stated that the eggs of *Aphidius smithii* Sharma & Subba Rao were killed within 28–30 h after *Ephedrus californicus* Baker had punctured the host without ovipositing. However, the effect of the physiological suppression described for the system *Ephedrus* spp.–*Aphidius* spp. is different from that occurring in the system *Trissolcus* spp.–*Ooencyrtus* spp. Further, in the case reported herein the ovipositing female of *O. telenomicida* seems not to affect the eggs of its competitor, which hatched, but instead impacts the larvae of *T. basalis* which were small and dark. In addition, when *N. viridula* eggs

contained a first instar of *T. basalis* and were subsequently punctured by *O. telenomicida*, it was found that *T. basalis* suffered high mortality and only few adults emerged. Our results are more similar to the recent findings of Bai et al. (2011) about the intrinsic competition between *Cotesia vestalis* (Haliday) and *Oomyzus sokolowskii* Kurdjumov, two endoparasitoids of the diamondback moth, *Plutella xylostella* (L.). They showed that the sterilized female of the superior intrinsic competitor, *C. vestalis*, injects wasp-associated factors capable of affecting the development of *O. sokolowskii* larvae within the host.

The influence of the ovipositing female in mediating interspecific intrinsic competition via physiological suppression may be underestimated due to the lack of experiments specifically developed to test this phenomenon (Conde & Rabinovich, 1979; Sjaarda, 1989; De Moraes et al., 1999; De Moraes & Mescher, 2005; Mucheru et al., 2009). Nevertheless, more experimental work based on the manipulation of the oviposition behavior or based on the oviposition of sterilized female wasps is required to clarify physiological suppression in insect parasitoids. In addition to the parasitoid–parasitoid interaction, the ovipositing female of *O. telenomicida* also plays a role in the host–parasitoid interaction. In fact, the alteration of the host ooplasm displayed in *N. viridula* eggs appears to be triggered by substances probably injected by the adult females and not released by their eggs, given that host yolk modifications and the production of a melanized-like substance were observed when wasp females were interrupted prior to laying eggs. The fact that *T. basalis* mortality was similar regardless whether *O. telenomicida* punctured the host egg before or after *T. basalis* oviposition, seems to suggest that *O. telenomicida* injects factor(s) to prepare the host for their offspring development, and which also indirectly affects the interspecific competitor's larvae. The evidence that hosts punctured by an *O. telenomicida* female die further supports this hypothesis. Interestingly enough, dissections of *N. viridula* hosts parasitized by *O. telenomicida* showed that the parasitoid eggs are enveloped in a melanized-like substance. Early morphological studies of *Ooencyrtus* species attacking pentatomid or coreid hosts did not report any melanization apart from the blackening of the egg stalk which occurs few hours after the parasitoid egg has been laid (Maple, 1937; Laraichi, 1977; Matterson, 1981). Interestingly, melanization and encapsulation have been recently reported in the encyrtid *Avetianella longoi* Siscaro, an egg parasitoid of *Phoracantha recurva* Newman, but in this case the melanization process aims at killing the parasitoid egg (Reed et al., 2007).

In conclusion, our results clarify the role of the *O. telenomicida* ovipositing female in mediating the intrinsic competition with the competitor, *T. basalis*, and the host, *N. viridula*. However, it would be interesting to identify the nature of the factors that induce the melanized-like area, and their origin, as it cannot be excluded that they could be released from the salivary glands of the ovipositing female during host feeding, or other factors.

Acknowledgements

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