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**INTRAGUILD INTERACTIONS BETWEEN EGG PARASITIDS:  
FROM LABORATORY TO FIELD INVESTIGATIONS**

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DOTTORATO



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# **CHAPTER 1**

## **1. Interspecific competitive interactions in egg parasitoids**

### **Abstract**

Egg parasitoids are organisms that kill the host before the eggs hatch and thereby prevent any feeding damage, this ability make them very attractive in applied biological control programs against insect pests. Understanding how interspecific competition between parasitoids can influence pest suppression is very important for improving biological pest control. In the present chapter interspecific competitive interactions between adult parasitoids searching/ exploiting hosts (extrinsic competition) or between parasitoid larvae developing inside of the same host (intrinsic competition) have been shortly reviewed. Finally the model study used in this PhD thesis to address interspecific interaction has been described.

### **Riassunto**

I parassitoidi oofagi sono organismi in grado di uccidere l'ospite ancora prima della schiusura delle uova, di conseguenza riescono a prevenire qualsiasi tipo di danno dovuto all'attività di nutrizione del fitofago. Questa loro caratteristica li rende molto adatti per essere utilizzati nei programmi di controllo biologico. Capire come la competizione interspecifica tra parassitoidi può influenzare la mortalità del fitofago è molto importante per migliorare il controllo biologico degli insetti dannosi in agricoltura. In questo capitolo sono state descritte brevemente le interazioni competitive tra parassitoidi adulti durante la ricerca/sfruttamento degli ospiti (competizione estrinseca) e tra parassitoidi allo stadio larvale che si sviluppano all'interno dello stesso ospite (competizione intrinseca). Infine viene presentato il modello di studio utilizzato per studiare le interazioni interspecifiche in questa tesi di dottorato.

### **1.1 Introduction**

Among biological control agents, egg parasitoids are the potentially most useful ones. In fact egg parasitoid species are organisms that kill the host before the eggs hatch and thereby prevent any feeding damage (early attack), this ability make them very attractive in applied biological control programs against insect pests. Some hosts are attacked by multiple species of parasitoids, and under certain conditions, competition for control of host resources may occur. The resulting competitive interactions that occur between members of the same "guild" (i.e. Intraguild interaction), are able to

modify the reproductive success of single species and consequently the impact on phytophagus population. Understanding how competitive interaction between egg parasitoids can influence pest suppression is very important for improving biological pest control. Competition, which can occur between organisms belonging to the same species (intraspecific competition) or different species (interspecific competition), falls into two broad categories: extrinsic (among free-living adults searching for host resources) and intrinsic (among immature parasitoids developing on or inside the host). The result of extrinsic competition is affected by differences in the parasitoids' host finding and dispersal abilities, reproductive capacities and phenological synchronization with the host (Hagvar 1989; Lewis et al. 1990; Tumlinson et al. 1993; Lei and Hanski 1998). In fact during the host location process, egg parasitoids can rely on cues originated from host eggs (=direct-related cues) and/or cues from different sources associated with the host egg (=indirect related cues). By exploiting these chemical cues, egg parasitoids are able to locate a patch in which host eggs have just been laid or are soon to be laid as adult herbivores are mating. (Fatouros et al. 2008b; Colazza et al. 2010).

Instead intrinsic competition refers to interactions among immature parasitoids, which occurs when female parasitoids of different species oviposit in the same host individual (multiparasitism). Since multiparasitism occurs there are two mechanisms involved in the suppression of competitors, during intrinsic interaction: physical attack (large sickle-shaped mandibles used to fight against competitors) and physiological suppression (multiple mechanisms including toxic secretion, anoxia induction, nutritional deprivation). Furthermore there are several factors affecting the outcome of intrinsic competition. In fact many studies have shown that both the order of oviposition and the interval between ovipositions are important in mediating the interspecific intrinsic competition (Mizutani 1994; Weber et al. 1996; Agboka et al. 2002; Irvin et al. 2006; Alim and Taek Lim 2011).

When there are time lags between the first and second oviposition, the first parasitoid to have oviposited generally outcompetes later parasitoids because its offspring can start ingesting limited food resources before competitors and this may prevent other larvae to develop within the same host. But other studies have shown different results, demonstrating that one species can be superior intrinsically with respect to another species and wins the contest regardless the order of ovipositions (Subba Rao and

Chacko 1961; Lee 1979; Kfir and van Hamburg 1988; Sjaarda 1989; Weber et al. 1996; Cusumano et al. 2011, 2012).

Further studies on interspecific competitive interactions can help to better understand how competition between parasitoids affects pest population density and consequently the efficiency of biological control programs.

## **1.2 The case study: Co-occurrence of the egg parasitoid *Trissolcus basalis* and *Ooencyrtus telenomicida* on the green stink bug *Nezara viridula***

The aim of this thesis was to address on extrinsic and intrinsic competitive interaction that occur between *Trissolcus basalis* (Hymenoptera: Platygasteridae) and *Ooencyrtus telenomicida* (Hymenoptera: Encyrtidae), two egg parasitoids of, *Nezara viridula* (Heteroptera: Pentatomidae), a serious pests for a wide number of crops in laboratory conditions and field and semifield conditions.

***Trissolcus basalis*:** It is a cosmopolitan species due to its use in classical biological control programs. It is probably native from Ethiopian/Palaearctic regions (Jones, 1988) but now has been recorded in N. and S. America, Europe, Africa, Australia, New Zealand in some Pacific Islands. It can successfully develop on several other pentatomid hosts but is mainly recorded as a natural enemy of the southern green stink bug (Photo 1).



**Photo 1.** *Trissolcus basalis*

***Ooencyrtus telenomicida***: It has a Palaearctic distribution occurring from West to East regions, across Europe, Asia, and Sub Saharan Africa. This species has very high host range and in fact it has been recorded from 44 hosts belonging to the order Heteroptera, Lepidoptera and Hymenoptera as facultative hyperparasitoid of platygastriids. Among heteropteran hosts, *O. telenomicida* is more closely associated with pentatomidae and coreidae species (Photo 2).



**Photo 2.** *Ooencyrtus telenomicida*



***Nezara viridula***: It is one of the most important insect pests of agricultural crops in the world. Its high polyphagous feeding habits, combined with passive spread via international commerce, have enabled the insect to become widely established around the world (Photo 3). It presently occurs in most of the warmer regions, damaging a wide variety of fruit, nut, grain and vegetable crops (Jones, 1988)



**Photo 3.** *Nezara viridula* adult and egg mass

Stink bugs (Heteroptera: Pentatomidae) can be considered as one of the most important families in the order Heteroptera because of the wide number of bug species and the large number of crops attacked (Panizzi et al., 2000).

Surveys on stink bug natural enemies carried out in several countries, have been shown that egg parasitoids appear to be the most common factors of natural mortality of these pests, (e.g. Hokyo et al., 1966; Yeargan, 1979; Buschman and Withcomb, 1980; Clarke, 1990; Correa-Ferreira and Moscardi, 1995; Ehler, 2002; Koppel et al., 2009) with the parasitoid belonging from the family Platygasteridae being most important, particularly parasitoid of the genus *Trissolcus*. However egg parasitoid guilds are often made up also of *Ooencyrtus* spp. wasps (Hymenoptera: Encyrtidae) even if they usually parasitize a lower amount of hosts in the field and thus considered of secondary relevance.

Some egg parasitoid species have been considered and used in many countries for biological control of stink bugs, especially the cosmopolitan *T. basalis* (Caltagirone, 1981; Clarke, 1990; Correea-Ferreira, 2002). This species has a long history of research as a natural enemy and biological control agent of the southern green stink bug, *N. viridula* in several countries (Cumber, 1951; Crouzel and Saini, 1983; Clarke, 1990; Hoffmann et al., 1991; Clarke and Walter, 1995; Colazza and Bin, 1995; Ehler, 2002; Johnson et al., 2005), with satisfactory to outstanding control of the stink bug in some countries (Caltagirone, 1981; Ehler, 2002), but dubious results in others (Clarke, 1990). *Ooencyrtus* spp. have also been used or evaluated for biological control programs of southern green stink bug (SGSB) but with no success. *Ooencyrtus submetallicus* (Howard) was imported from the West Indies and released in Luisiana, Hawaii, Australia and New Zeland (Wilson, 1960; Davis and Krauss 1963; Davis, 1967; Lee, 1979) but failed to become established. Other species, including *O. malayensis* Ferriere, *O. trinidadensis* Crawford, and *O. nezarae* Ishii have been imported and evaluated in quarantine facilities of USA but they were not released due to their wide host range and lack of efficient control in their native regions (Jones et al., 1983; Jones, 1988).

It has often been suggested that biological control programs should take into account the interactions among parasitoid guild species since competitive effects among them may change the reproductive success of each parasitoid species and thus, affecting the host mortality (Nechols et al., 1992; Follett et al., 2000). Hence understanding the competitive interactions among *Trissolcus* spp. and *Ooencyrtus* spp. species may be useful in order to improve biological control of stink bug pests.

In Sicily it was found that *T. basalis* and *O. telenomicida* are often collected together from eggs of the southern green stink bug *N. viridula*.

The study of both extrinsic and intrinsic competition may also give preliminary information about species coexistence. In fact it has been suggested that coexistence may be possible if inferior larval competitors evolved superior abilities in host finding or dispersal (counterbalanced competition) (Zwolfer 1971; De Moraes et al. 1999). It seems that the sympatric *T. basalis* and *O. telenomicida* fit such ecological requirements: it has been shown that, to locate *N. viridula* egg masses, *T. basalis* uses volatile synomones induced by egg deposition, volatile and contact kairomones, while *O. telenomicida* only exploits volatile kairomones (Colazza et al. 1999, 2004; Peri et al. 2011). On the other hand, *O. telenomicida* outcompetes *T. basalis* when multiparasitism

occurs, in fact interspecific larval competition between *O. telenomicida* and *T. basalis* was dominated by the former regardless of the sequence in which oviposition occurred and if parasitoids were, or were not, simultaneously released in the patch: *O. telenomicida* wins even when *T. basalis* parasitized an egg mass up to seven days before (Cusumano et al. 2011). In these circumstances, facultative hyperparasitoids can overcome the gap between ovipositions and develop on those parasitoid species that have totally consumed the host resources.

Thus, coexistence of these parasitoid species can be driven by a host finding-larval competition trade off: *T. basalis* is more efficient in host location (extrinsic superior competitor) while *O. telenomicida* is better at larval competition (intrinsic superior competitor) (Cusumano et al. 2011). However, such information are based on laboratory investigations and further studies under field or semi-field conditions should be carried out to confirm such findings. In fact, a key limiting aspect in understanding how interspecific competition affect pest suppression is the lack of experimental data from controlled population experiments carried out in more complex environments, such as greenhouses, despite several theoretical and laboratory studies.

### **1.3 Goals of the thesis**

The study was divided into several objectives, described in the following chapter.

**In the 2<sup>nd</sup> chapter**, in order to analyze costs and benefits for *O. telenomicida* that has the ability to be both a competitor and a facultative hyperparasitoids, experiments have been conducted under laboratory conditions to determine; 1) the suitability of *N. viridula* eggs for *O. telenomicida* as a function of time since eggs had been parasitized by *T. basalis* females; 2) the potential fitness costs, by comparing life history parameters of *O. telenomicida* when it developed in unparasitized *N. viridula* eggs, under interspecific competitive conditions (eggs containing a 1st instar *T. basalis* larva) or as a facultative hyperparasitoid (where all host resources had been totally exploited by a mature *T. basalis* larva); 3) the preferences of *O. telenomicida* females when provided unparasitized *N. viridula* eggs, and host eggs previously exploited by *T. basalis* that would result in either interspecific competition or facultative hyperparasitism.

**In the 3<sup>rd</sup> chapter**, in order to evaluated intraguild interactions between *T. basalis* and *O. telenomicida* under semi-field (in out-door mesh cages) and field conditions, the following parameters has been investigated: 1) the seasonal occurrence of competing

parasitoid species on sentinel egg masses; 2) the impact achieved by competing species on the shared host on naturally laid egg masses; and 3) the outcome of intraguild interactions under controlled conditions.

**In the 4<sup>th</sup> chapter** it has been studied how the presence of synthetic Methyl salicylate can influence the intraguild competitive interaction between *T. basalis* and *O. telenomicida*. The following parameters have been evaluated: 1) the proportion of parasitized egg masses out of the total number of egg masses to assess the ability of the parasitoid to locate the host; 2) the proportion of individual eggs exploited out of the total number of eggs of the located egg masses to evaluate the ability of the parasitoid to exploit the host.

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## **CHAPTER 2**

## 2. Intraguild Interactions between Egg Parasitoids: Window of Opportunity and Fitness Costs for a Facultative Hyperparasitoid<sup>1</sup>

### Abstract

We investigated intraguild interactions between two egg parasitoids of *Nezara viridula* (L.) (Heteroptera: Pentatomidae), *Ooencyrtus telenomicida* (Vassiliev) (Hymenoptera: Encyrtidae) and *Trissolcus basalis* (Wollaston) (Hymenoptera: Platygasteridae), as the former has the potential to be a facultative hyperparasitoid of the latter. We assessed the suitability of *N. viridula* eggs for the development of *O. telenomicida* as a function of egg age when they were unparasitized, or had been attacked by *T. basalis* at different times prior to exposure to *O. telenomicida* females. *Ooencyrtus telenomicida* can exploit healthy *N. viridula* host eggs up to 5 days of age, just prior to the emergence of *N. viridula*. This window of opportunity can be extended for an additional 6–7 days through interspecific competition or facultative hyperparasitism. While there are minor fitness costs for *O. telenomicida* as the result of interspecific larval competition, those costs are greater with facultative hyperparasitism. In choice assays *O. telenomicida* females discriminated between different quality *N. viridula* eggs, avoiding those where their progeny would have to develop as facultative hyperparasitoids of *T. basalis*. Results are discussed with respect to the possible effects that the costs of intraguild parasitism might have on biological control programmes.

### Riassunto

In questo capitolo sono state studiate le interazioni competitive tra due parassitoidi oofagi di *Nezara viridula* (L.) (Heteroptera: Pentatomidae), *Ooencyrtus telenomicida* (Vassiliev) (Hymenoptera: Encyrtidae) e *Trissolcus basalis* (Wollaston) (Hymenoptera: Platygasteridae) nel caso in cui il primo parassitoide si comporta da iperparassitoide facoltativo del secondo. Nel primo esperimento è stata valutata l'ideoneità delle uova di *N. viridula* per lo sviluppo di *O. telenomicida*, in funzione dell'età delle uova di *N. viridula* ed in funzione dello stato di parassitizzazione, in tempi differenti, da *T. basalis*. Nei casi in cui *O. telenomicida* si comporta da parassitoide primario, è in grado di parassitizzare le uova di *N. viridula* fino a 5 gg di età, poco prima della schiusura

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dell'ospite. Questo “periodo di opportunità per la parassitizzazione” può essere esteso per ulteriori 6 – 7 gg nel caso in cui si instaurino meccanismi o di competizione con *T. basalis* o di iperparassitismo facoltativo. In un secondo esperimento sono stati analizzati i costi ecologici connessi allo sviluppo di *O. telenomicida* come parassitoide primario, competitore interspecifico o iperparassitoide facoltativo. I risultati hanno evidenziato che, mentre ci sono costi minori in termini di fitness per *O. telenomicida* in condizioni di competizione interspecifica larvale, questi costi sono maggiori nel caso di iperparassitismo facoltativo. Infine è stata studiata la capacità di *O. telenomicida* di discriminare uova di *N. viridula* di qualità differente. Durante questi biosaggi, è stato osservato che femmine di *O. telenomicida* discriminano tra ospiti di qualità differente, evitando di ovideporre in quelli in cui la loro progenie si svilupperebbe come iperparassitoide facoltativo di *T. basalis*, in accordo con i risultati del secondo esperimento.

## 2.1 Introduction

Intraguild interactions occur among organisms sharing a common resource (Hawkins and MacMahon 1989) and “intraguild predation” (IGP), which is common in natural populations (Arim and Marquet 2004) occurs when two species that share a common host, under certain circumstances, prey upon each other (Polis et al. 1989). Most IGP studies have focused on prey-predator interactions but recently it has been recognized that similar ecological interactions occur between host-parasitoid and host-pathogen interactions (Borer et al. 2007).

In parasitoid guilds there can be interspecific competitive interactions, either between adult parasitoids searching/exploiting hosts (extrinsic competition) or between parasitoid larvae developing within the same host (intrinsic competition) (Zwolfer 1971, De Moraes et al. 1999, Harvey et al. 2013). However, Rosenheim et al. (1995) noted that intraguild parasitism can occur when one guild member is a facultative hyperparasitoid. Such species can act either as a primary parasitoid utilising some life stage of an herbivorous insect as a host, or as a hyperparasitoid where it uses a primary parasitoid as a host. Thus a facultative hyperparasitoid can exploit a healthy host but if it oviposits in a common host that has been already attacked by another species there are two possible outcomes: interspecific larval competition will occur if the competitor's offspring has not yet consumed all of the host resources, but if it has then hyperparasitism will occur (Rosenheim et al. 1995, Cusumano et al. 2012a). The

evolution of facultative hyperparasitism is poorly understood (Brodeur 2000) but may be key to the trophic shift from primary parasitism to obligatory hyperparasitism (Godfray 1994). There are several documented cases of facultative hyperparasitism but this phenomenon is probably underestimated (Brodeur 2000) and a real understanding of parasitoid trophic structure will only be achieved by very careful examination and dissection of host remains (Shaw 1994) and through the use of molecular techniques (Gariepy and Messing 2012). For example, *Trissolcus* spp. and *Ooencyrtus* spp. are parasitoids that exploit the eggs of the same stink bugs species and the latter group can develop as facultative hyperparasitoids of the former (Amarasekare 2000; Cusumano et al. 2011). Given that egg parasitoid guilds composed of *Ooencyrtus* and *Trissolcus* spp. have been reported in North America (Buschman and Whitcomb 1980; Hoffmann et al. 1991; Shepard et al. 1994; Ehler 2002), South America (Correa-Ferreira 1986; Correa-Ferreira and Moscardi 1995), Europe (Peri et al. 2011) and Japan (Hokyo 1965) it is possible that both interspecific competition and facultative hyperparasitism occur and deserve to be investigated further. This is not only important from a purely theoretical perspective, but also with respect to using parasitoids as biological control agents of important pests. There are benefits for a parasitoid that has the ability to be a facultative hyperparasitoid, such as an extended window of opportunity when it can successfully attack its host (Craig et al. 1990; Hayward and Stone 2005), as well as gaining additional food resources (Grandgirard et al. 2002). However, there could also be associated fitness costs. It has been well documented that interspecific competition may result in longer development times, as well as smaller adults with reduced longevity and fecundity (Harvey et al. 2009a). It is also possible that similar fitness costs may be associated with facultative hyperparasitism, due to the greater conversion costs when developing on entomophagous hosts, (Grandgirard et al. 2002; van Alphen and Thunissen 1983; Boivin and Brodeur 2006). While several studies have investigated intraguild predation (Rosenheim et al. 1993; Lucaset al. 1998; Nakashima et al 2006; Raak et al. 2012; Burgio et al. 2002; Finke and Denno 2002; Denno et al. 2004) few have experimentally looked at intraguild parasitism (Rosenheim et al. 1995; Rosenheim and Harmon 2006; Harvey et al. 2011; Wang and Messing 2004a,b). We, therefore, undertook a study to investigate interspecific interactions between *Trissolcus basalis* (Wollaston) (Hymenoptera: Platygasteridae) and *Ooencyrtus telenomicida* (Vassiliev) (Hymenoptera: Encyrtidae), two idiobiont egg parasitoids of the Southern Green Stink Bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae) that co-occur in cultivated crops

grown in Sicily. These parasitoid species differ in their host location and larval competitive abilities, with *T. basalis* being more efficient in host location (Peri et al. 2011; Colazza et al. 1999; Colazza et al. 2004; Conti and Colazza, 2012) while *O. telenomicida* largely dominates interspecific larval competition regardless of the order/time interval between oviposition events. Furthermore, *O. telenomicida* has the ability to develop as a facultative hyperparasitoid [Cusumano et al. 2011; Cusumano et al. 2012b). We conducted experiments to determine: 1) the suitability of *N. viridula* eggs as a host for *O. telenomicida* as a function of time since they had been parasitized by *T. basalis* females; 2) the potential fitness costs, by comparing life history parameters of *O. telenomicida* when it developed in unparasitized *N. viridula* eggs, under interspecific competitive conditions (eggs containing a 1st instar *T. basalis* larva) or as a facultative hyperparasitoid (where all host resources had been totally exploited by a mature *T. basalis* larva); 3) the preferences of *O. telenomicida* females when provided unparasitized *N. viridula* eggs, and host eggs previously exploited by *T. basalis* that would result in either interspecific competition or facultative hyperparasitism.

## **2.2 Materials and Methods**

### 2.2.1 Insect rearing

The *Nezara viridula* colony, augmented regularly with field collected material, was maintained at  $24\pm 1^{\circ}\text{C}$ ,  $70\pm 5\%$  RH, 16 h:8 h L:D on a diet of sunflower seeds and seasonal fresh vegetables that was changed every 2–3 days. All used insects were collected in the surroundings of Palermo, Italy. No specific permits were required for collection of insects. The collection sites were not privately owned or protected in any way and field samplings did not involve endangered or protected species.

Immatures and adults were kept in separate cages. Adult cages had paper towels as an ovipositional substrate and eggs were collected daily. The *O. telenomicida* and *T. basalis* colonies were established using wasps that emerged from naturally laid *N. viridula* egg masses or sentinel egg masses placed in the field. Colonies of each species were maintained at  $24\pm 2^{\circ}\text{C}$ ,  $80\pm 5\%$  RH, 16 L:8 D in 16-ml glass tubes and fed with a solution of honey–water. To maintain the colonies, newly laid *N. viridula* egg masses were exposed to five parasitoid females for 48 h, and the resulting male

and female parasitoids were kept together to ensure mating. In all the bioassays 4–5 day old, mated females of *O. telenomicida* and *T. basalis* were used, and in all cases, parasitoids were naïve with respect to oviposition. The wasps were isolated in small vials (1.5 x 5 cm) with a drop of honey–water solution one day before bioassays and transferred to the assay room at  $24\pm 1^{\circ}\text{C}$ ,  $60\pm 10\%$  RH 1 h before being tested. Tests were conducted from 8:30 to 14:00 h and females were only used once.

### 2.2.2 Bioassays

To test the window of opportunity of parasitism for *O. telenomicida* females a series of experiments was carried out. A female *O. telenomicida* was released at the center of a vertical, cylindrical Plexiglas arena (diameter: 1.8 cm, height: 0.5 cm) with an egg mass (5 *N. viridula* eggs on a small piece of Parafilm) located centrally on the floor. There were three different treatments: (I) unparasitized 1, 2, 3, 4 or 5 day old eggs; (II) 1 day old eggs parasitized by *T. basalis* and then exposed once to *O. telenomicida* 1, 2, 3, 4, 5, 6, 7 or 8 days later; and (III) 3 day old egg masses parasitized by *T. basalis* and then exposed once to *O. telenomicida* 1, 2, 3, 4, 5, 6, 7 or 8 days later. Each assay was observed and the *O. telenomicida* female was removed after she had parasitized all of the eggs. There were 10 replicates for each time interval of all three treatments and all egg masses held at  $24\pm 1^{\circ}\text{C}$ ,  $70\pm 5\%$  RH, 16L:8D so the number of *O. telenomicida* adults emerging from each egg mass could be recorded. The few host eggs that produced *T. basalis* adults or no parasitoid at all were not included in the subsequent analyses. The possible effects of interspecific larval competition and facultative hyperparasitism on *O. telenomicida* were determined by comparing the number and sex ratio (% males) of emerging adults, as well as the developmental time, and size (estimated from the length of the hind tibia as done by Wajnberg et al. [45]) of both sexes when females were allowed to oviposit in host egg masses that were: (I) 1 day old and unparasitized (II) 2 or (III) 4 days old that had been parasitized by *T. basalis* 24 h earlier, or (IV) 10 days old that had been parasitized by *T. basalis* 7 days earlier. When *O. telenomicida* oviposited 24 h after *T. basalis*, the latter is at the stage of young 1st instar larva but when *O. telenomicida* oviposited 7 days after *T. basalis*, the mature 3<sup>rd</sup> instar larva of *T. basalis* has consumed all ooplasm and is ready to pupate. Thus treatments (II) and (III) represent natural situations of interspecific larval competition, while (IV) would be facultative hyperparasitism. Egg masses were held at  $24\pm 1^{\circ}\text{C}$ ,  $70\pm 5\%$  RH, 16L:8D and checked daily.

Adults were frozen upon emergence (-18°C) then preserved in ethanol (70%) until the different measurements were taken.

Using the same experimental setup described above a choice bioassay was conducted to determine if *O. telenomicida* would exhibit an oviposition preference when simultaneously presented with different quality hosts. An *O. telenomicida* female was introduced in the arena containing a mass of 4 *N. viridula* eggs, one each of the following treatments: (I) a 1 day old unparasitized egg; (II) a 2 day old; and (III) a 4 day old egg that had been parasitized 24 h previously by *T. basalis*; and (IV) a 10 day old egg that had been parasitized 7 days previously by *T. basalis*. The oviposition preference was assessed in terms of “first oviposition”, i.e. the first host egg that has been parasitized by *O. telenomicida* under multiple choice conditions. There were 50 replicates and each was terminated after the *O. telenomicida* female had oviposited once.

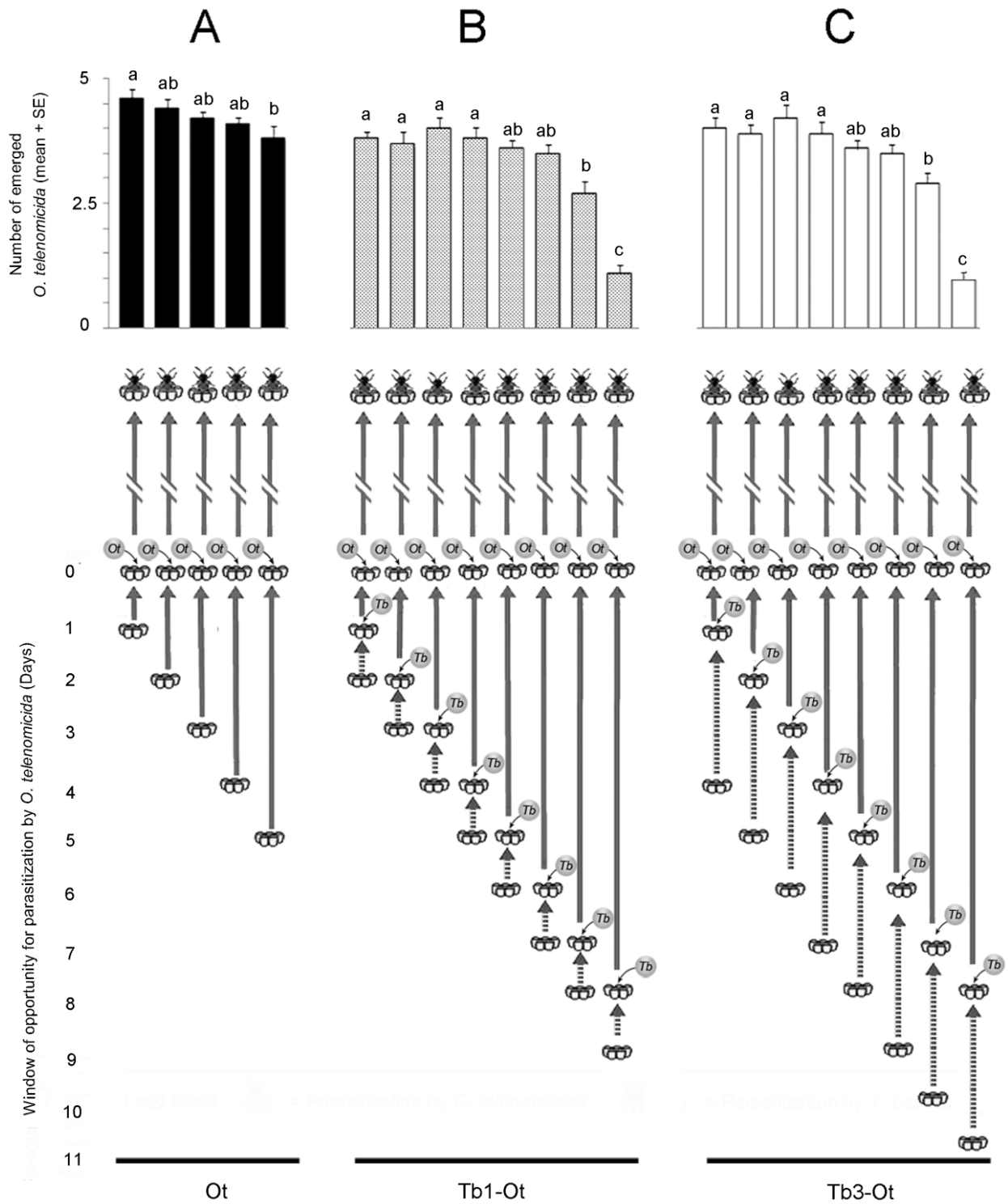
### 2.2.3 Statistical analysis

Data were tested for normality (Kolmogorov-Smirnov test) and if significantly different from a normal distribution were analyzed with non parametric tests. The effect of host age or time interval

between oviposition by the two parasitoid species on the number of *O. telenomicida* adults that emerged, as well as the effect of different host quality on developmental time and hind tibia length

were compared using a one-way ANOVA followed by Tukey test. The effect of host types on sex ratio was compared with the Kruskal-Wallis ANOVA and the Dunn test for multiple comparisons.

The ability of *O. telenomicida* females to discriminate among hosts of different quality was tested with a  $\chi^2$  test with Bonferroni correction. All statistical analyses were processed using STATISTICA7 software (StatSoft 2001).



**Figure 1. Window of opportunity for *Ooencyrtus telenomicida* as function of host egg age and interspecific parasitism status.** The emergence of *Ooencyrtus telenomicida* from (A) unparasitized 1 to 5 day old *Nezara viridula* eggs (Ot); (B) 1 day old *N. viridula* eggs parasitized by *Trissolcus basalis* that were then parasitized by *O. telenomicida* 1 to 8 days later (Tb1-Ot); and (C) 3 day old *N. viridula* eggs parasitized by *T. basalis* that were then parasitized by *O. telenomicida* 1 to 8 days later (Tb3-Ot).

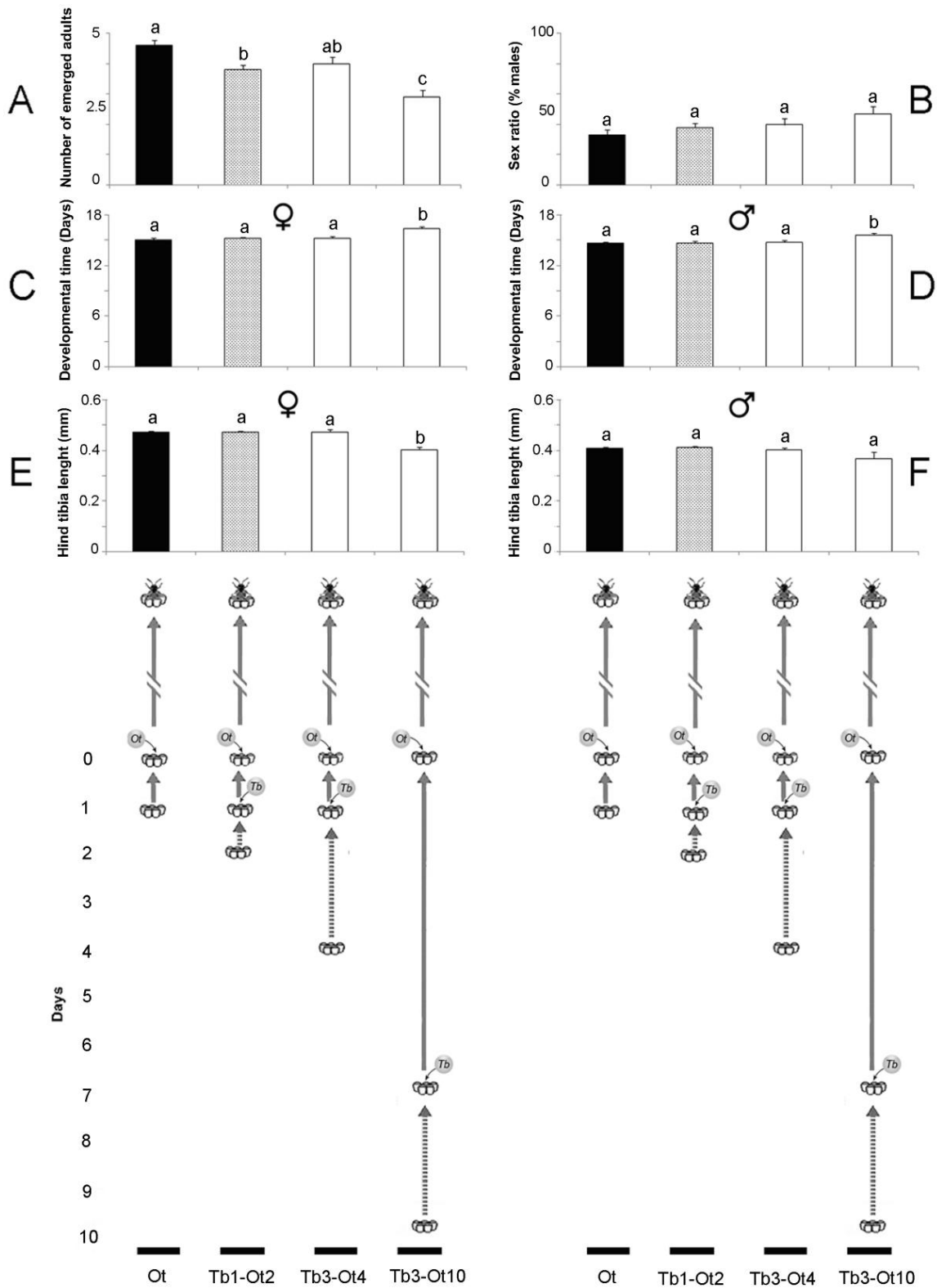


### 2.3 Results

There was a significant effect of host age on the number of adult *O. telenomicida* emerging from unparasitized *N. viridula* eggs (Fig. 1A;  $F = 3.21$ ,  $df = 4, 45$ ,  $P < 0.05$ ), being significantly lower from 5 than from 1 day old hosts. Similar temporal effects were observed when *O. telenomicida* oviposited in *N. viridula* eggs that had been attacked by *T. basalis* when the eggs were 1 day old (Fig. 1B;  $F = 20.26$ ,  $df = 7, 72$ ,  $P < 0.001$ ) or 3 days old (Fig. 1C;  $F = 23.41$ ,  $df = 7, 72$ ,  $P < 0.001$ ). In both cases there was a decrease in the number of *O. telenomicida* emerging from the oldest hosts. The average number of *O. telenomicida* adults produced was affected by the type of host exploited, (Fig. 2A;  $F = 13.84$ ,  $df = 3, 36$ ,  $P < 0.001$ ), generally being higher in previously unparasitized eggs than when in larval competition with, or as a facultative hyperparasitoid of *T. basalis*, although the proportion of males produced was similar in all treatments [Fig. 2B;  $H(3, N=37) = 4.31$ ,  $P = 0.229$ ]. When *O. telenomicida* was a facultative hyperparasitoid of *T. basalis* the developmental time of both females (Fig. 2C;  $F = 20.67$ ,  $df = 3, 36$ ,  $P < 0.001$ ) and males (Fig. 2D;  $F = 5.51$ ,  $df = 3, 33$ ,  $P < 0.001$ ) was longer. Being a facultative hyperparasitoid also resulted in smaller females (Fig. 2E;  $F = 23.69$ ,  $df = 3, 36$ ,  $P < 0.01$ ), although male size was not affected (Fig. 2F;  $F = 2.53$ ,  $df = 3, 33$ ,  $P = 0.074$ ). *Ooencyrtus telenomicida* females clearly discriminated between the different host egg types, avoiding host eggs that contained well developed *T. basalis* larvae where they would have to develop as a facultative hyperparasitoid (Table 1;  $\chi^2 = 17.68$ ,  $df = 3$ ,  $P < 0.001$ ). Interestingly, there was a marginal preference for eggs that had been attacked by *T. basalis* when they were 1 day old over unparasitized eggs ( $\chi^2 = 3.46$ ,  $df = 1$ ,  $P = 0.06$ ), or those attacked by *T. basalis* when they were 3 days old ( $\chi^2 = 2.78$ ,  $df = 1$ ,  $P = 0.09$ ).

<i>O. telenomicida</i> ovipositing in <i>N. viridula</i> egg mass assembled using 4 different egg types				
Egg types	Ot	Tb1 – Ot2	Tb3 – Ot4	Tb3 – Ot10
Egg age	1	2	4	10
Egg age when parasitized by Tb	-	1	3	3
Choice (%±SE)	24.0±6.0 a	46.0±7.1 a	26.0±6.2 a	4.0±2.8 b

**Table 1.** The proportion of *Ooencyrtus telenomicida* females selecting a (I) 1 day old, unparasitized *Nezara viridula* eggs (Ot), (II) 2 day old *N. viridula* eggs that had been parasitized by *Trissolcus basalis* when they were 1 day old (Tb1 - Ot2), (III) 4 day old *N. viridula* eggs that had been parasitized by *T. basalis* when they were 3 days old (Tb3 - Ot4), or (IV) 10 day old *N. viridula* eggs that had been parasitized by *T. basalis* 7 days earlier (Tb3 - Ot10) as their first oviposition site in a choice bioassay.



**Figure 2. Life history parameters of *Ooencyrtus telenomicida* when developing in different host types.** The number emerging (A), sex ratio (B), developmental time and size of female (C, E) and male (D, F) *Ooencyrtus telenomicida* adults developing in (I) 1 day old, unparasitized *Nezara viridula* eggs (Ot), (II) 2 day old *N. viridula* eggs that had been parasitized by *Trissolcus basalis* when they were 1 day old (Tb1 - Ot2), (III) 4 day old *N. viridula* eggs that had been parasitized by *T. basalis* when they were 3 days old (Tb3 - Ot4), or (IV) 10 day old *N. viridula* eggs that had been parasitized by *T. basalis* 7 days earlier (Tb3 - Ot10)

## 2.4 Discussion

In Sicily, more *T. basalis* adults emerge from parasitized field collected *N. viridula* eggs than *O. telenomicida*, (Amodeo personal observations), which is not particularly surprising given the superior abilities of the former to locate suitable hosts (Peri et al. 2011). Females of both *T. basalis* and *O. telenomicida* exploit volatile cues emitted by *N. viridula* virgin males and pre-ovipositing females (Peri et al. 2011; Colazza et al. 1999). In addition, *T. basalis* females use contact kairomones in host footprints and volatile oviposition-induced synomones (Peri et al. 2011; Colazza et al. 1999; Colazza et al. 2004; Colazza et al. 2009; Peri et al. 2006; Lo Giudice et al. 2011), so foraging females not only utilize more cues than *O. telenomicida*, but also ones that are more reliable indicators of the presence of host eggs. Furthermore, *T. basalis* females also have a higher total lifetime fecundity than *O. telenomicida* so the chances that *O. telenomicida* females find unparasitized egg masses may be quite low under field conditions.

However, as seen from the results of this study, *O. telenomicida* has evolved several strategies that increase the window of opportunity to exploit host eggs. For example, *N. viridula* eggs hatch after 5 days under our laboratory conditions and while *T. basalis* can only successfully develop on unparasitized *N. viridula* eggs that are 4 days old (Bin et al. 1993), *O. telenomicida* successfully exploits unparasitized *N. viridula* eggs up to the time of host emergence (Fig. 1A), similar to the congeneric, *O. nezarae* Ishii, an egg parasitoid of the bean bug *Riptortus clavatus* Thunberg (Heteroptera: Alydidae) (Takasu and Hirose, 1993). Furthermore, *O. telenomicida* is clearly superior under the conditions of interspecific larval competition, whether the eggs that have been attacked by *T. basalis* were 1 or 3 days old (Fig. 1), as in all of our experiments, 15% of all parasitoid adults were *T. basalis*. In addition, when acting as facultative hyperparasitoid (Fig. 1c), *O. telenomicida* can effectively exploit eggs for at least 10 days after they are laid by *N. viridula* females.

There are fitness costs for *O. telenomicida*, associated with both interspecific competition and facultative hyperparasitism (Fig. 2). In the case of competition the only significant effect observed was a lower number of *O. telenomicida* adults emerging when there was early-stage interspecific larval competition (Fig. 2A). Interestingly, in the choice bioassays, *O. telenomicida* showed a marginally significant preference for 2 day old eggs recently parasitized by *T. basalis*, over unparasitized ones and 4 day old eggs that *T. basalis* had attacked 1 day earlier, even though fewer adults emerged (Table

1, Fig. 2A). At oviposition, *T. basalis* injects substances that arrest embryonic development of the host and when the parasitoid's egg hatches teratocytes are released that alter the ooplasm (Volkoff and Colazza, 1992). To what extent these two events associated with the development of *T. basalis* affects the suitability of the eggs for *O. telenomicida*, when interspecific competition occurs, remains to be clarified.

In the case of facultative hyperparasitism the development time of both sexes was longer and females were significantly smaller (Fig. 2). This could be important as adult body size has been correlated with survival and reproductive success in many parasitoid species (Godfray 1994; Nicol and Mackauer 1999; Ueno 1999) although, as seen in the choice bioassays, *O. telenomicida* females will avoid hosts that result in facultative hyperparasitism if a choice is available (Table 1). If certain conditions resulted in high levels of facultative hyperparasitism this could impact on subsequent population dynamics at all trophic levels, and affect the efficacy of biological control programmes. As pointed out by Boivin and Brodeur (2006), assessing the impact of a species that act simultaneously as primary parasitoid, interspecific competitor and facultative hyperparasitoid is a huge challenge, both theoretically and experimentally. However, the few studies examining the potential fitness costs of facultative hyperparasitism have come up with quite varied findings, some showing there are fitness costs (Grandgirard 2002; Kfir and Rosen 1981), while others have found few or no effect (Perez-Lachaud et al. 2004; Harvey et al. 2009b). Therefore, it is clear that in order to understand the potential tradeoffs between the benefits accrued by a species that has the potential to be a facultative hyperparasitoid and the potential negative effects on all parasitoid species in the guild, both from basic and applied perspectives, considerably more information must be gathered from systems where interguild parasitism exists.

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## **CHAPTER 3**

### 3. Intraguild Interactions between Two Egg Parasitoids of a True Bug in Semi-Field and Field Conditions<sup>2</sup>

#### Abstract

Research on interspecific competitive interactions among insect parasitoids has often been characterized by laboratory studies in which host insects are exposed to female parasitoids of different species in various sequences and combinations. In the last years, an increasing number of studies have investigated interspecific interactions under field and semi-field conditions although just a few number of works focused on egg parasitoids. In this work, we undertook a two-year study to investigate interspecific interactions between *Trissolcus basalis* (Wollaston) (Hymenoptera: Platygasteridae) and *Ooencyrtus telenomicida* (Vassiliev) (Hymenoptera: Encyrtidae), two egg parasitoids of the pest *Nezara viridula* (L.) (Heteroptera: Pentatomidae) that co-occur in cultivated crops. Under semi-field (in out-door mesh cages) and field conditions, we investigated: 1) the seasonal occurrence of competing parasitoid species on sentinel egg masses; 2) the impact achieved by competing species on the shared host on naturally laid egg masses; 3) the outcome of intraguild interactions under controlled conditions. Results from sentinel egg masses showed that *T. basalis* occurs in May and successfully parasitizes hosts until the end of September/beginning of October, whereas *O. telenomicida* is mainly occurring in July-August. In both years, it was found that *T. basalis* is predominant. From naturally laid egg masses, results indicated that *T. basalis* achieves higher impact on the hosts, even in those egg masses which are parasitized by more than one female of different species (= multiparasitism). Results from manipulating intraguild interactions showed that *T. basalis* achieves higher impact on *N. viridula* when released alone, but it suffers from competition with *O. telenomicida*. The ecological factors that play a role in intraguild interactions in the context of biological control perspective are discussed.

#### Riassunto

Gli studi sulle interazioni interspecifiche tra insetti parassitoidi sono stati spesso caratterizzati da esperimenti di laboratorio nei quali gli insetti ospiti sono esposti alle femmine di parassitoidi in varie sequenze e combinazioni. Tuttavia, la mancanza di dati

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ottenuti in campo costituisce un aspetto limitante per comprendere come le interazioni interspecifiche tra nemici naturali possono influenzare la densità di popolazione degli insetti fitofagi. A tale scopo, sono state condotte delle ricerche di durata biennale utilizzando come sistema modello *Trissolcus basalis* (Wollaston) ed *Ooencyrtus telenomicida* (Vassiliev), due parassitoidi che coesistono naturalmente in campo su ovature della cimice verde *Nezara viridula* (L.). In dettaglio, in condizioni di campo e semi-campo si è voluto studiare: 1) la presenza stagionale delle specie di parassitoidi utilizzando la tecnica delle ovature sentinelle; 2) l'impatto che hanno le specie di parassitoidi nei confronti dell'ospite su ovature naturalmente deposte; 3) l'esito delle competizioni interspecifiche in condizioni di semi-campo. I risultati delle ovature sentinella hanno mostrato che, in entrambi gli anni, *T. basalis* è attivo in campo da Maggio a Settembre mentre *O. telenomicida* è presente principalmente nei mesi di Luglio-Agosto. I risultati delle ovature naturali hanno evidenziato un maggiore impatto sull'ospite da parte di *T. basalis* anche sulle ovature multiparassitizzate da *O. telenomicida*. Infine, i risultati di semi-campo hanno indicato che *T. basalis* è più efficiente quando rilasciato da solo, a causa dell'effetto negativo esercitato dal competitore *O. telenomicida*. Tali risultati sono discussi in una prospettiva di controllo biologico.

### **3.1 Introduction**

During the host location process, parasitoids may experience complex interactions with other parasitoids, hyperparasitoids, predators, and entomopathogens (Rosenheim et al. 1995; Rosenheim and Harmon 2006; Boivin and Brodeur 2006). There are many examples where a single insect host suffers attack from a range of parasitoid species (Hawkins 1994). Intraguild interactions between parasitoids can play an important role in species coexistence, in shaping community structures and can have important consequences for biological pest control (Godfray 1994).

When parasitoid species compete for the same host (= interspecific competitive interactions) the interactions can be divided into two broad categories: the interactions that occur among adult females searching for or exploiting hosts (=extrinsic competition) and the interactions that occur among supernumer-ary larvae developing in the same host (= intrinsic competition) (Cusumano et al. 2012; Harvey et al.2013).

In the context of biological control of phytophagous pests attacking crops, a key limiting aspect in understanding how interspecific competition affects pest suppression is the lack of experimental data from controlled experiments carried out in natural environments. Actually, due to the complexity of such investigations, research on interspecific competitive interactions among parasitoids has often been characterized by laboratory studies in which hosts are exposed to female parasitoids in various sequences and combinations [van Alebeek et al 1993; Jalali et al. 1988; De Moraes et al 1999; Agboka et al. 2002; Shi ZH et al 2004; De Moraes and Mescher 2005; Irvin et al. 2006; Harvey et al. 2009; Mucheru et al. 2009; Cingolani et al. 2013).

However, experimental laboratory conditions usually differ from field conditions, as the system is simpler, and it is therefore not easy to extrapolate results from laboratory experiments to field populations (Bogran et al. 2002) In the last years, however, an increasing number of studies investigated interspecific interactions in field and semi-field conditions although just a few number of works focused on egg parasitoids (Bogran et al. 2002; Lei and Hanski 1998; Borer et al. 2004). Such studies have shed light on the mechanisms explaining how competing parasitoid species can coexist. However, it is still unclear whether multiple biological control agents are more effective than single species in host suppression (Denoth et al. 2002; Stiling and Cornelissen 2005); nonetheless it has been recently shown that transient dynamics of host-parasitoid communities can play a major role in pest suppression (Kidd and Amarasekare 2012).

Surveys on egg parasitoid guilds of herbivorous stink bugs, that are serious pests for a wide number of crops worldwide, have shown that species belonging to the genera *Trissolcus* and *Ooencyrtus* can often naturally co-occur on the same hosts (Correa-Ferreira 1986; Hoffmann et al. 1991; Correa-Ferreira and Moscardi 1995; Amarasekare 2000; Amarasekare 2000).

In particular, on *Nezara viridula* (L.) eggs, such co-occurrence of parasitoids is widely reported in North America (Hoffmann et al. 1991; Buschman and Whitcomb 1980; Shepard et al.1994; Ehler 2002), South America (Correa-Ferreira 1986; Correa-Ferreira and Moscardi 1995), Europe (Cusumano et al. 2011) and Japan (Hokyo 1965). In many countries, in order to control stink bug populations, biological control programs based on egg parasitoids have obtained a variable degree of success (Hoffmann et al.1991; Ehler 2002; Caltagirone 1981; Clarke 1990). Thus, understanding the competitive interactions between *Trissolcus* and *Ooencyrtus* species attacking the same hosts under

natural conditions may be useful to improve biological control of such pests. Therefore, we undertook a study to investigate interspecific interactions between *Trissolcus basalis* (Wollaston) and *Ooencyrtus telenomicida* (Vassiliev), two egg parasitoids of *N. viridula* that co-occur in cultivated crops grown in Sicily, Italy. This model system has been extensively investigated under laboratory conditions and information about interspecific extrinsic and intrinsic competition, asymmetrical intraguild parasitism, as well as comparative host location strategies, is available (Cusumano et al. 2011; Colazza et al. 1999; Colazza et al. 2004; Peri et al. 2011; Cusumano et al. 2012; Cusumano et al. 2013). Actually, under laboratory conditions, it has been demonstrated that these parasitoid species differ in their host location and larval competitive abilities, with *T. basalis* being more efficient in host location (Colazza et al. 1999; Colazza et al. 2004; Peri et al. 2011) while *O. telenomicida* largely dominates interspecific larval competition regardless of the order/ time interval between ovipositions (Cusumano et al. 2011; Cusumano et al. 2012). Furthermore, *O. telenomicida* has the ability to develop as a facultative hyperparasitoid extending the window of opportunity for parasitism (Cusumano et al. 2013). This background information gained from laboratory experiments can be the basis for better understanding intraguild interactions in the field where such interactions naturally evolve and take place.

In this paper, under semi-field (in out-door mesh cages) and field conditions, we investigated: 1) the seasonal occurrence of competing parasitoid species on sentinel egg masses; 2) the impact achieved by competing species on the shared host on naturally laid egg masses; and 3) the outcome of intraguild interactions under controlled conditions.

## **3.2 Materials and Methods**

### 3.2.1 Insect rearing

The *N. viridula* colony was established from insects collected in crop areas around Palermo, South Italy. No specific permits were required for collection of insects. The collection sites were not privately owned or protected in any way and field samplings did not involve endangered or protected species. Bugs were reared in a climatic chamber ( $24\pm 1^{\circ}\text{C}$ ,  $70\pm 5\%$  r.h., and L16:D8 photoperiod), inside wooden cages (50x30x35 cm), with 5 cm diameter mesh-covered holes (200 holes/cm<sup>2</sup>). Immatures

and adults were reared in different cages and fed with a diet of seasonal vegetables, cabbages and sunflower seeds. Food was replaced every 2–3 days. Inside adults' cages, paper towels were hung as ovipositional substrate. Egg masses, that were daily collected to prevent adult cannibalism, were used to maintain the colony, to sustain parasitoids rearing and to carry out experiments.

The *T. basalis* and *O. telenomicida* colonies were established from wasps emerging from *N. viridula* egg masses found in crops located around Palermo, Italy. Adult parasitoids of each species were reared, in 85-ml glass tubes, fed with a honey-water solution, and kept in an incubator at  $24\pm 1^{\circ}\text{C}$ ,  $705\pm\%$  r.h., and L16:D8 photoperiod. Two-three times per week, 1–2-day-old egg masses of *N. viridula* were exposed to parasitoids for 48 h, and then stored for insect development under the same climatic conditions. After emergence, male and female parasitoids were kept together to allow mating. All female wasps used for the experiments were 4–5 days old, mated and naïve with respect to oviposition experience. All insect colonies were regularly refreshed with new field materials.

### 3.2.2 Seasonal occurrence under natural conditions

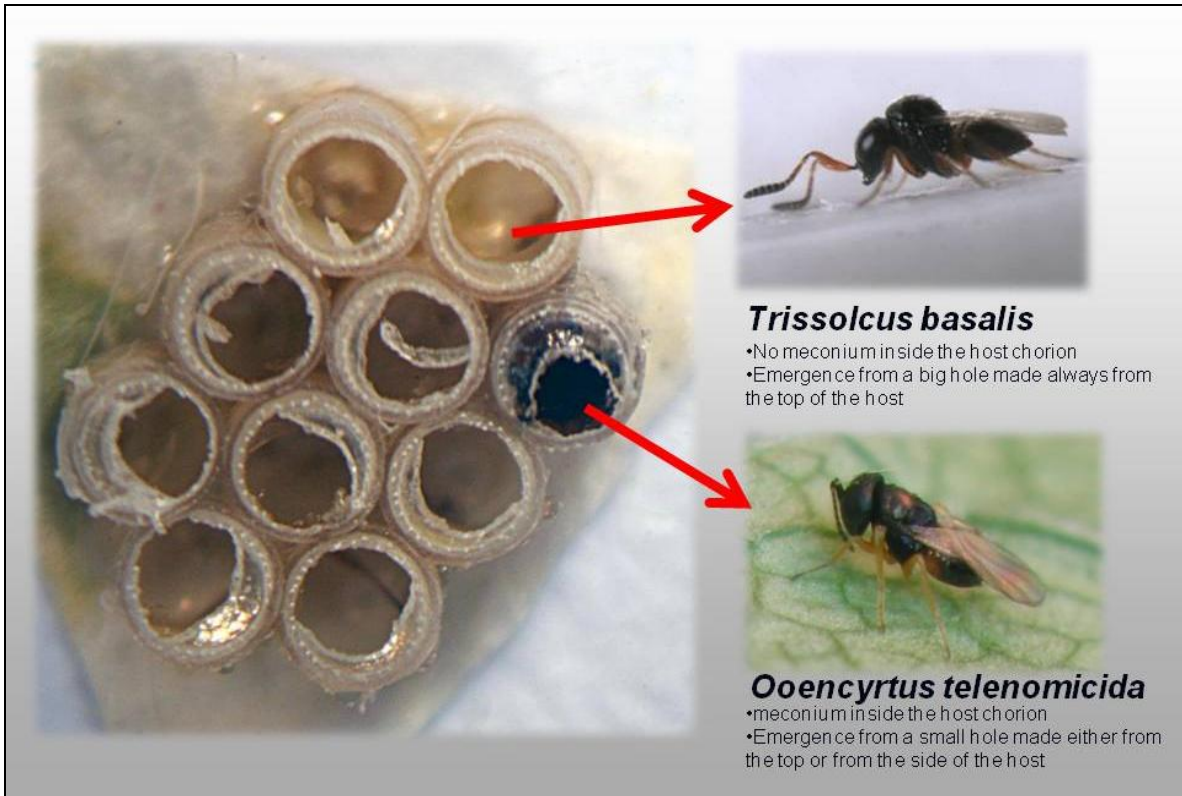
In order to monitor the occurrence of *T. basalis* and *O. telenomicida*, sentinel egg masses of *N. viridula* obtained under laboratory conditions were deployed in an experimental field located around Palermo. The research was carried out in 2011 and 2012 from May to October. An experimental field of 0.50 ha was cultivated with tomato, cultivar “Costoluto genovese”. Seedlings were transplanted every year at the beginning of April and arranged at distance of 0.8 m along the row and 1.2 m between rows. Mechanical weed removal and irrigation were carried out when needed. During the growing seasons, natural infestations of *N. viridula* and other minor pests were observed but not treated with pesticides. Using Metylan Normal wallpaper paste glue (Henkel, Dusseldorf, Germany), *N. viridula* sentinel egg masses were artificially assembled to consist of two lateral rows of three hosts each and a central row of four hosts for a total of ten 24 h-old eggs, on 2.5x2.0 cm white cardboards. Weekly, eight cardboards bearing sentinel egg masses were attached to the adaxial surface of the leaves using paperclips on randomly chosen plants that were marked with colored ribbons to better facilitate the recollection. After one week, sentinel egg masses were retrieved, placed individually in 85-ml glass tubes labeled with collection date, taken to laboratory and stored in an incubator ( $24\pm 1^{\circ}\text{C}$ , and  $70\pm 5\%$  r.h.) until the emergence of

parasitoids or the eclosion of stink bug nymphs. Investigations started on May, when no adults of *N. viridula* were yet observed in the field, and finished on October, when all sentinel egg masses were not parasitized for two consecutive weeks.

### 3.2.3 Host impact under natural conditions

In order to monitor the host impact achieved by competing parasitoid species, naturally laid host egg masses were sampled in another experimental field also located around Palermo. The research was carried out during the 2011 and 2012 growing seasons from June to September, when natural infestations of *N. viridula* occurred. Experimental tomato field was equal in terms of size, cultivar and agricultural practices to the one described above. The egg mass sampling procedure consisted of a careful visual examination of leaf surfaces of a random selection of plants for ~3 h. Egg masses discovered were individually placed in 85-ml glass tubes, labeled with collection date, taken to the laboratory and stored in an incubator ( $24 \pm 1^\circ\text{C}$ , and  $70 \pm 5\%$  r.h.) until the emergence of the parasitoids or the eclosion of stink bug nymphs. Egg masses from which stink bugs or parasitoids have already emerged were also taken into account. Indeed, previous observations had shown that host eggs that yield stink bug nymphs are clearly distinguishable from parasitized ones and that *T. basalis* and *O. telenomicida* are the only egg parasitoid species associated with *N. viridula* in Western Sicily (Peri et al. 2011) (Photo 1). Furthermore, hosts exploited by these two parasitoid species are also easily distinguishable: *T. basalis* chews a bigger hole from the top of the host egg without releasing meconium whereas *O. telenomicida* chews a smaller hole either from the top or from the side of the host egg leaving the meconium inside (Amodeo personal observations). No other parasitoid species was assumed to have emerged from empty egg masses.





**Photo 1.** Differences between eggs exploited by *T. basalis* and *O. telenomicida*

### 3.2.4 Outcome of intraguild interactions under controlled conditions

In order to evaluate the outcome of intraguild interactions between *T. basalis* and *O. telenomicida*, parasitoids were simultaneously released under semi-field conditions. The research was conducted on July 2012. Sentinel egg masses obtained under laboratory conditions were deployed in an experimental field located at the University of Palermo. The experimental field (100 m<sup>2</sup>) was prepared for seedlings by mechanically removing weeds to prevent competition for space, light and nutrients, and then cultivated with pepper plants, *Capsicum annum* var. “Quadrato d’Asti”. Seedlings were transplanted at the beginning of June and daily watered. Two weeks later, 18 cages of 150 × 150 × 70 cm made of wooden frame and mesh fabric net (200 holes/cm<sup>2</sup>) were placed on the field (Photos 2, 3). Each cage included five pepper plants, grown with the following arrangement: one plant in the center of the cage and four plants, which were equally distant 50 cm from the central one, near to the cage corners.



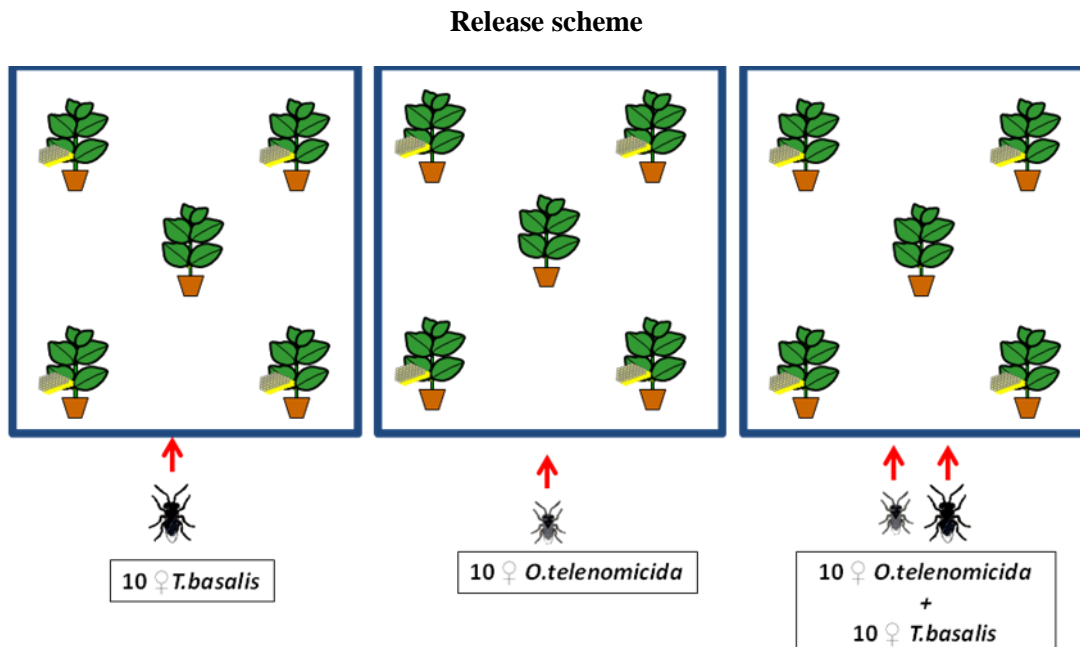
**Photo 2.** Cages for semi-field experiments under construction



**Photo 3.** Cages for semi field experiments completed



When the pepper plants reached about 45 cm of height, a 10-eggs sentinel egg mass of *N. viridula*, prepared as described above, was attached with paperclips to a medium sized leaf of each of the four lateral pepper plants. Parasitoids were then gently released on the central plant according to the following combinations: (1) single release of 10 females of *T. basalis*; (2) single release of 10 females of *O. telenomicida*; (3) simultaneous release of 10 females of both species (See: Release scheme). After a week, *N. viridula* egg masses were recollected and stored singly in 85-ml glass tubes into an incubator ( $24 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  r.h., and L16:D8 photoperiod) until the emergence of the parasitoids or the eclosion of stink bug nymphs. For each combination, six replicates were performed using a completely randomized design



### 3.2.5 Collection data

For both field and semi-field investigations, insect emergences from stored egg masses were used to compute the following efficiency indexes proposed by Bin and Vinson (1990) : 1) host location = number of egg masses from which at least one parasitoid emerged divided by the total number of sampled egg masses; 2) host exploitation = number of individually parasitized eggs divided by the total number of eggs from the located egg masses; 3) host impact = number of individually parasitized eggs divided by the total number of sampled eggs. The host location index assesses the parasitoids' searching ability whereas the host exploitation index estimates the parasitoids' reproductive capacity once an egg mass has been located. The host impact, which takes

into account all collected egg masses, either parasitized or unparasitized, assesses the overall parasitoids' efficiency as biological control agents. In order to better assess interspecific interactions, data were distinguished according to single (*T. basalis*; *O. telenomicida*) and concurrent (*T. basalis* + *O. telenomicida*) emergence of the egg parasitoid species from the same egg mass.

### 3.2.6 Statistical analysis

Host location data from naturally laid egg masses were analyzed with a contingency table  $\chi^2$  using Bonferroni correction to find significant differences. Such analysis was performed by pooling together data of 2011 and 2012 because there was no statistical difference between both years ( $\chi^2 = 0.55$ ,  $df = 3$ ,  $P = 0.907$ ). Data of host exploitation and host impact from naturally laid egg masses were analyzed, separately for each year, with a logistic regression which is a generalized linear model (GLM) designed for modeling binomial data with the logit link function. In this case, for host exploitation, the number of individually parasitized eggs and the non parasitized eggs from located egg masses were used as binomial dependent variables. For host impact, the number of individually parasitized eggs and the total number of non parasitized eggs from all egg masses were used as binomial dependent variables. In order to compare species abilities in locating and exploiting naturally laid egg masses, an additional analysis was conducted by pooling together all data from a single species regardless if parasitoids emerged singly or in egg mass also attacked by the interspecific competitor. More accurately, a logistic regression with year, species, egg mass size as factors and egg mass size  $\times$  species interaction, was performed to analyze host location whereas a GLM for Poisson distribution using the log link function was performed to analyze host exploitation data.

Data from semi-field experiments were also analyzed with a logistic regression using each egg mass as a different replicate unit. Logistic regression was followed by Tukey contrast for multiple comparisons. To assess the possible effect of interspecific interactions on the ability of parasitoids to impact the host population, the observed levels of *N. viridula* mortality in the multiple species release treatment (*T. basalis* + *O. telenomicida*) were compared to expected levels of *N. viridula* mortality calculated using data from the single species release treatments only (*T. basalis*; *O. telenomicida*). If interspecific interactions among parasitoid species have no effect on the host population (parasitoids have independent effects), the levels of host mortality should

follow a multiplicative risk model (Brogan et al 2002; Soluk and Collins 1988):

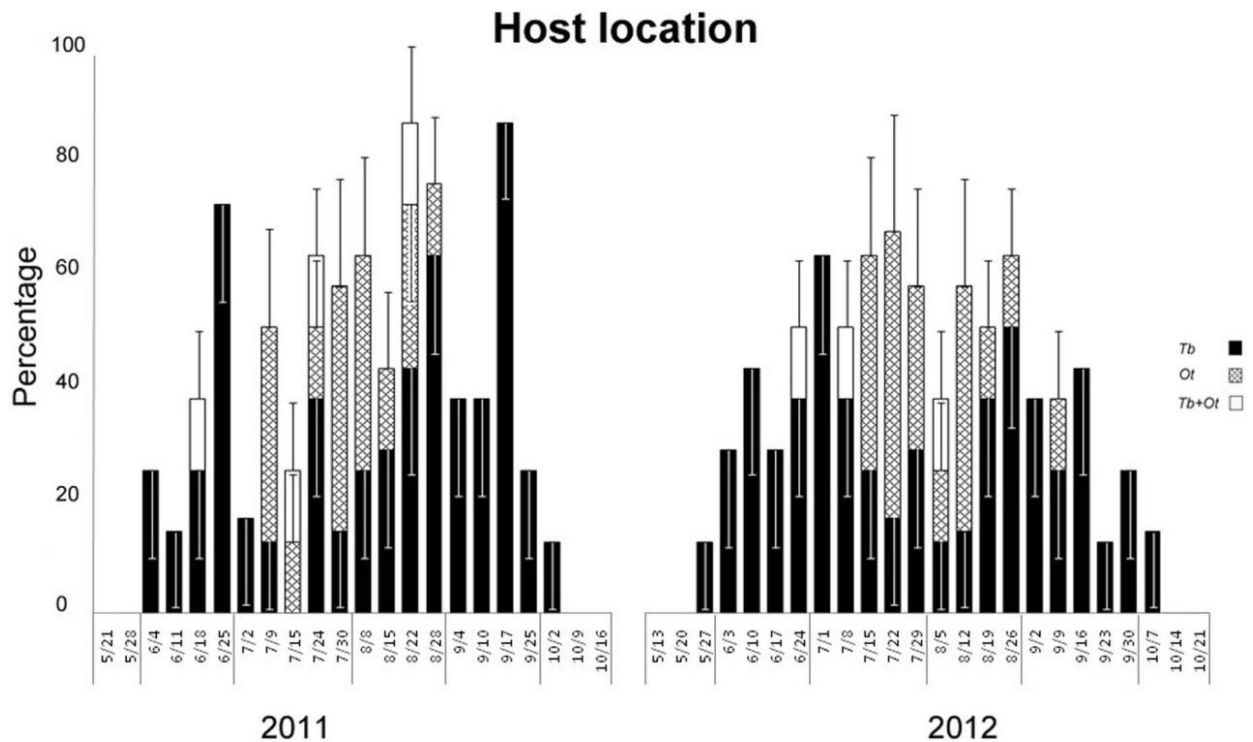
$$\hat{H}_{O_t+T_b} = (H_{O_t} + H_{T_b}) - (H_{O_t} \times H_{T_b})$$

where  $\hat{H}_{O_t+T_b}$  is the expected host mortality by parasitoids *T. basalis* and *O. telenomicida* together,  $H_{O_t}$  the observed host mortality by *O. telenomicida* alone and  $H_{T_b}$  the observed host mortality by *T. basalis* alone. The observed and expected levels of *N. viridula* mortality were compared, for each replicate, using simple  $\chi^2$  tests. Since data were independent, results of each of n replicate were combined using an Omnibus test to globally test the null hypothesis that there is no difference between observed and expected values leading to  $\chi^2$  with  $2n$  df (Fisher 1954; Koziol and Perlman 1978). Significant differences corresponding to a higher expected levels of host suppression compared to the observed levels would indicate a negative effect of interspecific competition on host population suppression. All statistical analyses were performed with the R 2.14.1 software (R Development Core Team 2011) and multiple comparisons were done with the *multcomp* package (Bretz et al.2010).

### 3.3 Results

#### 3.3.1 Seasonal occurrence under natural conditions

In 2011, successful parasitism by *T. basalis* was first recorded on sentinel eggs deployed on June 4 and this species always occurred until October 2 (fig. 1). Compared to *T. basalis*, occurrence of *O. telenomicida* alone was recorded for a shorter time during the season, from July 9 until August 28. Co-occurrence of both parasitoid species from one egg mass was also recorded in four sampling dates (June 18, July 15, July 24 and August 22). A similar seasonal pattern was reported in 2012 when *T. basalis* was again recorded before *O. telenomicida* and for a longer time throughout the season. Actually, emergence of *T. basalis* was first recorded on sentinel eggs placed in the field on May 27 and this species always occurred until October 7. Occurrence of *O. telenomicida* only was recorded for a shorter time during the season, from July 15 until September 9. Emergence of both parasitoid species from one egg mass was also recorded in three sampling dates (June 24, July 8 and August 5).



**Figure 1. Seasonal occurrence of parasitoid species (% ± SE) recorded from sentinel egg masses in 2011 and 2012 under field conditions.** Data are reported in terms of host location = number of egg masses from which at least one parasitoid emerged divided by the total number of recollected egg masses. Black, cross and white bars indicate egg masses discovered by *T. basalis* only (Tb), by *O. telenomicida* only (Ot) and by both species (Tb+Ot), respectively.

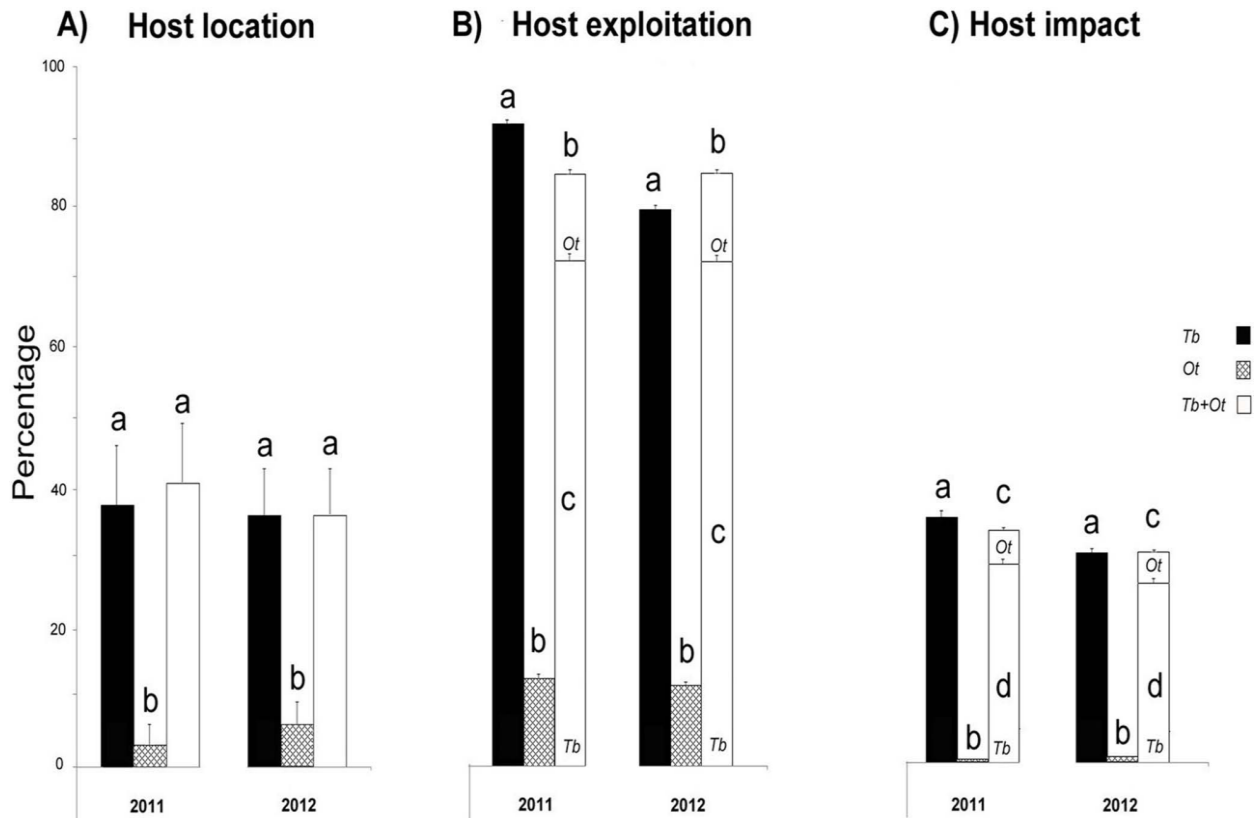
### 3.3.2 Host impact of parasitoid species under natural conditions

In both 2011 and 2012, a total of 32 and 50 egg masses naturally laid by *N. viridula* were collected, respectively. The proportion of egg masses that were unparasitized or located by parasitoid species was not significantly different between 2011 and 2012 ( $\chi^2 = 0.55$ ,  $df = 1$ ,  $P = 0.907$ ) but host location efficiency was affected by parasitoid emergence combination (*T. basalis*, *O. telenomicida*, *T. basalis* + *O. telenomicida*). More accurately, host location was not significantly different between *T. basalis* versus *T. basalis* + *O. telenomicida* ( $\chi^2 = 0.03$ ,  $df = 1$ ,  $P > 0.05$ ) but it was significantly different between *T. basalis* versus *O. telenomicida* ( $\chi^2 = 25.08$ ,  $df = 1$ ,  $P < 0.001$ ) as well as between *O. telenomicida* versus *T. basalis* + *O. telenomicida* ( $\chi^2 = 26.48$ ,  $df = 1$ ,  $P < 0.001$ ) (fig. 2A).

In both 2011 and 2012, a total of 2272 and 3480 individual eggs were exploited by parasitoids from the located egg masses, respectively. The proportion of individual host eggs that were exploited by parasitoid species was significantly affected by the

emergence combinations (GLM:  $\chi^2 = 3943.4$ ,  $df = 7$ ,  $P < 0.001$ ) (fig. 2B). Regardless of the year, host exploitation efficiency by *T. basalis* was reduced when this species exploited eggs in single versus concurrent exploitation whereas the host exploitation efficiency by *O. telenomicida* was not significantly affected (Tukey test,  $P < 0.05$ ). In both 2011 and 2012, a total of 2810 and 4371 individual eggs were sampled, respectively and the host impact index was significantly affected by the emergence combinations (GLM:  $\chi^2 = 4725.9$ ,  $df = 7$ ,  $P < 0.001$ ) (fig. 2C). Regardless of the year, the impact achieved by *T. basalis* and by *O. telenomicida* was significantly different when species exploited eggs in single versus concurrent exploitative conditions (Tukey test,  $P < 0.05$ ).

On naturally laid egg masses, a significant effect of the species (GLM:  $\chi^2 = 17.36$ ,  $df = 1$ ,  $P < 0.001$ ) was found on the host location efficiency, whereas the effect of the year (GLM:  $\chi^2 = 0.25$ ,  $df = 1$ ,  $P = 0.617$ ), of the eggs mass size (GLM:  $\chi^2 = 0.00$ ,  $df = 1$ ,  $P = 1.000$ ) and of the species  $\times$  eggs mass size interaction (GLM:  $\chi^2 = 0.30$ ,  $df = 1$ ,  $P = 0.584$ ) were all not significant. However, a significant effect of the species (GLM:  $\chi^2 = 1936.92$ ,  $df = 1$ ,  $P < 0.001$ ), of the egg mass size (GLM:  $\chi^2 = 288.21$ ,  $df = 1$ ,  $P < 0.001$ ) and of species  $\times$  egg mass size interaction (GLM:  $\chi^2 = 65.95$ ,  $df = 1$ ,  $P < 0.001$ ) was found on host exploitation.



**Figure 2. Parasitism data (% + SE) recorded from naturally laid egg masses in both 2011 and 2012 under field conditions.** All indexes are distinguished according to single or concurrent emergence of the egg parasitoid species from the same egg mass. (A) *host location* = number of egg masses from which at least one parasitoid emerged divided by the total number of egg masses. (B) *host exploitation* = number of individually parasitized eggs divided by the total number of eggs from the egg masses located by the wasps and (C) *host impact* = number of individually parasitized eggs divided by the total number of sampled eggs. In (B) and (C) black, cross and white bars indicate the proportion of individual eggs exploited from egg masses located by *T. basalis* only (Tb), by *O. telenomicida* only (Ot) and by both species (Tb+Ot), respectively. Different letters indicate significantly different percentages within the same year and index (C) (GLM,  $P < 0.05$ ).

### 3.3.3 Outcome of intraguild interactions under controlled conditions

The host location index of parasitoid species was significantly affected by the release and emergence combinations (GLM:  $\chi^2=25.22$ ,  $df=4$ ,  $P < 0.001$ ) (fig. 3A). The host location index of *T. basalis* only was significantly different in single versus simultaneous release whereas the host location index of *O. telenomicida* was not affected (Tukey test,  $P < 0.05$ ).

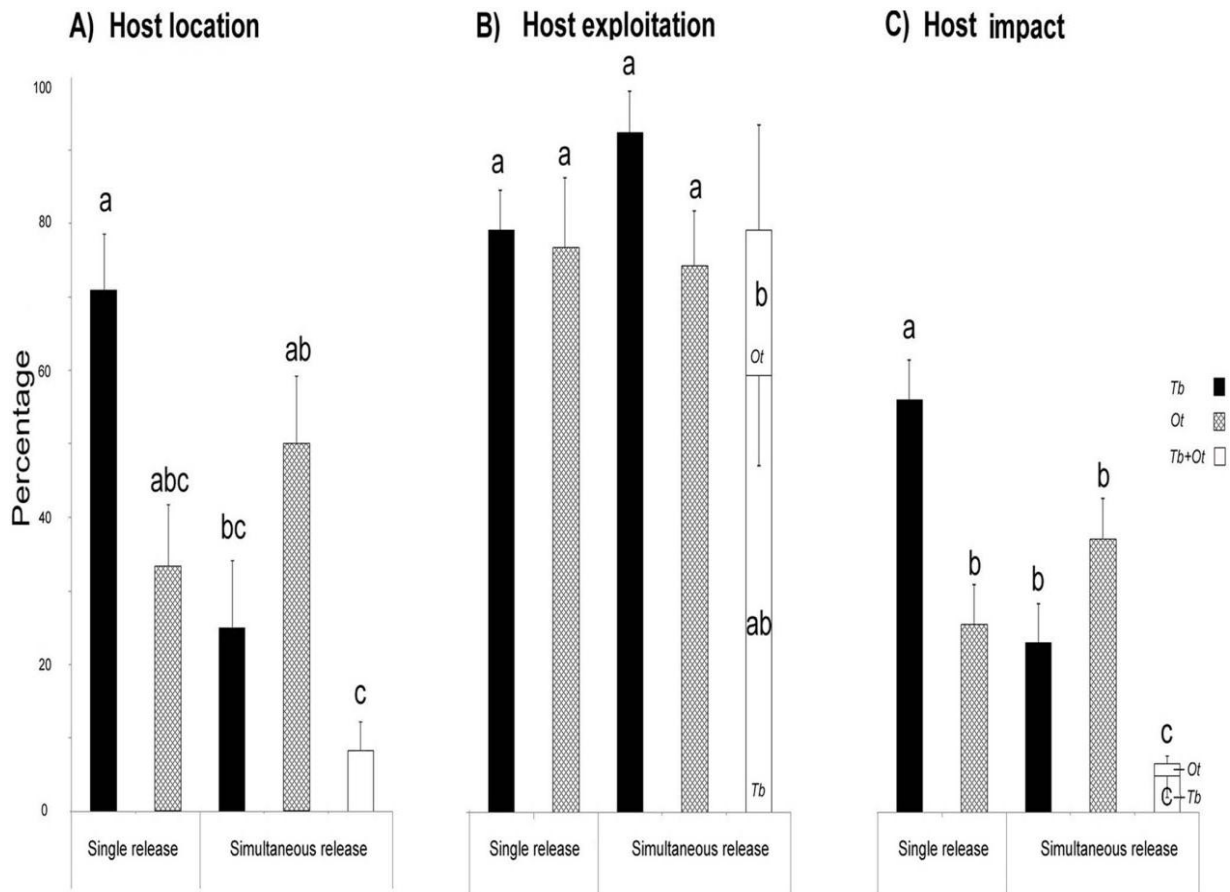
The proportion of individual host eggs that were exploited by parasitoid species was also significantly affected by the release and emergence combinations (GLM:  $\chi^2=22.37$ ,  $df=5$ ,  $P < 0.001$ ) (fig. 3B). Host exploitation efficiency of *T. basalis* was not significantly



different when this species exploited eggs in different release and emergence combinations (single release, single emergence in simultaneous release and concurrent exploitation), whereas the host exploitation efficiency of *O. telenomicida* decreased in condition of concurrent exploitation compared with single release or single emergence in simultaneous release (Tukey test,  $P < 0.05$ ).

Similarly to host location and exploitation, the host impact index was also significantly affected by the release and exploitation combinations (GLM:  $\chi^2 = 151.28$ ,  $df = 5$ ,  $P < 0.001$ ) (fig. 3C). The impact of *T. basalis* was significantly higher for single release, intermediate for single emergence in simultaneous release and very low in conditions of concurrent exploitation (Tukey test,  $P < 0.05$ ), whereas the impact of *O. telenomicida* was not statistically different between conditions of single release versus single exploitation in simultaneous release but it was much lower for concurrent exploitation in simultaneous release (Tukey test,  $P < 0.05$ ).

Regardless of the fact that data were compared separately for each replicate or combined together, no significant differences were found between observed and expected levels of *N. viridula* mortality inflicted by parasitoids in the two species combination ( $\hat{H}_{O+Tb} = 65.21\%$ ;  $H_{O+Tb} = 67.50\%$ ) ( $\chi^2 = 2.21$ ,  $df = 12$ ,  $P > 0.05$ ).



**Figure 3. Parasitism data (% + SE) obtained when species were released singly or simultaneously under semi-field conditions.** All indexes are distinguished according to single or concurrent emergence of the egg parasitoid species from the same egg mass. (A) *host location* = number of egg masses from which at least one parasitoid emerged divided by the total number of egg masses. (B) *host exploitation* = number of individually parasitized eggs divided by the total number of eggs from the located egg mass and (C) *host impact* = number of individually parasitized eggs divided by the total number of sampled eggs. In (B) and (C) black, cross and white bars indicate the proportion of individual eggs exploited by parasitoids from egg mass located by *T. basalis* only (Tb), by *O. telenomicida* only (Ot) and by both species (Tb+Ot), respectively. Different letters indicate significantly different percentages within the same index (GLM,  $P < 0.05$ )

### 3.4 Discussion

Egg parasitoids are organisms of particular interest for biological control due to their ability to kill insect pests before the crop-feeding stage (Wajnberg and Hassan 1994). In the perspective of biological control of stink bugs, an efficient egg parasitoid species must be characterized not only by a high host location index, i.e. a high ability to discover host egg masses, but also by a high exploitation efficiency in order to parasitize all the available eggs within an egg mass (Bin and Vinson 1990). Parasitoid species characterized by high host location and host exploitation abilities have consequently a high host impact index and are most likely interesting for biological control programs (Colazza and Bin 1995). However, such indexes can be affected by intraguild interactions due to the interference on reproductive success of each species (Nechols et al. 1992; Follett et al 2000). In the field, competitive interactions among parasitoids determine natural community structure and dynamics in the plant–herbivore–parasitoid systems, for example, by causing local displacement of inferior species or niche separation, and can play a relevant role in modifying the efficacy of parasitoids in biological control programs (Meiners and Peri 2013). In our system, although *T. basalis* and *O. telenomicida* compete for *N. viridula* egg mass, they coexist under field conditions, likely by adopting different strategies to exploit the shared resource.

The results from semi-field and field investigations, coupled with previous findings obtained under laboratory conditions aimed at better understanding intraguild interactions between egg parasitoids of a true bug. In particular, semi-field experiments investigated interspecific interactions under manipulative conditions of simultaneous species release in order to obtain high competition for hosts. Our results from sentinel egg masses placed in the field indicated a different pattern of occurrence of competing parasitoid species. In both years, *T. basalis* occurred in late May-early June and it was found parasitizing host eggs until late September-early October whereas *O. telenomicida* occurred mainly in July-August. Interestingly, similar temporal patterns of occurrence of stink bug egg parasitoid species have been found in other case studies confirming that *Ooencyrtus* species are more abundant later in the season whereas competing *Trissolcus* and *Gryon* species are commonly found earlier (Amarasekare 2000, Mainali and Lim 2012). The effects of abiotic conditions on the timing of occurrence of egg parasitoid guilds have already been reported, suggesting a role played by species' differences in cold tolerance and/or humidity requirements: for example,

*Trissolcus brochymenae* (= *murgantiae*) Ashmead is more cold-tolerant than its competitor *Ooencyrtus johnsonii* (Howard) (Sjaarda 1989) whereas *Gryon japonicum* (Ashmead) is likely to perform better with lower humidity than its competitor *Ooencyrtus nezarae* Ishii (Mainali and Lim 2012). The effects of weather conditions, especially cold tolerance, on the timing of occurrence of competing parasitoid species likely played a role also in our study system but further investigations are required in order to test whether *T. basalis* is able to parasitize host eggs under colder temperature conditions compared to *O. telenomicida*. In addition, these parasitoid species may have different overwintering requirements: it is possible that *T. basalis* completes overwintering period before *O. telenomicida* and, consequently, the former can occur earlier in the field. From a biological control perspective, the demonstration that the two species are active at different times of the season might suggest that the combination of the two parasitoids is likely to outcompete the level of control generated by each species alone. Such hypothesis could be supported by the results from the multiplicative risk model which indicated that host mortality when the two parasitoids are released together is not significant different than that one expected under independent action of the two species. However, short- and long-term population investigations taking into account host-parasitoid dynamics are required to confirm the results presented in this study.

Results obtained from naturally laid egg masses highlight a higher host location index and, thus, a superior host finding ability of *T. basalis* compared to *O. telenomicida*. These findings confirm previous laboratory data with Y-tube and open area bioassays. Indeed, *T. basalis* is known to exploit volatile oviposition-induced synomones, volatile cues from virgin males and preovipositing females, and contact kairomones in the host footprints (Colazza et al 1999; Colazza et al. 2004; Peri et al. 2006; Salerno et al. 2006; Peri et al. 2013), while *O. telenomicida* uses mainly volatile cues produced by host virgin males (Peri et al. 2011). Interestingly, *T. basalis* apparently exploits not only more cues than *O. telenomicida* but also the more reliable ones, i.e., cues that are highly associated with the host presence such as synomones-induced volatiles. Once an egg mass has been located, *T. basalis* is able to exploit a high proportion of the available host eggs whereas *O. telenomicida* is much less efficient and achieves similar efficiency regardless of single and concurrent exploitation. Such higher level of host exploitation by *T. basalis* has also been documented (Hoffmann et al. 1991; Ehler 2002; Colazza and Bin 1995; Jones et al. 1996). The main factors that are likely to affect species differences in host exploitation are reproductive abilities, such as egg load and total

lifetime fecundity, which are known to be higher in *T. basalis* compared to *O. telenomicida* (average egg loads: *T. basalis* = 76.2 eggs; *O. telenomicida* = 24.2 eggs, Cusumano, unpublished data). Such differences in terms of host exploitation are not evident in our semi-field investigations, where both species demonstrated similar performance in condition of single or simultaneous release, probably because both parasitoid species have a sufficient egg load for exploiting small-sized host egg masses. In these experiments, egg masses artificially assembled with 10 eggs only have been used to enhance interspecific competitive interactions, like in previous laboratory experiments (Cusumano et al. 2011; Cusumano et al. 2013). Therefore, under natural conditions, *T. basalis*, which is characterized by superior host location and host exploitation abilities than *O. telenomicida*, achieved a higher impact on *N. viridula* egg masses. As a consequence, chances that *O. telenomicida* females find unparasitized egg masses could remain quite low.

It has to be noted that the high proportion of egg masses located by both parasitoid species in the field shows that *O. telenomicida* has most likely evolved some strategies to overcome its inferior abilities to locate hosts. Actually, by being superior under the conditions of interspecific larval competition, and by being able to act as facultative hyperparasitoid, *O. telenomicida* can extend its window of opportunity to exploit host eggs for an additional 6–7 days. Indeed, *O. telenomicida* can successfully develop on host eggs laid by *N. viridula* females up to 10 days before, while *T. basalis* can only successfully develop on unparasitized host eggs that are 4 days old (Cusumano et al. 2013). Hence, the coexistence between these two species represents an example of counterbalanced competition (Zwolfer 1971). Counterbalanced competition suggests that coexistence among parasitoids attacking the same host can be possible when one species, in our case *O. telenomicida*, dominates the intrinsic competition, while the other, in our case *T. basalis*, fills the gap at the extrinsic level by having higher host finding or dispersal efficacies. The advantage of *O. telenomicida* over *T. basalis* under the conditions of interspecific larval competition is clearly showed by our results obtained in semi-field experiments. Under such conditions, *O. telenomicida* performs similarly regardless if it was released alone or in combination. On the contrary, *T. basalis* achieves higher host location efficiency and host impact when released alone, while it suffers from *O. telenomicida* competition when the two species are released in combination. Moreover, the proportion of egg masses located by both species under semi-field conditions is lower than in natural fields. Results from semi-field are in

apparent contrast with those obtained under natural conditions. However, this can be explained taking into account the small size of host egg masses used in the semi-field experiments. Under such conditions, competition for hosts is particularly severe and *T. basalis* can be excluded by *O. telenomicida*, due to the very low reproductive success of the former species when ovipositing into host eggs that are also attacked by its competitor, as was showed previously under laboratory conditions (Cusumano et al.2011; Cusumano et al. 2013). Consequently, it is possible that *T. basalis* has oviposited into multiparasitized host eggs yielding *O. telenomicida* offspring and, as a result, the host location index of the former species could have been reduced. Competitive exclusion of *Trissolcus* species by *Ooencyrtus* species under laboratory conditions has also been.

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## **CHAPTER 4**

#### **4. Field evaluation of Methyl salicylate efficacy for behavioral manipulation of egg parasitoids**

##### **Abstract**

In this chapter it has been studied how the presence of synthetic Methyl salicylate (MeSA) can affect the intraguild interactions between *Trissolcus basalis* (Wollaston) and *Ooencyrtus telenomicida* (Vassiliev). Field experiments have been conducted using sentinel egg masses placed randomly on pepper plants. The following parameters have been evaluated: 1) number of egg masses from which at least one parasitoid emerged divided by the total number of sentinel egg masses, to assess the ability of the parasitoid to locate the host (host location); 2) number of individually parasitized eggs divided by the total number of eggs from the located egg masses, to evaluate the ability of the parasitoid to exploit the host (host exploitation). The results demonstrated that *T. basalis* has a high ability to find hosts compared to *O. telenomicida* and that this ability is not affected by the presence of MeSA. Furthermore, it was shown that *T. basalis* is able to parasitize a higher number of eggs than *O. telenomicida*.

##### **Riassunto**

In questo capitolo è stata esaminata l'influenza del composto sintetico Metilsalicilato (MeSA) sulle interazioni competitive tra *Trissolcus basalis* (Wollaston) e *Ooencyrtus telenomicida* (Vassiliev). Sono stati condotti esperimenti di campo utilizzando ovature sentinella disposte in maniera casuale su piante di peperone. I risultati sono stati valutati sulla base dei seguenti parametri: 1) il rapporto tra ovature parassitizzate e il numero totale delle ovature sentinella, per stabilire la capacità del parassitoide di localizzare l'ospite (host location); 2) il rapporto tra le singole uova parassitizzate e il numero totale delle uova delle ovature localizzate, per valutare la capacità del parassitoide di sfruttare l'ospite (host exploitation). I risultati hanno mostrato che *T. basalis* ha una maggiore efficienza di localizzazione dell'ospite rispetto ad *O. telenomicida* e questa capacità non è influenzata dalla presenza di MeSA. Inoltre *T. basalis* possiede anche una maggiore efficienza di sfruttamento, essendo in grado di parassitizzare un numero di uova più elevato rispetto a *O. telenomicida*.

## 4.1 Introduction

Insect parasitoids can find their hosts in complex environments and reproduce through a series of behavioral steps that are regulated mainly by chemical cues, called semiochemicals.

Semiochemicals are chemical cues that mediate the relationship between two organisms by inducing behavioral and/or physiological responses to one or both organisms (Vet and Dicke 1992). According to their ecological roles, semiochemicals are classified as pheromones or allelochemicals (Nordlund and Lewis 1976, Dicke and Sabelis 1988, Vet and Dicke 1992). Pheromones are semiochemicals that mediate the relationships between individuals of the same species. In parasitic wasps, sex pheromones play an important role in allowing long-range orientation towards mates, or in mediating courtship behavior at close range. Allelochemicals are semiochemicals that mediate relationships between individuals belonging to different species. According to the benefits of the signal for the emitter or perceiver, these cues can be classified into different categories: allomones are favorable to the individual that emits the substance; both the emitter and the perceiver benefit from synomones; and kairomones are favorable to the perceiver.

These classifications are not strict, having the same compound multiple effects on the behavior. In several systems, for example, parasitoids use host pheromones as kairomones.

Chemical signals used by egg parasitoids searching for the host can be classified as follows:

*Direct host-related cues:* cues originated from the host eggs. The main direct host-related cues are: 1) synomones induced by egg deposition, 2) volatiles associated with the eggs, and 3) contact kairomones associated with host eggs. These cues can be exploited at long range, orienting wasp females towards the source, or at close range, inducing wasp females to evaluate the host egg surface by antennation. These chemicals are the most reliable cues, although they might be hard to detect except in the very close vicinity of the host.

*Indirect host-related cues:* cues originated from different sources associated with the host egg. The main indirect host-related cues that are exploited by egg parasitoid species are: 1) synomones induced by the feeding activity of the host herbivores (larvae and/or adults), 2) host pheromones and host allomones such as sex or aggregation pheromones of the adult host producer, and 3) kairomones from the egg laying adult, such as scales

of adult lepidopterous hosts, or from the traces left behind by adult hosts while moving on the plant. Exploiting indirect host-related cues represents the main strategy used by egg parasitoids female during the host location.

Recently the use of semiochemicals to improve biological control programs has been investigated and in this approach, the interest has mainly focused on *Herbivore – induced plant volatiles* (HIPVs). The use of these cues to manipulate the distribution and abundance of natural enemies offers important prospects for biological control and integrated pest management (Hare 2011; Vinson et al. 1998). Volatiles are able to recruit parasitoids in a particular area, thereby increasing host mortality rates and altering the relationships between hosts and natural enemies (Vet and Dicke 1992). Less recent studies showed an increase in aphid parasitization rates and/or number of wasps have been observed by Titayavan and Altieri (1990) in the system broccoli plants–*Brevicoryne brassicae* – *Diaeretiella rapae*, as a consequence of applying allyl isothiocyanate emulsion to plants.

Recently, it has been shown that synthetic HIPVs trigger enhancement of the naturally occurring densities of parasitoids in studies conducted on cotton plants. The mymarid *Anaphes iole* Girault parasitizes more eggs of the mirid bug *Lygus lineolaris* (Palisot de Beauvois) when dispensers containing (*Z*)-3-hexenyl acetate and  $\alpha$ -farnesene are placed near the host eggs (Williams et al. 2008), and the braconid *Microplitis mediator* Haliday parasitizes *Helicoverpa armigera* larvae in field cages treated with 3-7-dimethyl-1,3,6-octatriene (Yu et al. 2010).

In addition to increasing the recruitment of natural enemies, treatment with synthetic HIPVs seems to be able to trigger plants to produce endogenous volatiles that attract predator and parasitoid arthropods. For example, Simpson et al. (2011a) showed that grapevines and sweetcorn plants sprayed with synthetic HIPVs attracted predators and parasitoids for some days after application, suggesting that HIPV-treated plants may be induced to produce endogenous volatile synomones over an extended period of time. In a greenhouse study, Rodriguez-Saona et al. (2011) found that cranberry plants that normally released undetectable quantities of MeSA emitted large quantities of this compound when exposed to synthetic MeSA released by commercial dispensers.

An increasing number of studies is looking into how to apply these defense mechanisms to biological control, in particular whether herbivory- or oviposition-induced chemicals can be used as elicitors for plant defense activation. MeSA and jasmonic acid (JA) have been shown to induce plant defense and attract natural enemies, in a similar way to that

described for herbivore-induced defense (Heil, 2004; Zhu and Park, 2005; Ament et al., 2006; D'Alessandro et al., 2006; Bruinsma et al., 2008). Bruce et al. (2003a,b) reported that wheat, *Triticum aestivum* (L.), treated with *cis*-jasmone resulted in reduced aphid populations when compared to untreated crops, which was subsequently attributed to changes in benzoxazinoid levels (Moraes et al., 2008b). For soybean, *Glycine max* (L.) (Fabaceae), it has been shown that plants infested by soybean aphids, *Aphis glycines* (Matsumura), emit different profiles of volatile organic compounds (VOCs) when compared with undamaged soybean plants. In particular, infested plants release in higher quantities MeSA that elicits significant electrophysiological responses in seven-spot ladybirds, *Coccinella septempunctata* L. (Zhu and Park, 2005).

In order to enhance the efficacy of parasitoids, the application in the field of synthetic attractive compounds has been implemented. In this study, the attractive compound MeSA has been used to evaluate the influence on the intraguild interaction between egg parasitoids. The model studied regards *Trissolcus basalis* (Wollaston) and *Ooencyrtus telenomicida* (Vassiliev), two egg parasitoids of the southern green stink bug, *N. viridula*. Understanding in which way intraguild interaction can be affected by the presence of MeSA released in the field, may be important to improve biological control program.

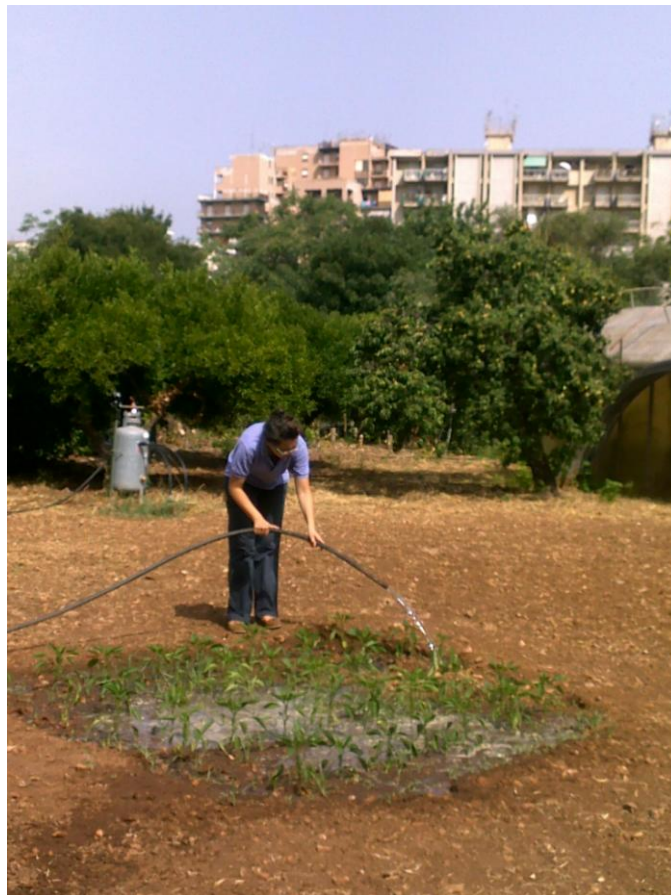
Actually, under laboratory, field and semi-field conditions, it has been demonstrated that these parasitoid species differ in their host location and larval competitive abilities, with *T. basalis* being more efficient in host location (extrinsic competition) while *O. telenomicida* largely dominates interspecific larval competition (intrinsic competition) regardless of the order/time interval between oviposition. This background information gained from laboratory experiments, and field and semi-field experiments, can be the basis for better understanding intraguild interaction in the field where such interactions naturally evolve and take place. Thus, the aim of this study was to understand if MeSA is able to influence intraguild interactions between *T. basalis* and *O. telenomicida*. With a HIPV released in the field, like MeSA, who will win the extrinsic competition? To answer this question experiments has been conducted to determine the following parameters: 1) *Host location*: number of egg masses from which at least one parasitoid emerged divided by the total number of sentinel egg masses, to assess the ability of the parasitoid to locate the host; 2) *Host exploitation*: number of individually parasitized eggs divided by the total number of eggs from the located egg masses, to evaluate the ability of the parasitoid to exploit the host (host exploitation



## 4.2 Materials and Methods

### 4.2.1 Crop

The research was conducted in July 2012. Sentinel egg masses obtained under laboratory conditions were deployed in an experimental field located at the University of Palermo. The experimental field was prepared for seedlings by mechanically removing weeds to prevent competition for space, light and nutrients, and then cultivated with pepper plants, *Capsicum annum* var. “Quadrato d’Asti”. Pepper seedlings were transplanted at the end of June and daily watered. Two parcels were created and, in each plot, 40 pepper seedlings were transplanted. Each parcel consists of five rows with eight plants each one. Pepper plants were grown according to the following arrangement: the distance within the row was 30 cm whereas the distance between the rows was 50 cm. The parcels were close to each other 4 m, a parcel was used for the test, the other for the control. (Photo 1)



**Photo 1.** Parcel with pepper's plants

#### 4.2.2 Insect rearing

The *Nezara viridula* colony, augmented regularly with field collected material, was maintained at  $24 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  RH, 16 h:8 h L:D on a diet of sunflower seeds and seasonal fresh vegetables that was changed every 2–3 days. All used insects were collected in the surroundings of Palermo, Italy. Immatures and adults were kept in separate cages. Adult cages had paper towels as an ovipositional substrate and eggs were collected daily. The *O. telenomicida* and *T. basalis* colonies were established using wasps that emerged from naturally laid *N. viridula* egg masses or sentinel egg masses placed in the field. Colonies of each species were maintained at  $24 \pm 2^\circ\text{C}$ ,  $80 \pm 5\%$  RH, 16 L:8 D in 16-ml glass tubes and fed with a solution of honey–water. To maintain the colonies, newly laid *N. viridula* egg masses were exposed to five parasitoid females for 48 h, and the resulting male and female parasitoids were kept together to ensure mating. In all the bioassays 4–5 day old, mated females of *O. telenomicida* and *T. basalis* were used, and in all cases, parasitoids were naïve with respect to oviposition. The females wasps were isolated in small vials (1.5 x 5 cm) half an hour before bioassays and *N. viridula* sentinel egg masses were artificially assembled to consist of two rows, one of two hosts and one of three hosts, for a total of five eggs.

#### 4.2.3 Bioassays

When the pepper plants reached about 45 cm of height, 10 egg masses, prepared as described above were placed randomly on the plants in both plots, using a paperclip. In the center of test plot a 100-ml bottle containing 40 ml of MeSA (purity 99%, Sigma Aldrich) was placed. At points equidistant to the two parcels 50 females of *O. telenomicida* and 50 females of *T. basalis* were gently released. After 3 days, the sentinel egg masses were collected, placed inside glass tubes and incubated in the conditioned room ( $24 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  RH, 16 h:8 h L:D) until emergence of parasitoids or stink bug nymphs. The replicates performed were 3 (20<sup>th</sup>, 24<sup>th</sup>, 30<sup>th</sup> July). 40 ml of MeSA, released by a 100 ml bottle have an average evaporation rate of 25.1 mg per day measured under field conditions at a mean daily temperature of  $25 \pm 4.0^\circ\text{C}$ . (Photo 2).



**Photo 2.** Dispenser of MeSA

#### 4.2.4 Statistical analysis

Data of host location were analyzed with a generalized linear model (GLM) for binomial distribution using the logit link function with treatment, species, and treatment x species interaction as factors. Data of host exploitation were analyzed with a GLM using a Poisson distribution and the logit link function with treatment, species, and treatment x species interaction as factors. All statistical analyses were performed with the R 2.14.1 software (R Development Core Team 2011).

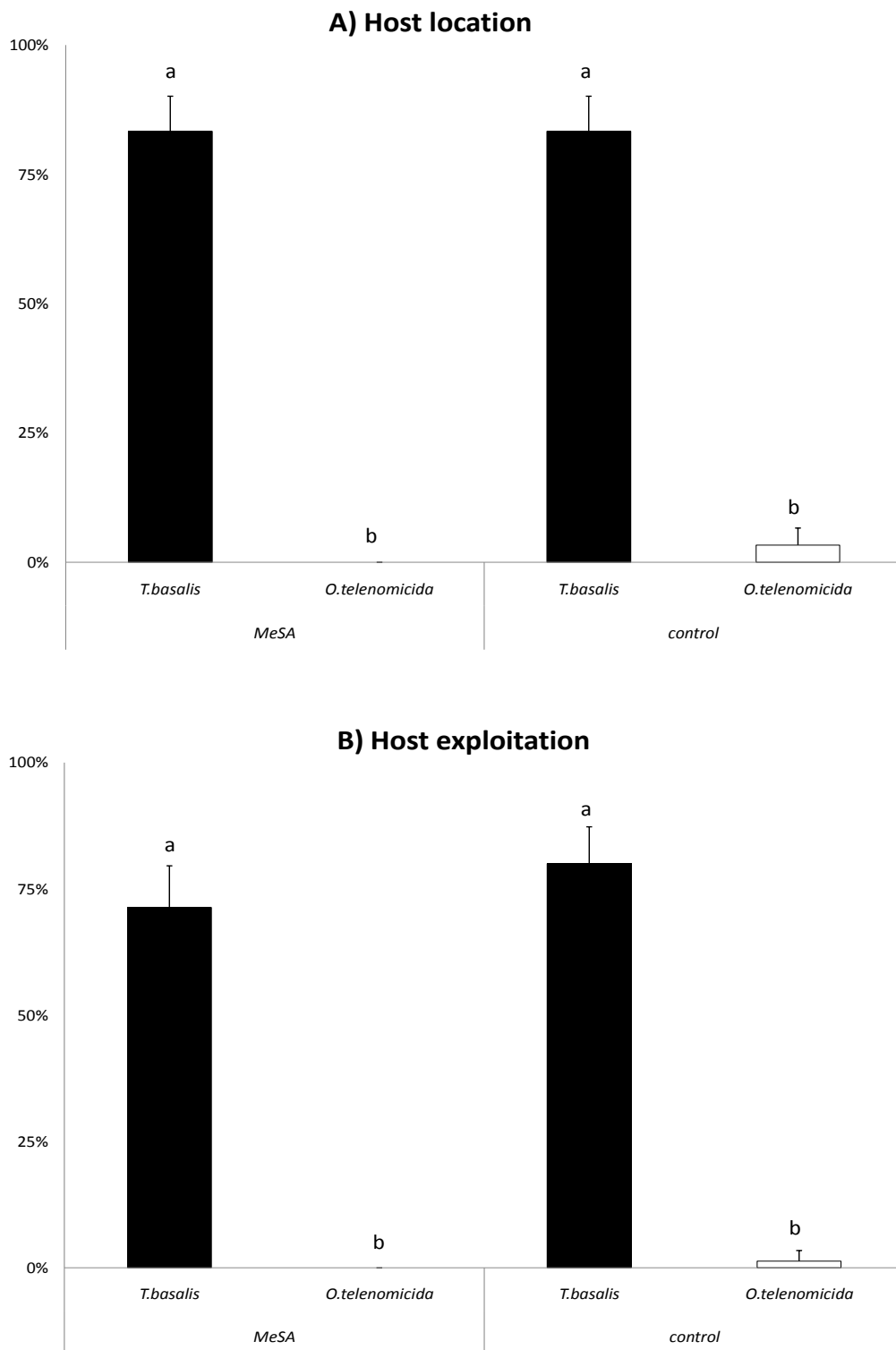
#### **4.3 Results**

The results obtained in laboratory, counting the number of emerged wasps, showed a distinct prevalence of *T. basalis* on *O. telenomicida*.

The proportion of egg masses that were located (Fig.1) by parasitoids was not affected either by the treatment (i.e. MeSA release) (GLM:  $\chi^2=0.035$ ,  $df=1$ ,  $P=0.852$ ) or the treatment x species interaction ( $\chi^2=1.197$ ,  $df=1$ ,  $P=0.274$ ). However *T. basalis* located significantly more egg masses than *O. telenomicida* (GLM:  $\chi^2=104.025$ ,  $df=1$ ,  $P<0.001$ ). *T. basalis* parasitized more of 83,33% ( $\pm 6.80$  SE) of the egg masses in both

plots, test and control. Instead *O. telenomicida* resulted absent in test plot and in small percentage ( $3.33\% \pm 3.28\% \text{SE}$ ) in control plot.

Similarly, the proportion of individual host eggs that were exploited (Fig. 2 Host exploitation) was not affected either by the treatment (GLM:  $\chi^2=0.983$ ,  $\text{df}=1$ ,  $P=0.321$ ) or the treatment x species interaction ( $\chi^2=2.773$ ,  $\text{df}=1$ ,  $P=0.096$ ). After locating an egg mass, *T. basalis* exploited significantly more eggs than *O. telenomicida* (GLM:  $\chi^2=294.517$ ,  $\text{df}=1$ ,  $P<0.001$ ). The percentage of parasitized eggs by *T. basalis* respect of the total number of the parasitized sentinel egg masses is  $71.33\pm 8.26\%$  in the plot with MeSA and  $80.00 \pm 7.30\%$  in the control plot The percentage of parasitized eggs by *O. telenomicida* resulted equal to  $1.33 \pm 2.09\%$  in the control plot. No eggs have been parasitized by *O. telenomicida* in the test plot.



**Fig. 1 Parasitism data (% + SE) obtained when species were released simultaneously under semi-field conditions. (A) host location** = number of egg masses from which at least one parasitoid emerged divided by the total number of egg masses. **(B) host exploitation** = number of individually parasitized eggs divided by the total number of eggs from the located egg mass. Different letters indicate significantly different percentages within the same index (GLM,  $P < 0.05$ )

#### 4.4 Discussion

In our model system, the results showed that *T. basalis* has a high ability to locate host egg masses compared to *O. telenomicida* and it is able to parasitize a number of eggs higher than the competitor. These abilities are not affected by the presence of MeSA; as a consequence, in field conditions, the extrinsic competition, which is dominated by *T. basalis*, is not influenced by the MeSA applying.

MeSA is released by some herbivore-attacked plants and serves as a foraging cue to the natural enemies of some herbivores (De Boer and Dicke 2004, Zhu and Park 2005, Ishiwari et al. 2007). For example, MeSA played an important role in enabling the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) to discriminate between the volatile blends induced by its prey *Tetranychus urticae* Koch and the nonprey caterpillar *Spodoptera exigua* Hubner (De Boer et al. 2004). James and Grasswitz (2005) showed that significantly greater numbers of *Anagrus* spp. (Mymaridae) and *Metaphycus* sp. (Encyrtidae) were captured in blocks of juice grapes baited with MeSA than in unbaited blocks. Five species of predatory insects, *Chrysopa nigricornis*, *Hemerobius* sp., *Deraeocoris brevis* Knight, *Stethorus punctum picipes* Casey, and *Orius tristicolor*, were attracted to MeSA (James and Price 2004). Furthermore MeSA is able to attract the predators *Erigonidium graminicolum* (Sundevall) and *Orius similis* (Zheng). This study is the first demonstration of the attraction of beneficial insects to MeSA in a cotton field.

In field, *T. basalis* and *O. telenomicida* can occur on a common host, the pentatomid bug *N. viridula*, according to counterbalanced competition. Surveys of *N. viridula* egg masses conducted in the field showed that these species can compete for the same host egg mass, and that *T. basalis* achieves a higher level of parasitization (Peri et al. 2011). Laboratory bioassays demonstrated that *T. basalis* is the superior extrinsic competitor in this system.

*Trissolcus basalis* not only exploits more cues than *O. telenomicida* to locate *N. viridula* egg masses, but also chooses more reliable ones. It eavesdrops on volatile oviposition-induced synomones, volatile cues from virgin males and preovipositing females, and contact kairomones in the host footprints (Colazza et al. 1999, 2004), while *O. telenomicida* exploits volatile kairomones from host virgin males only (Peri et al. 2011). On the other hand, when both parasitoid species parasitize the same host egg mass, interspecific larval competition is dominated by *O. telenomicida* regardless of the sequence in which oviposition occurs and whether the parasitoids simultaneously find

the host patch (Cusumano *et al.* 2011, 2012). Moreover, *O. telenomicida* can develop as a facultative hyperparasitoid, ovipositing in host eggs already parasitized by *T. basalis* up to 7 days earlier, and developing on the primary parasitoid larva that has totally consumed the host resources (Cusumano *et al.* 2011). These interactions do not seem affected by MeSA, in fact, in our model system, the attraction of egg parasitoids to MeSA released by dispensers placed in pepper field was not found. However, additional experiments with replication that take into account differences in environmental conditions (temperature, air relative humidity, precipitation, area size, habitat complexity) and biotic stress (presence of damage by other herbivores, physiological stress of the plants) should be conducted to verify the role of these variables on the fluctuation of parasitoids and their host populations, to achieve a more solid data base and to test how different dosage or application technique could help to extend the effects of MeSA on parasitoid recruitment and parasitism rates. So, in the next experiments, it is necessary to optimize MeSA concentration, adjust distance between plots and take into account that the environment was not totally isolated so the presence of other plants around the plots could affect the results.

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## Concluding remarks

Studies on interspecific competitive interactions can help to better understand how competition between parasitoids affects pest population density and consequently the efficiency of biological control programs. It is important that both extrinsic and intrinsic competitions are deeply explored in order to have a more complete view about the interactions that can be established between parasitoid specie, and such interspecific interactions are important to assess the value of parasitoids as biological control agents

In the chapter 2 of this thesis it has been shown that interspecific larval competition between *O. telenomicida* and *T. basalis* was dominated by the former regardless of the sequence in which oviposition occurred and if parasitoids were, or were not, simultaneously released in the patch: *O. telenomicida* wins even when *T. basalis* parasitized an egg mass up to seven days before. In these circumstances, facultative hyperparasitoids can overcome the gap between ovipositions and develop on those parasitoid species that have totally consumed the host resources.

In the chapter 3 it has been shown that *T. basalis* occurs in May and successfully parasitizes hosts until the end of September/beginning of October, whereas *O. telenomicida* is mainly occurring in July-August. In both years, it was found that *T. basalis* is predominant. From naturally laid egg masses, results indicated that *T. basalis* achieves the highest parasitism impact on the hosts, even in those egg masses which are parasitized by more than one female of different species (= multiparasitism). Results from manipulating intraguild interactions showed that *T. basalis* achieves the highest parasitism impact on *N. viridula* when released alone, but it suffers from competition with *O. telenomicida*.

In the chapter 4 it has been shown that *T. basalis* has a high ability to find host compared to *O. telenomicida* and that this ability is not affected by the presence of MeSA. Furthermore, it was shown that *T. basalis* is able to parasitize a higher number of eggs than *O. telenomicida*.

Finally the results confirm the concept of counterbalanced competition that is, coexistence among parasitoids attacking the same host is practicable when one species dominates in the intrinsic competition and the other compensates at the extrinsic level by having higher host finding or dispersal efficacy. In our model system, the intrinsic competition is dominated by *O. telenomicida*, while the extrinsic one by *T. basalis*.

### **Future works**

In this thesis, in order to force parasitoid species to compete for hosts, the majority of the experiments were developed using only small patches consisting of 10 eggs only; however naturally laid egg masses of *Nezara viridula* are much bigger, carrying usually 60-90 eggs each. In the results of the field experiments using naturally laid egg mass, a strong effect of the egg mass size was found indicating an important role for coexistence of species that should be better investigated. It is known that the egg load of a single *T. basalis* female is adequate to parasitize a whole egg mass but it may be unlikely that *O. telenomicida* has similar biological traits due mainly to synovigenic egg maturation and very high host handling time. In terms of egg production, it is also interesting to consider that eggs of *O. telenomicida* are rich of nutrients compared with those of *T. basalis* suggesting that the cost of egg production and maturation can be different between these egg parasitoid species. Host handling time can also play a role in coexistence, as *T. basalis* is very efficient at exploiting suitable hosts, showing a higher attack rate and parasitizing a higher fraction of host eggs than *O. telenomicida*. Therefore further studies will address if host egg mass size, and parasitoids' reproductive costs can also play a role in species coexistence. It may be possible that *T. basalis* evolved different strategies to counteract its inferiority in larval competition and to mitigate the negative effect of hyperparasitism.

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