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Intraguild interactions between two egg parasitoids exploring host patches

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Abstract Intraguild interactions between two egg parasitoids, *Trissolcus basalis* (Hymenoptera: Scelionidae) and *Ooencyrtus telenomicida* (Hymenoptera: Encyrtidae), exploring egg masses of the Southern Green Stink Bug (SGSB) *Nezara viridula* (Heteroptera: Pentatomidae), were investigated in laboratory conditions by single, simultaneous and sequential host attack experiments. Mortality of *N. viridula* eggs was higher in simultaneous and sequential releases compared to single species releases. In simultaneous host exploitations, *T. basalis* females displayed an aggressive behavior against *O. telenomicida* females. The outcome of multiparasitism showed that interspecific larval competition was dominated by *O. telenomicida* regardless of the sequence in which oviposition occurred and which parasitoid was or was not simultaneously released in the patch. Finally, *O. telenomicida* can successfully develop in hosts already parasitized by *T. basalis* up to seven days earlier, acting as a facultative hyperparasitoid, so that intraguild predation (IGP) also occurred. The ecological factors that allow species coexistence and the role played by competition in biological control programs are discussed.

Keywords *Trissolcus basalis* · *Ooencyrtus telenomicida* · *Nezara viridula* · Interspecific competition · Counter-balanced competition

Introduction

Interspecific competition between parasitoids can play a role in species coexistence and in sizing and shaping community structures (Godfray 1994). In addition to being important for ecological studies, understanding how interspecific competition between parasitoids can affect pest suppression may also improve biological control (Murdoch et al. 1998). There is a long standing debate about the wisdom of multiple species release with some authors arguing that the more species that are introduced the greater the effect of reducing pest density (Smith 1929; DeBach 1966; Stiling and Cornelissen 2005). Others suggest that multiple species release may disrupt biological control particularly when parasitoids interact directly through lethal interference competition or facultative hyperparasitism (Turnbull and Chant 1961; Briggs 1993; Murdoch et al. 1998; Denoth et al. 2002). The issue raised against multiple species introduction is based mainly on the assumption that, for each situation, there is a

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potentially best natural enemy able of achieving maximum impact on the target pests. Consequently, it has been discussed that several species competing for the same host may have a lower impact than the single best species would in the absence of competition (Ehler and Hall 1982; Denoth et al. 2002). Some works have demonstrated that the species most effective in controlling the pest when alone is also the mostly negatively affected by competitive interactions (Force 1974; Leveque et al. 1993). Nevertheless, other studies support multiple releases because it was also demonstrated that two species together may have a higher impact on the pest than each species alone, although each species in the combined situation is less efficient when it is alone (Ehler 1979; Ismail and Long 1982). In fact, when parasitoid niches do not or partially overlap due to different ecological traits, interspecific discrimination or different host stage/instar preferences, multiple releases may be the right choice. Obviously the degree of niche separation and competition between species are key ecological factors that must be important in the question of single versus multiple releases.

Interspecific competitive effects can occur between adult parasitoids, i.e. the indirect interactions between adult females searching for or exploiting hosts, as well as between immature parasitoids, i.e. the competition that occurs between larvae developing in the same host (Zwolfer 1971). When more than one parasitoid species oviposits in the same host (= multiparasitism *sensu* Mackauer 1990), two main outcompeting mechanisms can take place: physical attack and physiological suppression (Fisher 1961; Godfray 1994). The former mechanism has been successfully described and explained (Salt 1961; Vinson and Iwantsch 1980) but what is referred to as physiological suppression is still not clearly understood because, when there is no evidence of combat between immatures, authors are often forced to assume that a physiological suppressive factor is involved (Mackauer 1990; Vinson and Hegazi 1998). Practically, when the suppression of supernumery larvae is mediated by physical combat, the order in which oviposition occurs and the time interval between ovipositions have been shown to be important for the outcome of larval competition while such aspects need further investigations if the suppression is mediated by physiological mechanism (Chow and Mackauer 1984; Mackauer 1990).

It has been suggested that biological control programs should consider the interactions between parasitoid guilds since competitive effects among them may change the reproductive success of each parasitoid species and thus may affect the host mortality (Nechols et al. 1992; Follett et al. 2000). Field studies on egg parasitoid guilds of herbivorous Stink Bugs, serious pests for a wide number of crops, have shown that *Trissolcus* spp. and *Ooencyrtus* spp. can often naturally co-occur on the same host, and generally the former parasitize more eggs than the latter (Correa-Ferreira 1986; Hoffmann et al. 1991; Correa-Ferreira and Moscardi 1995; Amarasekare 2000b). In particular the co-occurrence of *Trissolcus* spp. and *Ooencyrtus* spp., on *N. viridula* eggs is widely 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. 2010) and Japan (Hokyo 1965).

In many countries to control Stink Bug populations, biological control programs based on egg parasitoids have obtained a variable degree of success (Caltagirone 1981; Clarke 1990; Hoffmann et al. 1991; Ehler 2002). Thus, understanding the competitive interactions between *Trissolcus* and *Ooencyrtus* species attacking the same host may be useful to improve the biological control of such pests. However only a few researchers have investigated the effects of competitive interactions between these egg parasitoids (Lairichi 1978; Lee 1979; Amarasekare 2000a, b).

Females in the genus *Trissolcus* typically fight among conspecifics and congenerics for possession of newly discovered host eggs (Safavi 1968; Field 1998; Wajnberg et al. 2004) but it is unknown if interspecific aggressive behavior occurs between *Trissolcus* and *Ooencyrtus* species.

In this paper we address the interspecific interactions that occur between *Trissolcus basalis* (Hymenoptera: Scelionidae) and *Ooencyrtus telenomicida* (Hymenoptera: Encyrtidae), two egg parasitoids of the Southern Green Stink Bug (SGSB), *Nezara viridula* (Heteroptera: Pentatomidae) that naturally have been found to co-occur from cultivated crops located in western Sicily. Hence, in order to clarify intraguild interactions between *T. basalis* and *O. telenomicida*, a series of experiments were developed to investigate (1) if the simultaneous or sequential release of either parasitoid species plays a role in the outcome of multiparasitism; (2) if interspecific aggressive behaviors occur; (3) which species is superior in larval competition and (4) if direct trophic interactions between these egg parasitoids occur.

Materials and methods

Study organisms

Ooencyrtus telenomicida is a minute encyrtid egg parasitoid of several phytophagous bugs of different heteropteran (Coreidae, Pentatomidae, Scutelliridae) and lepidopteran (Lymantriidae, Notodontidae, Thaumetopoeidae) families (Japoshvili and Noyes 2006). *Ooencyrtus telenomicida* is also reported as a facultative secondary parasitoid of Scelionidae (Hymenoptera) (Catalán and Verdù 2005). Further, even if it is usually solitary when developing in SGSB eggs, when engages in superparasitism two *O. telenomicida* can occasionally emerge from the same host (Cusumano unpublished). During the oviposition and before laying an egg, females feed on host fluids following drilling of the host chorion (host feeding). This species is native from the Palaearctic region and it has been recorded to occur from West to East across Europe, Asia, and Sub Saharan Africa (Zhang et al. 2005; Japoshvili and Noyes 2006).

Trissolcus basalis can successfully develop on several pentatomid hosts but it is mainly recorded as a natural enemy of the SGSB (Jones 1988). In fact, it is the most important and the most widely distributed species of parasitoids of SGSB egg masses. This solitary parasitoid, probably native from the Ethiopian-Palaearctic region (Jones 1988), is now cosmopolitan and has been recorded in America, Europe, Africa, Australia, New Zealand and some Pacific Islands (Jones 1988 and references within).

Comparing both parasitoid species in terms of host breadth, *O. telenomicida* should be considered more generalist (Steidle and van Loon 2003) since it attacks and successfully develops on several hosts belonging to three different insect orders, even if it is more closely associated with Heteroptera.

Nezara viridula is a highly polyphagous pest that attacks both perennial and annual plants. It occurs in most of the warmer regions of the world, damaging a wide variety of fruit, nut, grain and vegetable crops (Todd 1989).

Insect colonies and bioassay setup

Nezara viridula colony was held in wooden cages (50 × 30 × 35 cm), ventilated with mesh-covered holes (5 cm in diameter), in a environmental room (24 ± 1°C, 70 ± 5% RH, 16 h:8 h L:D), and fed with a diet of sunflower seeds and seasonal fresh vegetables. Food was changed every 2–3 days, and separate cages were used for immatures and adults. Paper towels were placed inside each adult cage as an ovipositional substrate. Daily collected egg masses were used to maintain the colony which was also regularly augmented by field-collected bugs.

The *O. telenomicida* and *T. basalis* colonies were established from wasps emerging from sentinel and/or naturally laid *N. viridula* egg masses on cultivated and un-cultivated crops. Adult parasitoids were reared in 16-ml glass tubes (density = 50–60 wasps tube^{-1}), fed with a solution of honey–water, and kept in an incubator (24 ± 2°C, 80 ± 5% RH, 16 h:8 h L:D). Collected *N. viridula* egg masses were bi-weekly exposed to parasitoids for 48 h, then the eggs were removed and stored for incubation. After

Table. 1 Percentages of total dead host eggs obtained by pooling together data scored during single, simultaneous, and sequential releases of the egg parasitoid species

Total *N. viridula* dead eggs (%) ± SE scored during				
Single release		Simultaneous release	Sequential release	
Tb	*Ot*	*Tb* + *Ot*	*Tb*→*Ot*	*Ot*→*Tb*
3.75 ± 1.07 a	15.25 ± 2.09 b	26.75 ± 3.03 c	27.00 ± 3.20 c	27.75 ± 2.94 c

Different letter indicates significantly different percentages between treatments (ANOVA, Tukey test, $P < 0.05$)

Tb T. basalis; *Ot O. telenomicida*

emergence, male and female parasitoids were kept together to allow for mating.

In all the bioassays females of *O. telenomicida* and *T. basalis* were 4–5 day old, mated, and naïve in regards to host oviposition and were used only once. About 24 h before the experiments, wasp females were isolated in small vials (1.5 × 5 cm) with a drop of honey–water solution. About 1 h before bioassays, parasitoids were transferred into the experimental room (24 ± 1°C, 60 ± 10% RH) to acclimatize them. The experimental arenas consisted of a small Petri dish (4 cm in diameter) containing at the centre an egg mass placed on a piece of Parafilm®. All egg masses used in bioassays were up to 24 h old and artificially assembled to consist of two lateral rows of three hosts each and a central row of four hosts for a total of ten eggs. A video recorder connected to a video camera was used to record the details of parasitoid oviposition sequences.

Single, simultaneous and sequential release

The objective of the experiment was to investigate if the order of the ovipositing species and the time interval between ovipositions play a role in parasitoid species emergences. In addition, single species release was also performed to evaluate species exploitative ability and to compare host mortality that occurred as a consequence of single, simultaneous or sequential releases.

Experiments were conducted in the arena described above according to the following releases: (1) a single *T. basalis* female was introduced (*Tb* only); (2) a single *O. telenomicida* female was introduced (*Ot* only); (3) one female of each species was simultaneously released (*Tb* plus *Ot*); (4) a *T. basalis* female was released first for half of the total time trial, then it was removed and subsequently an *O. telenomicida* female was introduced for the second half time (*Tb* then *Ot*); (5) same pair combination as in (4) but in reversed order (*Ot* then *Tb*). All these combinations were replicated according to the following four duration times: 3 h, 6 h, 12 h and 24 h. Time started when a female displayed an ovipositing posture. When this situation did not happen during 10 min from release into the arena, the trial was discarded.

For each of the five set of trials and the four duration times, ten replicates were performed. After wasps were removed following the test, the parasitized egg masses were placed into incubators (24 ± 1°C, 70 ± 5% RH, 16 h:8 h L:D) until the emergence of the parasitoids or the eclosion of SGSB nymphs. To record parasitoid offspring emergence we employed two criteria: for the solitary *T. basalis* it was scored as the number of adults that emerged while for the "occasionally gregarious" *O. telenomicida* we counted the number of host eggs from which adults emerged. All the SGSB eggs that failed to hatch were counted as "dead host eggs".

Data were tested for normality (Kolmogorov–Smirnov test). Because there was no significant deviation from normal distribution, values were then analyzed with parametric tests. The number of parasitoid's that emerged in the single, simultaneous and sequential releases were analyzed by a two-way ANOVA with parasitoid species, time exposure and their interaction as factors. Post-hoc comparison was made using Tukey test. The effect of the exposure time on parasitoid emergences in single release was analyzed with a one-way ANOVA followed by a post-hoc Tukey test. The mortalities of *N. viridula* eggs recorded during the single, simultaneous or sequential releases of the egg parasitoids were pooled for the four duration times, analyzed by one-way ANOVA followed by post-hoc Tukey, and then presented as percentages.

Behavioral interactions

The purpose of the experiment was to investigate if *T. basalis* displays aggressive behavior versus *O. telenomicida* for possession of a host egg mass as reported when interacting with conspecifics. One female of each species was simultaneously introduced inside the experimental arena described above. Recordings started as soon as both parasitoids were found simultaneously on the egg mass showing the oviposition posture. If a female did not show the ovipositing posture within 10 min from her release, the trial was discarded. Behavioral interactions were recorded for 1 h, and the results were reported in terms of number of confrontations. A total of 15 replicates was performed.

Outcome of multiparasitism

The purpose of the experiment was to investigate the competitive larval ability of both species when multiparasitism occurred. Experiments were similar to those of the previous set of bioassays relatively to simultaneous (*Tb* plus *Ot*) and sequential (*Tb* then *Ot*; *Ot* then *Tb*) release for an exposure time of 24 h. However, once the parasitoids were removed, only host eggs parasitized by both species were isolated singly into gelatine capsules and were placed into a climatic chamber (24 ± 1°C, 70 ± 5% RH, 16 h:8 h L:D) until the emergence of the parasitoids while host eggs parasitized by a single species were eliminated. The host eggs in which parasitoids have successfully oviposited are distinguishable because *O. telenomicida*'s egg is provided with a respiratory stalk protruding externally from the host, while *T. basalis* use to mark the parasitized host by sweeping its ovipositor on the chorion surface. Such marking behavior is highly correlated (99%) with egg deposition. For each of the three trials 20 replicates were carried out. Number of emerged *T. basalis* and *O. telenomicida* were compared using a χ^2-test discarding from analysis all hosts that did not produce a parasitoid.

Intraguild predation

The objective of the experiment was to explore how parasitoids species emergences were affected when *O. telenomicida* acts as a hyperparasitoid, interacting with *T. basalis* not only via competition but also via trophic interaction so that intraguild predation (IGP) occurs. A single *T. basalis* female was introduced into the arena described above and allowed to parasitize a 10-eggs *N. viridula* egg mass for 24 h. If female did not display the oviposition posture after 10 min from release the trial was discarded. Then she was removed and the egg mass was stored into a climatic chamber (24 ± 1°C, 70 ± 5% RH, 16 h:8 h L:D). After one day, the stored egg mass was exposed to a *O. telenomicida* female for 24 h. The bioassay was repeated at the following time interval between *T. basalis* removal and *O. telenomicida* introduction, 2, 3, 4, 5, 6, 7, 8, 9, and 10 days. After the females of *O. telenomicida* were removed, egg masses were stored into a climatic chamber (24 ± 1°C, 70 ± 5% RH, 16 h: 8 h L:D) until emergence of parasitoids. Each exposure was done between 10 and 12 a.m. The 24-hour egg mass exposure was started soon after the female showed an ovipositing posture within 10 min after release, if not the bioassay was discarded.

The number of parasitoid emerged was tested for normality (Kolmogorov–Smirnov test). Because there was no significant deviation from normal distribution, values were then analyzed with parametric tests. Data were processed by a two-way ANOVA with parasitoid species, time of exposure and their interaction as factors. Post-hoc comparison was performed using Tukey test. All statistical analyses were processed using Statistica 6.0 [Statsoft, 2001, Vigonza (PD), Italy].

Results

Single, simultaneous and sequential release

In the single species release the average number of emerged *T. basalis* (*Tb* only) was not influenced by exposure time (ANOVA: $F = 1.87$, $df = 3{,}36$, $P = 0.152$), but it was very high in all treatments [3 h = 9.8(mean) ± 0.13(SE); 6 h = 9.4 ± 0.22; 12 h = 9.4 ± 0.27; 24 h = 9.9 ± 0.10] (Fig. 1a). In contrast, the average number of emerged *O. telenomicida* (*Ot* only) was affected by exposure time ($F = 59.26$, $df = 3{,}36$, $P < 0.001$) with longer time trials yielding more parasitoids [12 h = 7.7 ± 0.99; 24 h = 8.6 ± 0.31] (Fig. 1b). The average number of emerged *T. basalis* was significantly higher than *O. telenomicida* for 3 h and 6 h exposition time treatments ($P < 0.01$) while no difference was shown for 12 h and 24 h (Fig. 1).

When both species were released simultaneously (*Tb* plus *Ot*), a significant effect of species ($F = 719.22$, $df = 1{,}72$, $P < 0.001$) was found as well as with the time × species interaction effect ($F = 30.28$, $df = 3{,}72$, $P < 0.001$) on parasitoid emergence. In fact, the average number of emerged *T. basalis* was significantly higher than *O. telenomicida* for the 3, 6 and 12 h treatments ($P < 0.01$) while no difference was shown for the 24 h treatment (Fig. 2). A higher emergence value was recorded for *T. basalis* from the shorter exposition time trial [3 h = 8.3 ± 0.42] but values progressively dropped as the duration time increased (Fig. 2). An opposite trend was found for *O. telenomicida* since the proportion of wasps was very low for 3 h [0.10 ± 0.10] and higher when the experiment lasted longer [24 h = 3.1 ± 0.38] (Fig. 2).

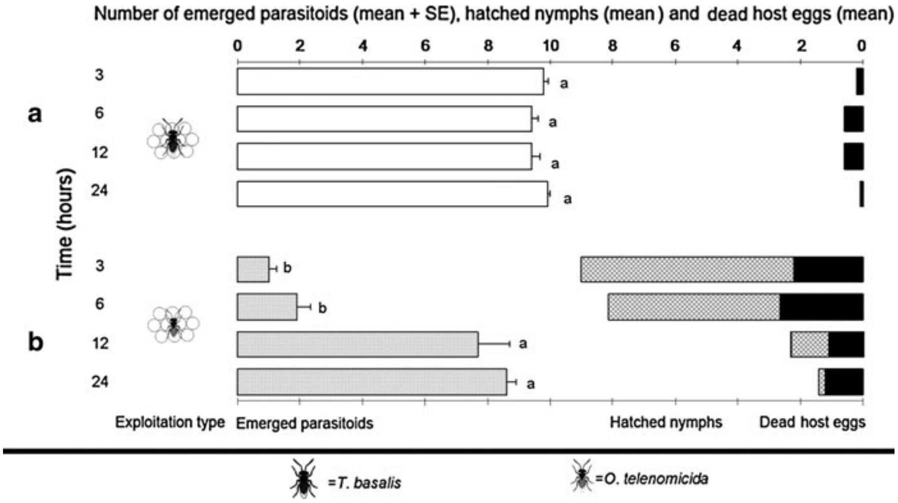

Fig. 1 Mean number (+ SE) of emerged parasitoids (white and dotted bars), hatched stink bug nymphs (cross bars) and dead host eggs (black bars) observed from 10-eggs *N. viridula* egg mass exposed to *T. basalis* female (**a**) or *O. telenomicida* female (**b**). Time indicates the duration of the experiment. Different letters indicate significantly different means between *T. basalis* and *O. telenomicida* within the same experimental time (ANOVA, Tukey test, $P < 0.05$)

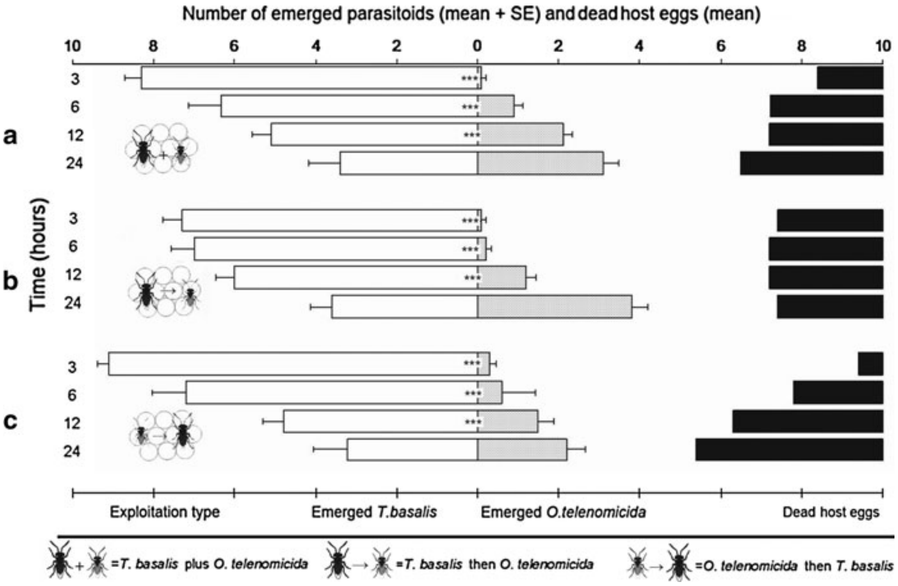

Fig. 2 Mean number (+ SE) of emerged *T. basalis* (white bars), emerged *O. telenomicida* (dotted bars) and dead host eggs (black bars) observed from 10-eggs *N. viridula* egg mass exposed to a pair of parasitoid species. **a** Eggs exposed to females of *T. basalis* and *O. telenomicida* released at the same time. **b** Eggs exposed for the first half of the experiment to *T. basalis* female and then exposed to *O. telenomicida* female for the second half of the experiment. **c** Same as **b** but eggs exposed first to *O. telenomicida* and then to *T. basalis*. Time indicates the duration of the experiment. Asterisks indicate significantly different means between *T. basalis* and *O. telenomicida* within each experimental time (ANOVA, Tukey test, $P < 0.05$)

Results of the sequential exploitative releases (*Tb* then *Ot*; *Ot* then *Tb*) were similar to those obtained from the simultaneous experiment (Fig. 2). A significant effect of species was also found (*Tb* then *Ot*: $F = 500.35$, $df = 1{,}72$, $P < 0.001$; *Ot* then *Tb*: $F = 369.56$, $df = 1{,}72$, $P < 0.001$) as well as with the time × species interaction effect (*Tb* then *Ot*: $F = 37.03$, $df = 3{,}72$, $P < 0.001$; *Ot* then *Tb*: $F = 27.39$, $df = 3{,}72$, $P < 0.001$) on parasitoid emergence. In both sequential releases, regardless which species was introduced first in the arena, the average number of *T. basalis* that emerged declined while the number of *O. telenomicida* that emerged increased. However, *T. basalis* adult emergence still remained significantly higher than that of *O. telenomicida* for 3, 6 and 12 h duration time trials ($P < 0.01$) while no difference was shown for 24 h trials (Fig. 2).

SGSB nymphs only hatched when *O. telenomicida* was released alone whereas none of the other single, simultaneous or sequential releases produced a SGSB nymph (Figs. 1, 2). Statistically significant differences in the number of total dead host eggs were found between single, simultaneous and sequential releases (ANOVA: $F = 300.50$, $df = 4{,}45$, $P < 0.001$) (Table 1). Number of dead host eggs was lower when *T. basalis* was released alone [*Tb* only = 3.75% ± 1.07] intermediate when *O. telenomicida* was released alone [*Ot* only = 15.25% ± 2.09] and higher for simultaneous release [*Tb* plus *Ot* = 26.75% ± 3.03] and both sequential releases [*Tb* then *Ot* = 27.00% ± 3.20; *Ot* then *Tb* = 27.75% ± 2.94] (Table 1).

Behavioral observations

When both species were simultaneously exploiting the patch, interspecific aggressive behavior occurred. As soon as *T. basalis* contacted or noted *O. telenomicida* on the egg mass, it displayed aggressive behavior, with a frequency of $2.13 \pm 0.23\ \mathrm{h}^{-1}$. In all encounters, *T. basalis* moved its wing quickly, appearing ready to attack and lunged towards its competitor which retreated.

Outcome of multiparasitism

From a total of 389 multi-parasitized *N. viridula* eggs, 47% yielded a parasitoid and none produced a SGSB nymph. Significantly more *O. telenomicida* survived in the simultaneous exploitation experiment (*Tb* plus *Ot*) ($\chi^2 = 41.14$, $df = 1$, $P < 0.0001$), and in both sequential experiments (*Tb* then *Ot*: $\chi^2 = 26.06$, $df = 1$, $P < 0.0001$; *Ot* then *Tb*: $\chi^2 = 51.20$, $df = 1$, $P < 0.001$) (Fig. 3). Data from the simultaneous experiments (*Tb* plus *Ot*) showed that from 44% ± 4.6 of the hosts an *O. telenomicida* emerged while from 3% ± 1.7 of the hosts a *T. basalis* emerged (Fig. 3). When *T. basalis* was released first (*Tb* then *Ot*), the percentage of

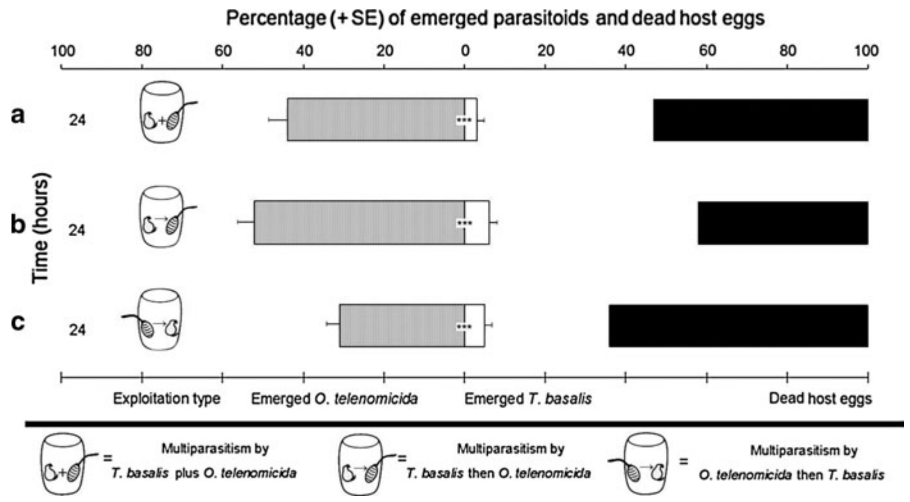

Fig. 3 Percentage (+ SE) of emerged *T. basalis* (white bars), emerged *O. telenomicida* (dotted bars) and dead host eggs (black bars) obtained from only multiparasitized hosts observed from 10-eggs *N. viridula* egg mass exposed to a pair of parasitoid species. **a** Eggs exposed to females of *T. basalis* and *O. telenomicida* released at the same time. **b** Eggs exposed for the first half of the experiment to *T. basalis* female and then exposed to *O. telenomicida* female for the second half of the experiment. **c** Same as **b** but eggs exposed first to *O. telenomicida* and then to *T. basalis*. Time indicates the duration of the experiment. Asterisks indicate significantly differences between the percentage of emerged *T. basalis* and the percentage of emerged *O. telenomicida* within each release sequences (χ^2 test, $P < 0.05$)

O. telenomicida wasps obtained was 52% ± 4.2 and the percentage of *T. basalis* wasps obtained was 6% ± 2.0 (Fig. 3). Reversing the order of oviposition (*Ot* then *Tb*) resulted in 31% ± 4.0 of the mutiparasitized eggs producing *O. telenomicida* while only 5% ± 1.8 produced *T. basalis* (Fig. 3).

Intraguild predation

A significant effect of species (ANOVA: $F = 65.01$, $df = 1{,}180$, $P < 0.001$) and of time × species interaction ($F = 34.52$, $df = 9{,}180$, $P < 0.001$) was found on the emergence of parasitoids. Comparing the average number of emerged wasps, significantly more *O. telenomicida* emerged when it was exposed to hosts already parasitized by *T. basalis* 1–7 days earlier ($P < 0.005$) (Fig. 4). No difference was shown for the eight days trial while significantly more *T. basalis* emerged only if it oviposited nine or more days before *O. telenomicida* ($P < 0.001$) (Fig. 4).

Discussion

Intraguild interactions between *T. basalis* and *O. telenomicida* occurred when species shared the same SGSB host egg mass. Our results showed that *T. basalis* exploits very efficiently the host patch when it was alone. In fact, due to its low host handling time (Bin et al. 1993), it is capable of parasitizing all the available hosts in less than 3 h with no trials yielding SGSB nymphs. On the other hand, *O. telenomicida* requires more than 6 h but less then 12 h to parasitize the whole patch, consequently a large proportion of SBSG nymphs emerged from 3 h and 6 h treatments. In addition, *O. telenomicida* may also leave the patch before all the hosts have been exploited since a small proportion of nymphs emerged from 12 h and even from 24 h treatments. A similar strategy was already observed for *O. nezarae* (Ishii) females which leave the egg mass of *Megacopta punctatissimum* Montandon before they completely oviposit in all the host eggs (Takasu and Hirose 1991). Such unusual behavior can be explained considering that *O. telenomicida* has a small egg load (Cusumano unpublished) so it is possible that especially for younger females, some eggs could be saved for high quality hosts, if ever encountered.

When both species were released, the number of parasitoids that emerged was similar for each species regardless if species were released simultaneously or sequentially, with no host producing a SGSB nymph. Differences in parasitoid's competitive ability and host handling times played a key role in the wasp

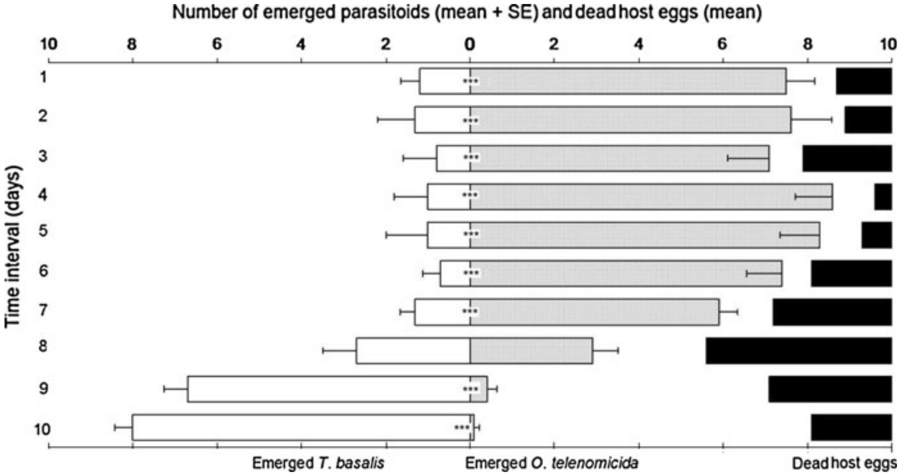

Fig. 4 Mean number (+ SE) of emerged *T. basalis* (white bars), emerged *O. telenomicida* (dotted bars) and dead host eggs (black bars) observed from 10-eggs *N. viridula* egg mass exposed to a pair of parasitoid species. Eggs already parasitized by *T. basalis* female for 24 h exposed to *O. telenomicida* female for 24 h. Time interval (days) indicates the interval between the removal of *T. basalis* female and the introduction of *O. telenomicida* female. Asterisks indicate significantly different means between emerged *T. basalis* and *O. telenomicida* within the same time interval (ANOVA, Tukey test, $P < 0.05$)

average emergence patterns found: *T. basalis* was the species that emerged in high number from 3, 6 and 12 h thanks to its superior exploitation ability. However, as the exploitation time increased, *T. basalis* suffered interspecific competition with *O. telenomicida* and the proportion of the former gradually decreased from 3 h to 24 h treatment while the latter showed an opposite trend, with longer time trials yielding more wasps. Even if *O. telenomicida* is superior when competition occurred, the number of wasps emerged in the 24 h treatments was not statistically higher compared to the number of *T. basalis*: this might be due to the habits of *O. telenomicida* to leave the patch not completely exploited so it is possible that some hosts were only parasitized by *T. basalis*. Furthermore, it is also possible to argue that *O. telenomicida* can suffer, even to a minor extent, from competition with *T. basalis*.

Comparing data of host mortality, a lower value was found when *T. basalis* was released alone whereas higher value was recorded for *O. telenomicida*, probably because the latter engages in host feeding and occasional superparasitsm which may cause a reduction of food available for the offspring. However the highest host mortality values were observed when both species were introduced, regardless if simultaneously or sequentially, suggesting that interspecific competition for host resources may play a key role especially for longer time trials when both species can exploit all the hosts.

The outcome of multiparasitism indicates that asymmetric larval competition occurs between *T. basalis* and *O. telenomicida,* with the latter outcompeting the former regardless the sequence in which oviposition occurred and regardless if the parasitoids were or were not simultaneously present in the patch. The first instar larva of *T. basalis* is equipped with sickle-shaped mandibles used to fight conspecifics when superparasitism occurs (Volkoff and Colazza 1992), while the first instar larva of *O. telenomicida* is provided with small mandibles unlikely to cause serious physical damage to its competitor (Cusumano 2010). Taking into account that *O. telenomicida* is superior at interspecific larval competition despite the physical disadvantage of its first instar larva, it is possible to suggest that a physiological suppressive factor is probably involved even though further studies are required to better clarify the mechanism of suppression.

When both species simultaneously exploit a host patch, interference competition (aggressive behavior) occurs. In fact, it has been observed that *T. basalis* engages in fighting conspecifics for the ownership of the host patch "host patch defense" (Field 1998; Wajnberg et al. 2004). A similar behavior was observed against *O. telenomicida* as it tried to get closer to the egg mass controlled by *T. basalis.* Interestingly, *T. basalis* seems to adjust its behavior according to the larval competitive abilities of the intruder. When interacting with *O. telenomicida*, a superior larval competitor, we demonstrated that *T. basalis* displays a distinct aggressive behavior. In contrast, Sujii et al. (2002) showed that *T. basalis* adopts a fugitive strategy when interacting with *Trissolcus ulrichi* Crawford.

Ooencyrtus telenomicida wins through its larval competition, so it should be relatively less important to be the first species exploiting unparasitized hosts. However, the lack of fighting in *O. telenomicida* could be also related to its smaller size in comparison to *T. basalis* (Petersen and Hardy 1996; Irvin and Hoddle 2005).

Zwolfer (1971, 1979) studying a parasitoid complex of forest pests argued that coexistence may be allowed by differences in the competitive abilities of the species. He suggested that coexistence may be possible if inferior larval competitors evolved superior abilities in host finding or dispersal (counterbalanced competition) and classified parasitoids in "intrinsically" or "extrinsically" superior if they have superior abilities at larval competition or at host finding/dispersal respectively. It seems that *T. basalis* and *O. telenomicida* have similar ecological requirements. In fact it has been shown that, to locate SGSB 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. 2010). Thus, coexistence of parasitoid species can be driven by a host finding-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).

To date, the competitive interactions occurring between *Trissolcus* and *Ooencyrtus* species have been investigated in two similar host-multiparasitoid systems which results corroborate our findings. Lee (1979) studied the potential abilities of *T. basalis* and

O. submetallicus (Howard) in SBSG biological control programs and concluded that, in the field, *T. basalis* was more efficient in host finding and dispersal, but that *O. submetallicus* was clearly superior in parasitizing host eggs in laboratory studies. Amarasekare (2000a, b) focusing on species coexistence between *Trissolcus murgantiae* (Ashmad) and *Ooencyrtus johnsonii* (Howard), egg parasitoids of the harlequin bug *Murgantia histrionica* (Hahn), found that *O. johnsonii* was absent from "low productive hosts patches" while *T. murgantiae* was always present suggesting that the latter may be better at exploiting unparasitized hosts. However in laboratory studies, both parasitoid species engage in strong larval competition and, again, *O. johnsonii* was superior to *T. murgantiae* (Sjaarda 1989).

Finally the interactions between the two egg parasitoids are not limited to competition, but also direct trophic relations occur, i.e. intraguild predation (IGP). In fact, *O. telenomicida* is a facultative hyperparasitoid which is able to complete its development on egg masses parasitized up to seven days before by *T. basalis*, when larvae of *T. basalis* have previously pupated (Volkoff and Colazza 1992). Clarke and Seymour (1992) already reported hyperparasitism by two *Acroclisoides* species on *N. viridula* egg masses parasitized by *T. basalis*. However laboratory tests, exposing both parasitized and unparasitized hosts, showed that *Acroclisoides* spp. are parasitoids of *T. basalis* and not primary parasitoids of the SGSB (Clarke and Seymour 1992).

It is straightforward stating that secondary parasitoids disrupt biological control, while ecological systems involving IGP display more complexity. In fact such systems are currently under intense investigation in order to understand whether IGP will cause an increase or decrease in long term herbivore population densities (Rosenheim et al. 1995; Rosenheim and Harmon 2006). In conclusion our findings suggest superior host exploitation abilities of *T. basalis* compared to *O. telenomicida*. In addition, no SGSB nymphs hatched from all the releases in which the former species was introduced. *Trissolcus basalis* suffered intraguild interaction with *O. telenomicida* due to inferior ability at larval competition when multiparasitism occurred and due to the negative effect of facultative hyperparasitism. Even though our results indicate that *T. basalis* could achieve higher impact on the host when released alone, laboratory conditions differ from field conditions so it is not easy to infer results from the former to field populations. Hence controlled population experiments under field or semi field conditions to assess the extent of competition and IGP in egg parasitoid guilds of Stink Bugs are required.

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