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Studies on interactions between parasitoids: the case of the idiobiont *Trichogramma cacoeciae* and the koinobiont *Ascogaster quadridentata* on *Cydia pomonella* eggs

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Abstract

To optimize biological control of the codling moth *Cydia pomonella* (L.) (Lepidoptera Tortricidae), two of its natural enemies, the egg parasitoid *Trichogramma cacoeciae* Marchal (Hymenoptera Trichogrammatidae) and the egg-larval parasitoid *Ascogaster quadridentata* Wesmael (Hymenoptera Braconidae), exploiting the same host resource were investigated for potential interference. Laboratory experiments revealed that in general both parasitoids could not reliably differentiate between unparasitized and previously parasitized eggs whatever quality or combination was provided. This situation was beneficial for *T. cacoeciae*, as all eggs successfully parasitized by both parasitoids developed solely into *T. cacoeciae* adults. Nonetheless, the latter's parasitism rate was much lower in the case of simultaneous presence of a *A. quadridentata* female than alone showing *A. quadridentata* capability to defend its own offspring. Although further tests under more natural conditions are necessary, this study suggests that regarding control of the codling moth, inundative releases of *T. cacoeciae* may disturb the natural control made by the endemic parasitoid *A. quadridentata*.

Key words: biological control, interspecific interactions, *Ascogaster quadridentata*, *Trichogramma cacoeciae*, intra-guild predation.

Introduction

As a response on increasing pest resistance and declining public acceptance of chemical pesticides, the number of farmers adopting organic growing concepts is increasing. Also these concepts mainly rely on an arthropod pest management combining a variety of strategies (Zehnder *et al.*, 2007). Reinforcing the action of endemic parasitoids by the release of additional species in order to maintain pests at acceptable levels may be one appropriate approach. The occurrence of spatial, temporal or behavioural refuges from parasitism can allow individuals in a pest population to escape from the natural enemy's attack, but the identification of additional parasitoid species that can break the host refuge from parasitism may lead to greater success in biological control (Mills, 2005). However, it is important to ensure that these natural enemies are compatible or synergistic in action and that interspecific interactions are not detrimental to the suppression of the host population over time (Wang *et al.*, 2008). For this reason, the biology of biological control agents should be carefully explored to avoid the potentially disruptive effects of interference, competition or intraguild predation (Collier *et al.*, 2002).

Understanding the role of intraguild interactions may contribute to an effective pest management strategy when multiple natural enemies may be necessary to control a given pest (Zang and Liu, 2007). Different pest species have different biologies and different arrays of natural enemies. As a consequence, pest control solutions need largely to be developed on a case-by-case basis (Batchelor *et al.*, 2006). For example, in an attempt to control the whitefly *Bemisia argentifolii* Bellows et Perring (Homoptera Aleyrodidae), Heinz and Nelson (1996) found that interspecific interactions between the two parasitoids *Encarsia pergandiella* Howard (Hymenoptera Aphelinidae) and *Encarsia formosa* Gahan (Hymenoptera Aphelinidae) may facilitate biological control rather than hinder it. However, analysis of the interactions between two other natural enemies clearly showed that the predator *Delphastus catalinae* Horn (Coleoptera Coccinellidae) could exhibit a devastating impact on the parasitoid *Encarsia sophia* (Girault et Dodd) (Hymenoptera Aphelinidae) when used both species to control the whitefly *Bemisia tabaci* (Gennadius) (Homoptera Aleyrodidae) (Zang and Liu, 2007). Thus, it is important to ensure that the different natural enemies are able to choose the appropriate host. In this context, Ardeh *et al*. (2005) insisted to the fact that an important element of host selection in parasitoids is "host discrimination", which is the capability to distinguish between parasitized and unparasitized hosts.

In our study, we explored interactions between two natural enemies of the codling moth *Cydia pomonella* (L.) (Lepidoptera Tortricidae), namely the egg-larval parasitoid *Ascogaster quadridentata* Wesmael (Hymenoptera Braconidae) and the egg parasitoid *Trichogramma cacoeciae* Marchal (Hymenoptera Trichogrammatidae). Codling moth is a notorious apple fruits boring pest worldwide (Mills, 2005), due to direct damage of fruits and increasingly resistance against insecticides as well as viral biopesticides (Bosch *et al.*, 2007). New strategies preferably built on biological control methods have to be developed for sustainable regulation of this pest, also taking into account that the parasitoid community of the codling moth is highly effective, when not disturbed by the use of pesticides (Maini and Mosti, 1988; Hassan *et al.*, 1998;

Cross *et al.*, 1999). Previous research showed that *A. quadridentata* and the larval parasitoid *Hyssopus pallidus* (Askew) (Hymenoptera Eulophidae) were incompatible in using *C. pomonella* as host. *H. pallidus* had competitive superiority and also experienced a distinct fitness advantage from parasitizing host larvae previously parasitized by *A. quadridentata* (Zaviezo and Mills, 2001). In our approach we focused on the question: whether there is an interaction between the egg parasitoid *T. cacoeciae* and the egg-larval parasitoid *A. quadridentata* that allows them the partition of the host resource or not, thus facilitating or hindering *C. pomonella* biological control in the case of *Trichogramma* mass-releases and natural control provided by endemic *A. quadridentata*. We conducted non-choice and choice experiments to explore the ability of females for interspecific host discrimination between parasitized and unparasitized hosts as well as to examine their antagonistic behaviour during parasitization of hosts.

Materials and methods

Insect origin

Rearing of *C. pomonella*

C. pomonella eggs used during the experiments were provided from a permanent rearing maintained under controlled conditions (22 ± 1 °C; 60 ± 10% RH; 16/8 L/D) at our laboratory. Adult moths were held in cage cylinders (3 litres volume) and were provided with sugar solution for nutrition and with smooth transparent plastic sheets for egg laying. Eggs were harvested daily by changing the plastic sheets and served either for maintaining the codling moth and *A. quadridentata* rearing or to provide the experiments.

Rearing of *A. quadridentata*

Adults were obtained from a permanent rearing maintained at controlled conditions (22 ± 1 °C; 60 ± 10% RH; 16/8 L/D) at our laboratory. *A. quadridentata* were put upon their emergence in a glass cage (30 × 30 × 30 cm) and supplied with pure honey and also water. Plastic sheets with fresh *C. pomonella* eggs were put into the cage for parasitization by female wasps and replaced after two days. Parasitized eggs were further incubated until emergence of larvae, which were subsequently put on diet for further rearing. Parasitized larvae left the diet after completion of the development and these were transferred in boxes supplied with corrugated cardboard in which they could hide for pupation until *A. quadridentata* adults' emergence. Prior to the experiments, newly emerged adults were put all together in a specific glass cage (30 × 30 × 30 cm), fed on water and honey and mated about one day. For sex determination, wasps were shortly anaesthetized with CO_2 and examined under the binocular for characteristics of their abdomen (DeLury, 1998) and antennae (A. Herz, personal observation), and then females were transferred singly into glass vials.

Rearing of *T. cacoeciae*

The stock population of *T. cacoeciae* was maintained on the eggs of the factitious host *Sitotroga cereallela* (Olivier) (Lepidoptera Gelechiidae) in incubators at alternating conditions (seven days at 25 ± 1 °C; 60 ± 10% RH; 16/8 L/D, following seven days at 18 ± 1 °C; 60 ± 10% RH; 16/8 L/D until emergence of the new generation. *S. cereallela* eggs were purchased from a supplier, glued on paper with diluted Arabic gum and kept at (3 ± 0.1 °C; 60 ± 10% RH; 0/24 L/D) up to three weeks without any negative effects on egg quality until their use in parasitoid rearing. Prior to the test, and for at least two generations, *T. cacoeciae* species was maintained on *S. cereallela* eggs under laboratory conditions at permanent conditions at 25 ± 1 °C; 60 ± 10% RH; 16/8 L/D until their emergence. *T. cacoeciae* is an obligatory thelytokous species (Vavre *et al.*, 2004) and no sex differentiation of adults was necessary. Newly emerged females were placed singly in glass test tubes (50 mm long × 12 mm in diameter, closed by a cotton plug) with a small drop of honey under laboratory conditions (25 ± 1 °C; 60 ± 10% RH; 16/8 L/D) for a day before being used in experiments.

Experiments to test interspecific host discrimination of *T. cacoeciae* and *A. quadridentata* females

Non-choice situation (parasitized egg)

Parasitoid females were singly held in glass test tubes (50 mm long × 12 mm in diameter, closed by a cotton plug) for the experiment. Two *C. pomonella* egg qualities were provided: fresh or one-day old eggs. First, only a single egg - in order to force the female to consider it - (either a fresh or a one-day old one) was exposed to each female (either *T. cacoeciae* or *A. quadridentata*) during 90 min or 30 min, respectively. The different duration of the exposure period to one or the other parasitoid species was chosen due to large differences in their size, activity range and egg parasitization behaviour. *T. cacoeciae* was allocated triple the experimental time granted to *A. quadridentata* because *T. cacoeciae* needed more time to start parasitizing a host egg, while *A. quadridentata* oviposited faster (Ksentini and Herz, unpublished data). During this exposure time, the oviposition behaviour of the respective female was observed and it was noted if the parasitoid effectively parasitized the egg or not. The egg was categorized as "parasitized" in the case the oviposition was observed and as "exposed" in the case that no oviposition occurred. Then the parasitoid of the first species was removed, discarded from further experimentation, and a female of the other species was introduced into the test tube after a specific time (0, 4, 8, 12, 24 or 48 hours after removal of the first parasitoid female). Then, the parasitoid of second species was in contact with the egg for either 30 min (*A. quadridentata*) or 90 min (*T. cacoeciae*), and then discarded from further experimentation. Again, during exposure time, the oviposition behaviour of the respective female was observed and it was noted if the parasitoid effectively oviposited in the egg or not. All the experiments were fully inspected, conducted under controlled conditions (25 ± 1 °C; 60 ± 10% RH), and the number of tested wasps ranged from 16 to 27 per parasitoid/egg combination (table 1). After the experiment, eggs were further incubated and the develop-

Table 1. Number of replicates per combination and egg category, used in the non-choice situation.

Time of presentation	Eggs parasitized initially by *T. cacoeciae*, then presented to *A. quadridentata* after						Eggs parasitized initially by *A. quadridentata*, then presented to *T. cacoeciae* after					
	0h	4h	8h	12h	24h	48h	0h	4h	8h	12h	24h	48h
	N replicates per egg quality						N replicates per egg quality					
Fresh	18	17	20	18	20	19	20	20	16	18	19	27
1 day old	20	20	20	16	20	20	19	24	22	21	22	24

ing organism (*C. pomonella*, *A. quadridentata* or *T. cacoeciae*) was determined by examination of the egg (blackened if *T. cacoeciae* was developing) or dissection of the newly emerged larva under the binocular (as explained below).

Choice situation (parasitized versus unparasitized egg)

The first species (either a female of *T. cacoeciae* or *A. quadridentata*) was allowed to effectively oviposit in a single *C. pomonella* egg (either a fresh or a one-day old one) for either 30 min (*A. quadridentata*) or 90 min (*T. cacoeciae*). This was performed with so many females of parasitoid species 1 until ten parasitized eggs were obtained. Each already parasitized egg was combined with an unparasitized egg of the same age and this pair of eggs was presented to a female of the second species (either *A. quadridentata* or *T. cacoeciae*). The egg pair was presented to the second parasitoid after a specific time following the first oviposition event (0, 24 or 48 hours). Parasitoid females were singly held in glass test tubes (50 mm long × 12 mm in diameter, closed by a cotton plug) for all experimental steps. *T. cacoeciae* was allowed to contact the egg(s) during 90 min and *A. quadridentata* during 30 min and the behaviour of each female was permanently observed during the experimental exposure. Each parasitoid/egg combination was replicated 10 times and every wasp was used only once. All tests were fully inspected and conducted under controlled conditions (25 ± 1 °C; 60 ± 10% RH). After the experiment, eggs were further incubated and the developing organism (*C. pomonella*, *A. quadridentata* or *T. cacoeciae*) was determined by examination of the egg (blackened if *T. cacoeciae* was developing) or by larva dissection under the binocular (as explained below).

Experiments to test antagonistic behaviour between *T. cacoeciae* and *A. quadridentata* females

Single females of both species were either introduced together, or the second species was introduced when the first species started parasitizing the single provided *C. pomonella* egg (fresh or one-day old eggs). Testing vial was again a small glass tube (50 mm long × 12 mm in diameter, closed by a cotton plug). Afterwards, the parasitoid females were left together over night in an incubator at controlled conditions (25 ± 1 °C; 60 ± 10% RH; 16/8 L/D). Ten replicates were undertaken for each experiment, and every wasp was used once.

In all experiments, the attack and the possible oviposition (Kainoh and Hiyori, 1982; Schmidt, 1994; Wang *et al.*, 2004; Paraiso *et al.*, 2013) of each tested female was observed during the duration of the given parasitization time. Rosenberg (1934) described explicitly the oviposition behaviour of *A. quadridentata* and these descriptions were used in our case to conclude if oviposition had occurred. In the case of *Trichogramma*, we relied on the description by Klomp *et al.* (1979), Blanche *et al.* (1996) and Paraiso *et al.* (2013) to distinguish oviposition from other behaviours. All tested eggs were kept in an incubator at controlled conditions (25 ± 1 °C; 60 ± 10% RH; 16/8 L/D) for development. Developing *T. cacoeciae* inside the codling moth was recognized by blackening of the egg. If codling moth larvae instead emerged, these were dissected to check for parasitism by *A. quadridentata*. In addition, the development of the various organisms in the host egg was also documented by examination of single eggs in dark field illumination under a Zeiss Axioplan microscope.

Data analyses

Graphical evaluation and statistical analysis of data were performed using the software package SigmaPlot (version 13.0). Parasitoid females which had been observed ovipositing into offered host eggs were considered as "responding females". In the non-choice experiment, the number of responding females of the first parasitoid (species 1) was determined. It indicated the number of eggs parasitized by this species which were subsequently offered to females of the second parasitoid (species 2). The number of responding females of the second parasitoid species was also obtained based on observations. Their host acceptance of previously unparasitized codling moth eggs versus those previously parasitized by parasitoid species 1 was estimated by comparing the proportion of females responding to one egg category versus the other. These proportions were compared by Fisher's exact test for potential significant differences for each parasitoid/egg (quality and parasitization age) combination.

Data of the choice-experiment, where unparasitized and parasitized eggs (by parasitoid species 1) had been offered simultaneously, were analysed by comparing the proportion of eggs of both categories which were parasitized by parasitoid species 2 using the McNemar's test for comparing paired proportions.

Results of the experiment testing antagonistic behaviour of parasitoid females, where unparasitized eggs had been offered simultaneously to both parasitoids or immediately to parasitoid species 2 after their examination by parasitoid species 1, were presented by measuring the number of the following events in the different treatments (age of offered eggs): 1) the egg was not parasitized by any of the two parasitoids (CP), 2) it was resorbed due to superparasitism by one or both parasitoids (RE) or 3) it developed into one of the tested parasitoids (TC/AQ). By the end of each experiment, the physical state of the tested parasitoids was not taken into consideration when treating the results. Successful parasitism by *T. cacoeciae* (event TC) in the three different scenarios was compared using Fisher's exact test with Freeman-Halton extension for 2 × 3 contingency tables.

Results

Host discrimination

Non-choice situation (parasitized egg): being the first parasitoid, on average eleven females of *A. quadridentata* responded positively to the provided fresh or one-day old host eggs (figure 1), and corresponded to around 59% (fresh eggs) and 52% (one-day old eggs) of the tested females (n = 20 ± 3.7 and n = 22.0 ± 1.9 respectively). Even with more time, only 26% (fresh eggs) and 17% (one-day old) of tested females of *T. cacoeciae* (n = 18.7 ± 1.2 and n = 19.3 ± 1.6 respectively) responded to the unparasitized codling moth eggs (figure 1), which means that most of these eggs that had been exposed to *T. cacoeciae*, were left unparasitized. Both parasitoids did not show any significant preference for a particular host age.

When offering these eggs (effectively parasitized by parasitoid 1 or exposed, but unparasitized) to females of the second parasitoid species, their host acceptance was evaluated by comparing the proportion of females responding positively to one egg category versus the other. This was done for each host egg age (fresh eggs, one day old egg) and parasitization age (0, 4, 8, 12, 24 and 48 hours) separately. *T. cacoeciae* as second parasitoid did not display any difference between acceptance of one or the other egg category in the case fresh host eggs were offered (figure 2a, Fisher's exact test, P ranging between 0.380 and 1). When exposed to one-day-old eggs, females of *T. cacoeciae* did not oviposit into eggs eight hours after parasitization by *A. quadridentata* (figure 2b, Fisher's exact test, P = 0.046). In all other trials there was no significant difference between acceptance of previously parasitized and unparasitized host eggs. Multiparasitism did always lead to the successful development of *T. cacoeciae* offspring and the abortion of the *A. quadridentata*-first instar larva inside the host egg (figures 3 and 4).

Females of *A. quadridentata* seemed to be more attracted to eggs, which had not been parasitized by *T. cacoeciae* previously (figure 5), although this tendency was significant only at four hours after first oviposition in fresh eggs (Fisher's exact test, P = 0.022) and 12 hours after first oviposition in one day old host eggs (Fisher's exact test, P = 0.026). In most trials, less than 50% of the tested *A. quadridentata* females were confronted to eggs previously parasitized by *T. cacoeciae*, so results were certainly biased due to the unequal presentation of unparasitized and parasitized eggs. Multiparasitism always led to the successful development of *T. cacoeciae* offspring and abortion of the 1st instar larva of *A. quadridentata* (figures 3 and 4).

In the choice experiment, host acceptance was analysed by comparing the proportion of eggs of both categories (unparasitized and parasitized by parasitoid 1) which were parasitized by parasitoid species 2, for each host egg age (fresh eggs, one day old eggs) and parasitization age (0, 1, 2 days) separately. No significant differences were observed in the acceptance of the two egg categories by parasitoid 2, being *A. quadridentata* (figure 6a) or *T. cacoeciae* (figure 6b).

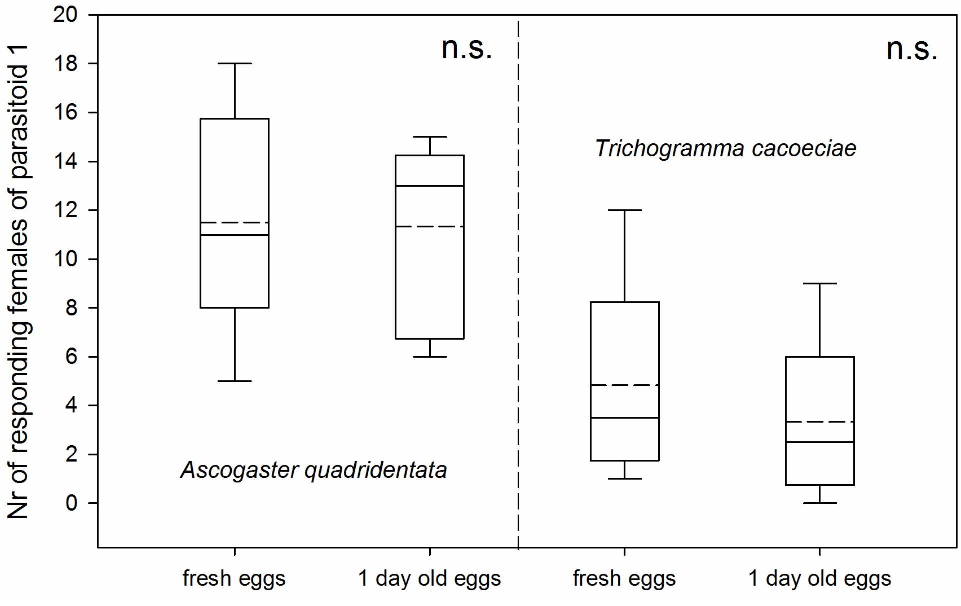

Figure 1. Non-choice test: Number of responding females of parasitoid species 1 (either *A. quadridentata* or *T. cacoeciae*) to fresh and one-day old eggs of *C. pomonella* in the non-choice situation of the host discrimination experiment. n.s.: no significant difference was found according to t-test or Mann-Whitney Rank Sum Test. In box, dashed line: mean, solid line: median. Data were obtained from six experimental combinations per egg category, each with 16 to 27 replicates.

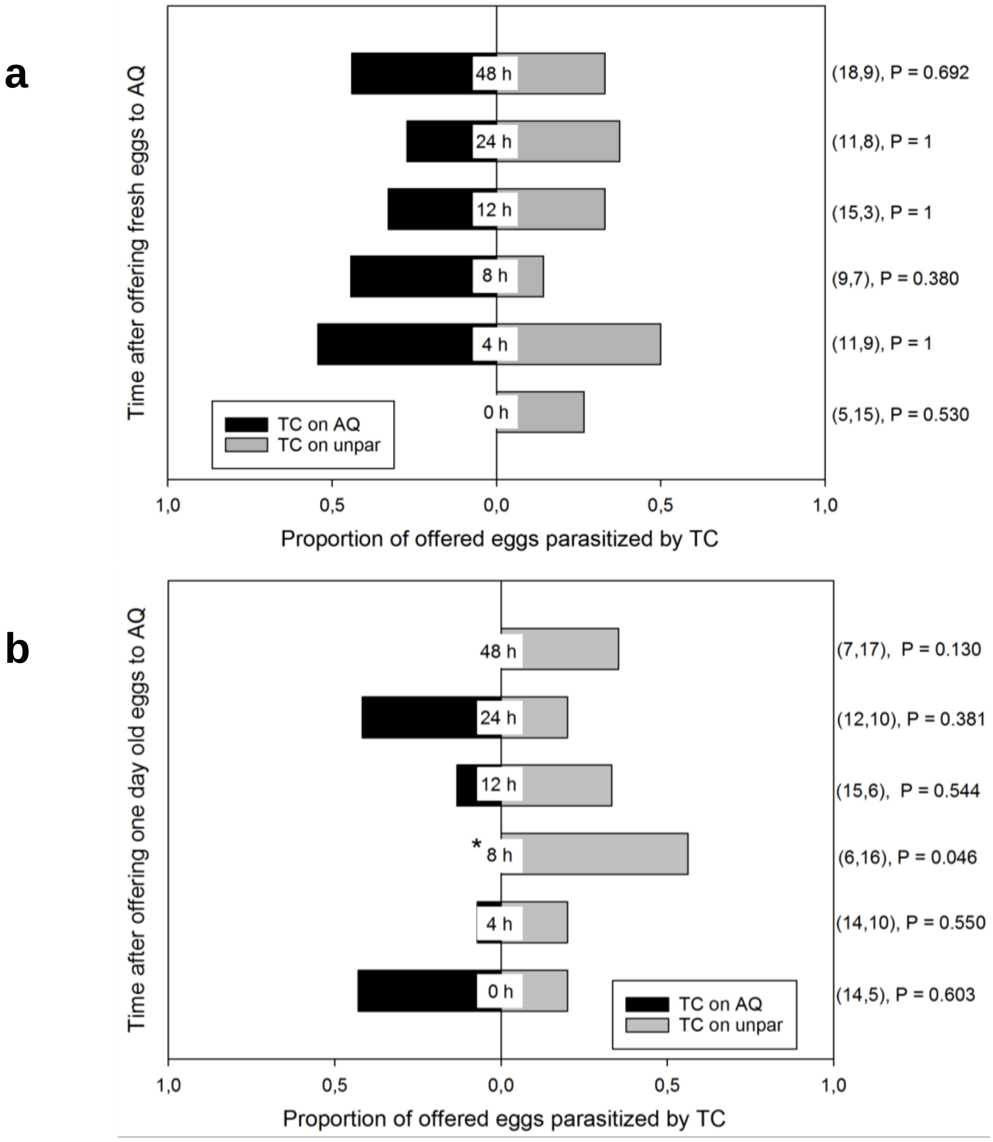

Figure 2. Non-choice test: proportion of eggs parasitized by *T. cacoeciae* females (TC) after exposure of unparasitized (grey bars: TC on unpar) or parasitized *C. pomonella* eggs which had been previously offered to females of *A. quadridentata* (AQ) (black bars: TC on AQ) for 30 min and then provided at particular times (0-48 hours) to *T. cacoeciae*. Numbers in parentheses give numbers (replicates) of AQ parasitized versus unparasitized eggs. Each egg was presented to one female. Proportions were compared with Fisher's exact test and P-values are given. *: significant at $P \leq 0.05$. **a**: offering fresh eggs to parasitoid 1 (AQ), **b**: offering one day old eggs to parasitoid 1 (AQ).

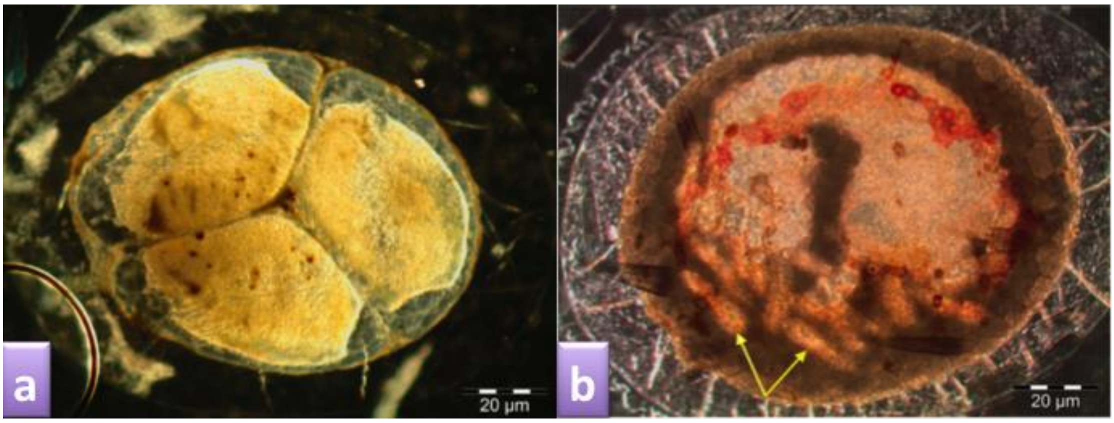

Figure 3. One day old *C. pomonella* egg; **a**: parasitized by *T. cacoeciae* since 3 days. Inside the egg, 3 *Trichogramma* nymphs are visible. **b**: parasitized by *A. quadridentata*. Arrows point to *A. quadridentata* larvae near *C. pomonella* embryo.

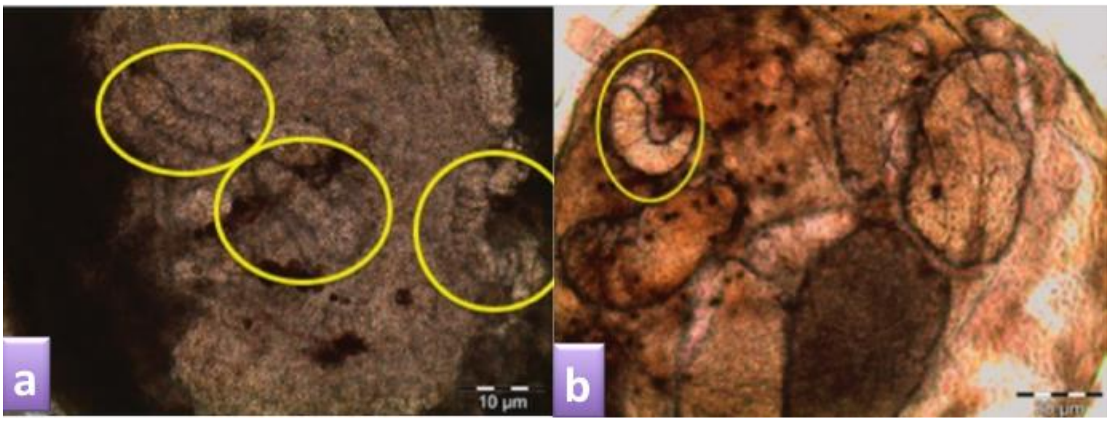

Figure 4. *C. pomonella* egg parasitized first by *A. quadridentata* and then by *T. cacoeciae*: **a**: circles show *A. quadridentata* larvae surrounded by dissolved cells. **b**: (**a** after one day) the circle shows the last surviving *A. quadridentata* larva near the developing *T. cacoeciae* larvae.

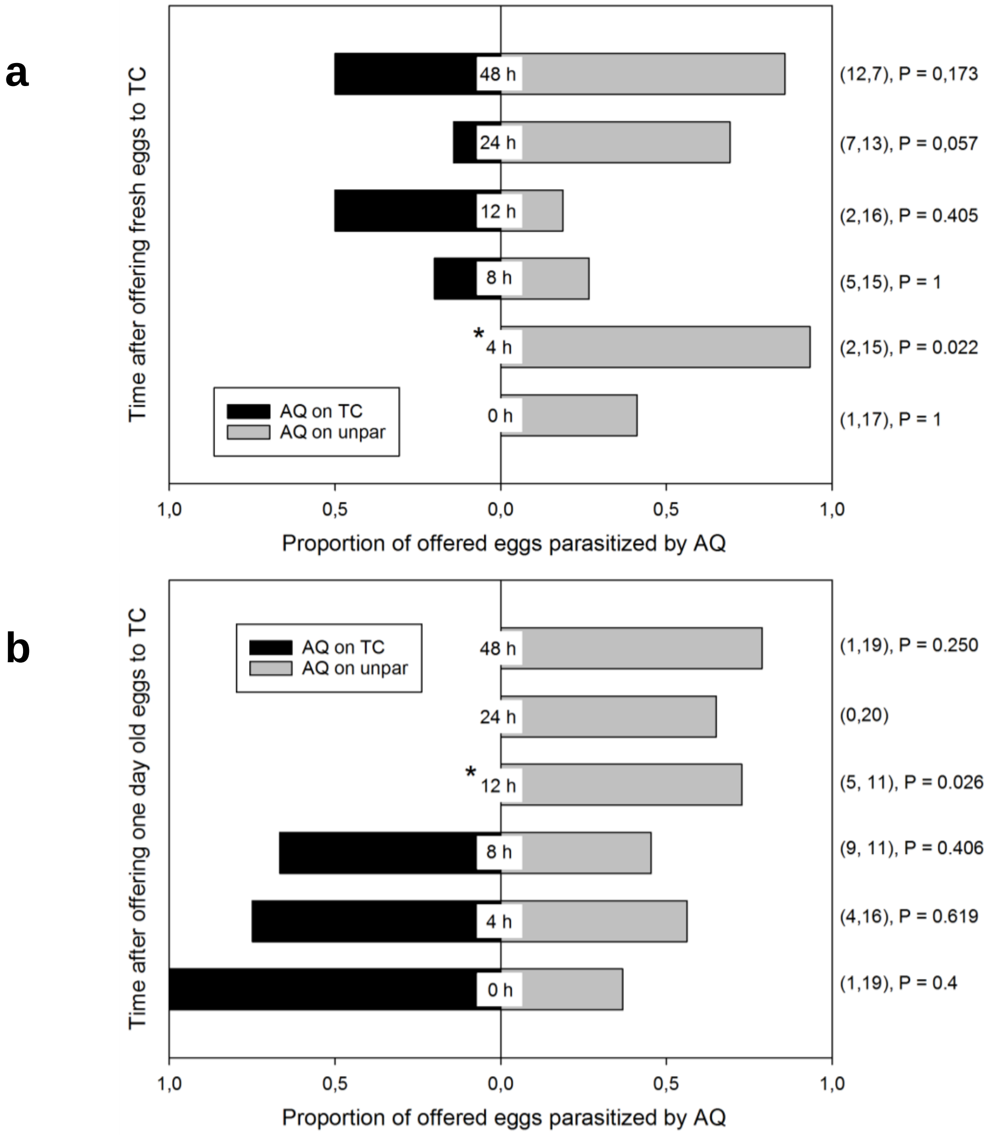

Figure 5. Non-choice test: Proportion of eggs parasitized by *A. quadridentata* females (AQ) after exposure of unparasitized (grey bars: AQ on unpar) or parasitized *C. pomonella* eggs which had been previously offered to females of *T. cacoeciae* (TC) (black bars: AQ on TC) for 90 min and then provided at particular times (0-48 hours) to *A. quadridentata*. Numbers in parentheses give numbers (replicates) of TC parasitized versus unparasitized eggs. Each egg was presented to one female. Proportions were compared with Fisher's exact test and P-values are given. *: significant at $P \leq 0.05$. **a**: offering fresh eggs to parasitoid 1 (TC), **b**: offering one day old eggs to parasitoid 1 (TC).

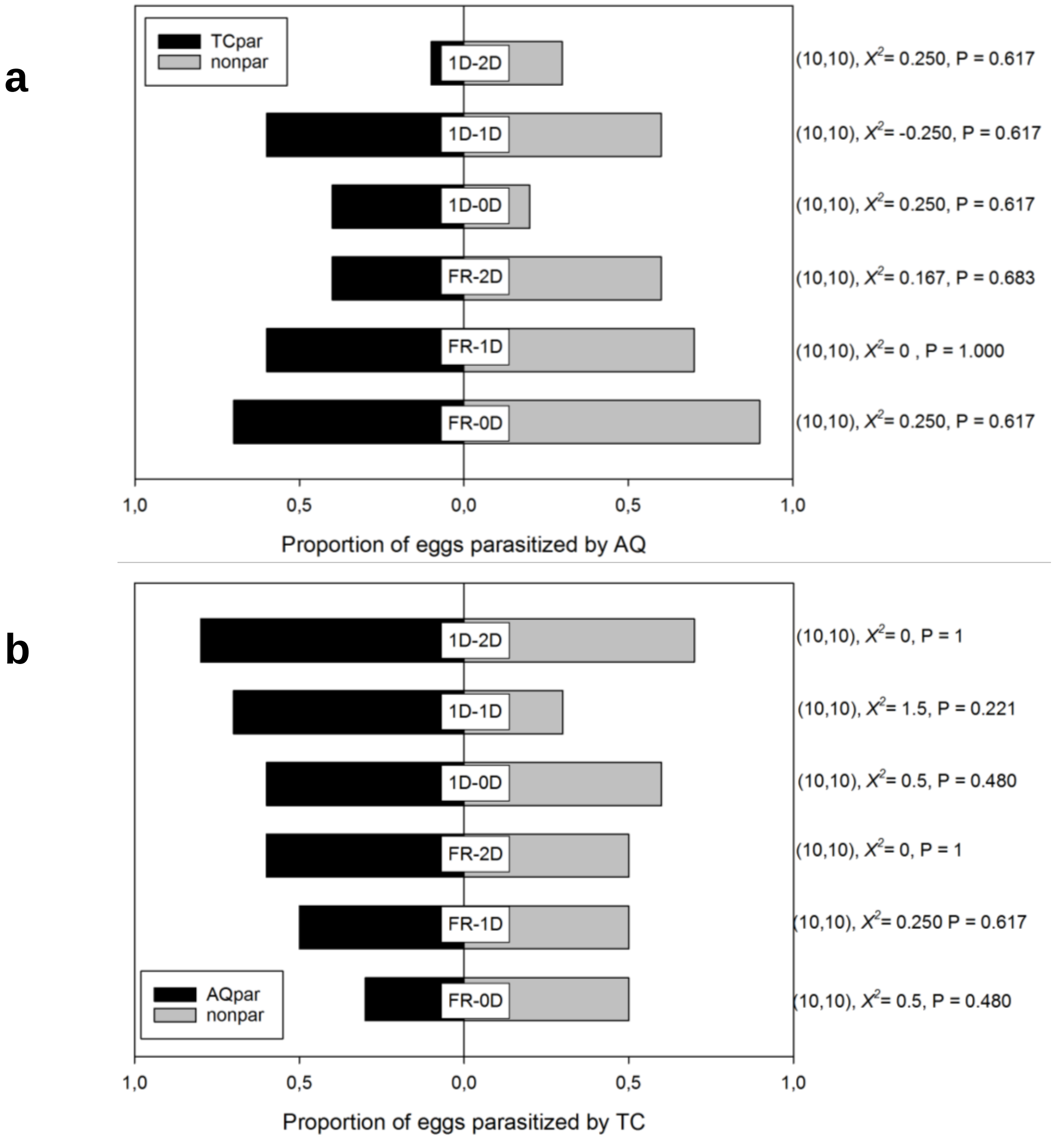

Figure 6. Choice test: proportion of eggs parasitized by parasitoid species 2 (*A. quadridentata* females (**6a**) or *T. cacoeciae* (**6b**)) when simultaneously provided with one unparasitized (nonpar) and one parasitized host egg (**6a**: TCpar: by *T. cacoeciae*; **6b**: AQpar: by *A. quadridentata*). Numbers in partentheses give numbers (replicates) of AQ- or TC-parasitized versus unparasitized eggs. Each egg pair was presented to one female of parasitoid species 2. FR: fresh eggs. 1D: one day old eggs. Time of presenting eggs to parasitoid species 2 after parasitization by parasitoid species 1: directly (0D), one day delay (1D), two days delay (2D). Proportions were compared with McNemar's test and χ^2 and P-values are given.

Antagonistic behaviour competition between *T. cacoeciae* and *A. quadridentata* females

In experiments one and two, where both parasitoids were introduced together or where *A. quadridentata* was allowed to encounter eggs first, less than 50% of the eggs were successfully parasitized by *T. cacoeciae* afterwards. In contrast, in the third experiment, where *T. cacoeciae* was allowed to start the parasitization, the success of *T. cacoeciae* was higher (on average 75%, event "TC"). Considering each host egg age separately (figure 7), parasitism on one day old eggs by *T. cacoeciae* was significantly less in the scenario "together" or "AQ first" in comparison to "TC first" (Fisher's exact test, P = 0.0084). For fresh host eggs it was also less, but the difference was not significant (Fisher's exact test, P = 0.4667).

Discussion and conclusions

A. quadridentata is a solitary koinobiont egg-larval endoparasitoid, with some potential for the control of the codling moth *C. pomonella* (Mohamad *et al.*, 2015). Its life cycle is well synchronised with that of the codling moth (Brunner, 1993). Female *A. quadridentata* places a single egg, just under the chorion or into the yolk of a *C. pomonella* egg that hatches and penetrates into the developing host embryo egg two to three days after oviposition (Brown *et al.*, 1990). This first instar larva remains inside the host larva but does not begin to develop and consume it entirely before the host larva reaches the fourth instar (Brunner, 1993). Although *A. quadridentata* is a biological control agent that shows promise for reducing *C. pomonella* populations (DeLury, 1998), the

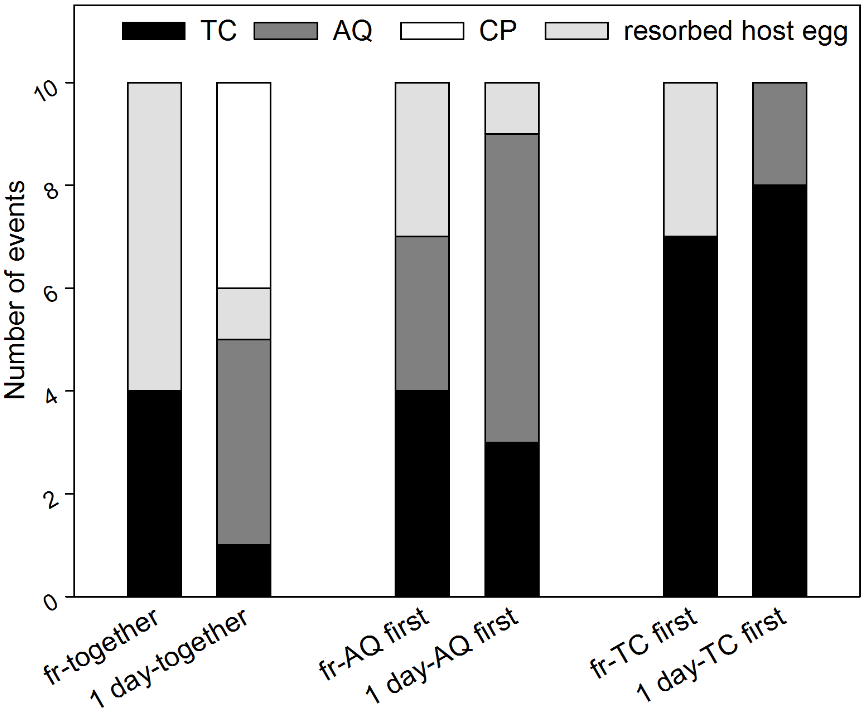

Figure 7. Antagonistic behaviour between females: number of developing organisms from fresh and 1 day old *C. pomonella* eggs after their exposition simultaneously to *T. cacoeciae* and *A. quadridentata* (treatment: fr-together, 1day-together), to *T. cacoeciae* immediately when *A. quadridentata* started examining the host (treatment: fr-AQfirst, 1day-AQfirst) and to *A. quadridentata* immediately when *T. cacoeciae* started examining the host egg (treatment: fr-TCfirst, 1day-TCfirst). Eggs development were classified as the following: the egg was not parasitized by both parasitoids and resulted in a healthy codling moth larva (CP), was resorbed due to superparasitism by one or both parasitoids (RE) or developed into one of the tested parasitoids (TC/AQ). Data were obtained from ten replicates per treatment.

identification of an additional parasitoid species that can break the host refuge from parasitism and shows no evidence of antagonistic interactions may provide a powerful opportunity to achieve greater success in biological control (Mills, 2005). However, being a koinobiont that allows the host to continue its development after oviposition (Santos and Quicke, 2011), *A. quadridentata* is vulnerable as another parasitoid species could find, attack, and exploit the same resource (Magdaraog *et al.*, 2012). Hence it is necessary that the second species to be released is selective in the sense that it avoids the hosts already parasitized by *A. quadridentata* and vice versa. The ability for host discrimination gives an advantage to parasitoid females by reducing time and eggs wasting, and by minimizing the mortality risk for their offspring (van Lentern, 1981; Ardeh *et al.*, 2005).

In an attempt to control the Emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera Buprestidae), Yang *et al.* (2013) reported that the subsequent exposure of parasitized hosts to *Tetrastichus planipennisi* Yang (Hymenoptera Eulophidae) did not affect either parasitism rates by or progeny production of *Spathius agrili* Yang (Hymenoptera Braconidae) because *T. planipennisi* was able to host discriminate previously parasitized from healthy hosts. The authors concluded that competitive interactions between these two parasitoids species could occur, but were less important in the case of simultaneous releases in a biological control programme against *A. planipennis*. Also, Ardeh *et al.* (2005) mentioned that *Eretmocerus eremicus* Rose et Zolnerowich (Hymenoptera Aphelinidae) females avoided to multiparasitize *B. tabaci* hosts which had been already parasitized by *Eretmocerus mundus* Mercet (Hymenoptera Aphelinidae). However, hosts already parasitized by *E. eremicus* were found to be multi-parasitized by *E. mundus* females, making the latter detrimental to the first one's survival after few generations whenever they share the same ecological niche. In our study, *T. cacoeciae* did not differentiate between unparasitized and parasitized host eggs except in one case, (figure 2b). In the non-choice situation, *A. quadridentata* tended to avoid the already parasitized host eggs to some extent, but this was not the case in the choice experiment. Host discrimination might be possible due to modifications inside the parasitized host egg, and/or the effect of some changing cues during egg development. Female wasps accept or reject host eggs based on visual, tactile, olfactory and gustatory stimuli (Vinson, 1975; Calvin and Losey, 1991; Huang and Gordh, 1998), and for instance *A. quadridentata* is known to exploit host derived stimuli that change throughout the host development (DeLury, 1998). In this study, we offered fresh and 1-day old eggs for the first parasitization. Neither *A. quadridentata* nor *T. cacoeciae* showed preference for any of these host ages (figure 1). Ables *et al.* (1981) found that *Chelonus insularis* (Cresson) (Hymenoptera

Braconidae) preferred host eggs older than 24 hours, while *Telenomus heliothidis* Ashmead (Hymenoptera Scelionidae) more frequently attacked host eggs ≤ 24 hours old and that *Trichogramma pretiosum* Riley (Hymenoptera Trichogrammatidae) was less selective than the other two species for host age. On the other hand, previous studies found that *T. cacoeciae* preferred fresh eggs (Hegazi *et al.*, 2000; Ksentini *et al.*, 2014), in comparison to old eggs, because of their better suitability (Ruberson and Kring, 1993). In fact, the contents of young host eggs, consisting primarily of yolk, appear to be well suited to ingestion by *Trichogramma* larvae (Jarjees and Merritt, 2003). Older host eggs can be more cuticularized, apparently resistant to penetration of venom, and do not break down easily into dissociated cells (Jarjees and Merritt, 2004). Female wasps may be able to recognize the older hosts as a less suitable resource for production of their progeny than younger hosts (Honda and Luck, 2000). But this supposed relationship between host age, duration since the first parasitization and host acceptance by the second parasitoid species need to be investigated in additional experiments with more replicates and probably less constrained conditions.

The idiobiont *T. cacoeciae* female immediately kills the host (Jarjees and Merritt, 2003), so that the venoms and toxins injected during oviposition, enables parasitization of hosts and produces an appropriate milieu for larval development (Jarjees and Merritt, 2004), via host necrosis (Olson, 1998), and cells dissociation (Jarjees and Merritt, 2004). Although Frilli (1968) previously denied *A. quadridentata* females' ability to distinguish between already parasitized eggs, the results in the non-choice experiment let us assume that *A. quadridentata* females could somehow detect host egg metabolic changes, because some tendency to non-acceptance of previously parasitized hosts was observed (fresh eggs: 0 and 4 hours observations, figure 5a; one day old eggs: 12, 24 and 48 hours observations, figure 5b; not all significant,). In this context, Vinson (1998) proposed that female parasitoids might perceive changes in the chemistry of the parasitized host due to venoms and to other secretions. On the other hand, *A. quadridentata* females were observed attacking fresh eggs already parasitized by *T. cacoeciae* since 2 days although the host larva cells were completely dissociated. In the same context, they rejected one-day old eggs already 12 hours after their parasitization by *T. cacoeciae* although the latter egg hatched around 28-29 hours at 25 °C after oviposition (Volkoff *et al.*, 1995). Jarjees and Merritt (2004) mentioned that in one-day old *Helicoverpa armigera* (Hubner) (Lepidoptera Noctuidae) eggs examined 21 hours after their parasitization by *T. australicum* (at 29 °C), the embryonic segments remain discernible, indicating that the structure of host embryo is not completely lost. Boyce (1936 in DeLury, 1998) mentioned that *A. quadridentata* females had been observed attempting to oviposit into shells of hatched eggs or where host eggs have been removed. The observation of this somewhat erratic behaviour of *A. quadridentata* females lead us supposing that host acceptance and host discrimination may also be affected by experience and oviposition pressure.

Although Reed-Larsen and Brown (1990) and DeLury (1998) indicated that *A. quadridentata* females rely on chemical cues to detect the presence of host eggs of any age, it is not clear whether they could exploit these stimuli effectively to recognize already parasitized eggs by *T. cacoeciae*. We further supposed that *A. quadridentata* could detect some marking substance left by *T. cacoeciae* during egg parasitization, *A. quadridentata* exhibits some marking behaviour on the host egg after oviposition (Herz, personal observation; Rosenberg, 1934), however it is not known whether any marking substance is left and, if yes, such a substance would dissuade the second parasitoid female from exploiting the same resource. Ables *et al.* (1981) noticed during interspecific tests, that none of *T. heliothidis* and *T. pretiosum* or *C. insularis* showed any tendency to reject eggs parasitized by the other two species in favour of unparasitized eggs, thus confirming the non-effect of host marking pheromones and other stimuli on females of the three different species, although they were effective towards conspecific females. In fact, the ability to identify hosts attacked by other species is less frequent than discrimination against hosts attacked by conspecifics (Fellowes *et al.*, 2007). However, it is advantageous for a superior parasitoid to oviposit in hosts parasitized by the inferior species (van Alphen and Visser, 1990). In our study, *A. quadridentata* accepted already parasitized eggs, thus eventually opting for the same adaptative strategy as found in reported cases of superparasitism in other parasitoid species (Charnov and Skinner, 1984), when hosts are scare or searching for unparasitized hosts would be inefficient (van Velzen *et al.*, 2016).

Such behaviour was also observed in the choice tests, where only two eggs (parasitized versus unparasitized) were presented to each female. Both parasitoids did not discriminate between the host egg conditions and parasitized the eggs in same proportions. The idiobiont *T. cacoeciae* is considered to be a superior intrinsic competitor in comparison to the koinobiont *A. quadridentata* as it kills the host egg - and as a consequence *A. quadridentata* embryo - regardless of its previous parasitization. On the contrary, Cabello *et al.* (2011) avoided classifying species into superior and inferior competitors as they observed a reduction in the emergence rate of *Trichogramma brassicae* Bezdenko (Hymenoptera Trichogrammatidae) offspring, particularly when the egg-larval parasitoid *Chelonus oculator* (F.) (Hymenoptera Braconidae) was the last to parasitize, probably due to the toxins and polydnaviruses injected by *C. oculator* female. This finding implies that the harmful effect of competition does not only affect the competitive inferior species, but may also have a marked effect on the competitive superior species. However, in our study, we did not observe a negative effect of the subsequent parasitization by *A. quadridentata* on previously parasitized host eggs on *T. cacoeciae*, as those host eggs evolved solely in viable *Trichogramma* adults. Nonetheless, Collier *et al.* (2002) assure that species that tend to win in intrinsic competition should lose in extrinsic competition and vice versa. Indeed, Mahmoud and Lim (2008) assure that *Trissolcus nigripedius* (Nakagawa) (Hymenoptera Scelionidae) always win in adult competition against *Teleno-*

mus gifuensis Ashmead (Hymenoptera Scelionidae) (extrinsic competition) while the latter species may defeat the former species at all times in immature competition inside multiparasitized host eggs (intrinsic competition). In this context, our results showed that although superior in interspecific competition, females of *T. cacoeciae* had apparently difficulties to oviposit in the presence of adult *A. quadridentata* (see video in supplemental material). In fact, the amount of eggs successfully evolving into *T. cacoeciae* offspring was inferior when its introduction was simultaneous or following that of *A. quadridentata*. However, we think that *T. cacoeciae* ability to oviposit in the presence of an impressive parasitoid such as *A. quadridentata* was a possible task. In reality, *T. cacoeciae* could be subject to *A. quadridentata* aggressiveness, although unintentional. Yet, such behaviour could be more possible if both parasitoids were of equal sizes. In this context and in an attempt to control the coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera Scolytidae) with three Bethylidae wasps, Perez-Lachaud *et al.* (2002) found that *Cephalonomia stephanoderis* Betrem (Hymenoptera Bethylidae), *Cephalonomia hyalinipennis* Ashmead (Hymenoptera Bethylidae), and *Prorops nasuta* Waterston (Hymenoptera Bethylidae) females actively defend their hosts and progeny. In fact, these resources defence generally consisted of pursuit, biting and stinging which often resulted in paralysis and death (Batchelor *et al.*, 2005). Moreover, *C. stephanoderis* was found to transport the intruder's corpse to the farthest cell of the apparatus (Perez-Lachaud *et al.*, 2002). This kind of behaviour was not recorded in our study, and the death of a few *A. quadridentata* and *T. cacoeciae* females by the end of the tests (data not shown) could be a simple consequence of a prolonged captivity in a small arena or an unintentional killing, especially that *A. quadridentata* could easily harm *T. cacoeciae* when actively tapping and examining *C. pomonella* eggs with its antennae. However, Mahmoud and Lim (2008) found - when attempting to study two parasitoids interaction - that *T. nigripedius* adults who are superior competitors in possessing and guarding the host eggs, could also fight with their antennae against *T. gifuensis*. Yet, such behaviour was unclear in our study against *T. cacoeciae*, but *A. quadridentata* female may be able to exhibit an aggressive behaviour and to engage in a fight against a conspecific female using its mandibles, as some corpses were found dismembered (Ksentini, personal observation). Nonetheless its aggressiveness towards *T. cacoeciae* was not proven and *A. quadridentata* was found to lose in intrinsic competition with *T. cacoeciae*. However, it is important to point out to the fact that in our experiments conditions, parasitoids were held in constrained artificial conditions. Besides both parasitoids were left together over night in a small arena; a circumstance that probably never occurs under natural conditions. Although the occurrence for interspecific competition may arise since each host supports the complete development of only one species (Uka *et al.*, 2006), these insect parasitoids will probably experience much lower rates of encounter under natural conditions. In this context, Ulyshen *et al.* (2010) insist that it remains difficult to predict how the two species will interact in nature based on a study in which the parasitoids were confined to small enclosures with limited resources. In conclusion, despite the fact that the combination of both parasitoids was proven to be detrimental to *A. quadridentata* offspring survival, further research should focus on the real impact of the simultaneous use of these parasitoids under field conditions.

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References

ABLES J. R., VINSON S. B., ELLIS J. S., 1981.- Host discrimination by *Chelonus insularis* (Hym.: Braconidae), *Telenomus heliothidis* (Hym.: Scelionidae), and *Trichogramma pretiosum* (Hym.: Trichogrammatidae).- *Entomophaga*, 26 (2): 149-156.

ARDEH M. J., DE JONG P. W., VAN LENTEREN J. C., 2005.- Intra- and interspecific host discrimination in arrhenotokous and thelytokous *Eretmocerus* spp.- *Biological Control*, 33: 74-80.

BATCHELOR T. P., HARDY I. C. W., BARRERA J. F., PÉREZ-LACHAUD G., 2005.- Insect gladiators II: competitive interactions within and between bethylid parasitoid species of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae).- *Biological Control*, 33: 194-202.

BATCHELOR T. P., HARDY I. C. W., BARRERA J. F., 2006.- Interactions among bethylid parasitoid species attacking the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae).- *Biological Control*, 36: 106-118.

BLANCHE S., CASAS J., BIGLER F., JANSSEN-VAN BERGEIJK K. E., 1996.- An individual-based model of *Trichogramma* foraging behaviour: parameter estimation for single females.- *Journal of Applied Ecology*, 33: 425-434.

BOSCH D., RODRIGUEZ M., AVILLA J., 2007.- A new bioassay to test insecticide resistance of *Cydia pomonella* (L.) first instar larvae: results from some field populations of Lleida (Spain).- *IOBC/wprs Bulletin*, 30 (4): 195-199.

BOYCE H. R., 1936.- Laboratory breeding of *Ascogaster carpocapsae* Vier. with notes on biology and morphology.- *The Canadian Entomologist*, 68: 241-246.

BROWN J. J., REED-LARSEN D., AHL J., 1990.- Physiological relationship between a diapausing endoparasitoid (*Ascogaster quadridentata*) and its dormant host (*Cydia pomonella*).- *Archives of Insect Biochemistry and Physiology*, 13: 229-238.

BRUNNER J. F., 1993.- *Ascogaster quadridentata* Wesmael (Hymenoptera: Braconidae). In: *Orchard pest management online* (BEERS E. H., BRUNNER J. F., WILLET M. J., WARNER G. M., Eds).- Good Fruit Grower, Yakima, WA, USA. [online] URL: http://jenny.tfrec.wsu.edu/opm/displayspecies.php?pn=927 (Accessed 31 January 2019).

CABELLO T., GÁMEZ M., TORRES A., GARAY J., 2011.- Possible effects of inter-specific competition on the coexistence of two parasitoid species: *Trichogramma brassicae* Bezdenko and *Chelonus oculator* (F.) (Hymenoptera: Trichogrammatidae, Braconidae).- *Community Ecology*, 12 (1): 78-88.

CALVIN D. D., LOSEY J. E., 1991.- Preferences of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) for three age classe of southwestern corn borer eggs, pp. 59-62. In: *Les colloques n° 56* (WAJNBERG E., VINSON S. B., Eds), Institut National de la Recherche Agronomique, Paris, France.

CHARNOV E. L., SKINNER S. W., 1984.- Evolution of host selection and clutch size in parasitoid wasps.- *Florida Entomology*, 67: 5-21.

COLLIER T., KELLY S., HUNTER M., 2002.- Egg size, intrinsic competition, and lethal interference in the parasitoids *Encarsia pergandiella* and *Encarsia Formosa*.- *Biological Control*, 23: 254-261.

CROSS J. V., SOLOMON M. G., BABANDREIER D., BLOOMERS L., EASTERBROOK M. A., JAY C. N., JENSER G., JOLLY R. L., KUHLMANN U., LILLEY R., OLIVELLA E., TOEPFERT S., VIDAL S., 1999.- Biocontrol of pests of apples and pears in Northern and Central Europe: 2. Parasitoids.- *Biocontrol Science and Technology*, 9 (3): 277-314.

DELURY N. C., 1998.- Pheromonal and kairomonal attraction of *Ascogaster quadridentata* Wesmael (Hymenoptera: Braconidae), a parasitoid of *Cydia pomonella* L. (Lepidoptera: Tortricidae), 95 pp. *Thesis*, Simon Fraser University, National Library of Canada, Ottawa, Canada.

FELLOWES M. D. E., VAN ALPHEN J. J. M., JERVIS M. A., 2007.- Foraging behaviour, pp. 1-71. In: *Insectes as natural enemies: a practical perspective* (JERVIS M., Ed.).- Springer, Dordrecht, The Netherlands.

FRILLI F., 1968.- Allevamento sperimentale di *Carpocapsa pomonella* L. e del suo parassita *Ascogaster quadridentatus* Wesm.- *Bollettino di Zoologia Agraria e di Bachicoltura*, 8: 182-190.

HASSAN S. A., HAFES B., DEGRANDE P. E., HERAI K., 1998.- The side-effects of pesticides on the egg parasitoid *Trichogramma cacoeciae* Marchal (Hym., Trichogrammatidae), acute dose-response and persistence tests.- *Journal of Applied Entomology*, 122 (9): 569-573.

HEGAZI E. M., KHAFAGI W. E., HASSAN S. A., 2000.- Studies on three species of *Trichogramma*. I. Foraging behaviour for food or hosts.- *Journal of Applied Entomology*, 124: 145-149.

HEINZ K. M., NELSON J. M., 1996.- Interspecific interactions among natural enemies of *Bemisia* in an inundative biological control program.- *Biological Control*, 6: 384-393.

HONDA J. Y., LUCK R. F., 2000.- Age and suitability of *Amorbia cuneana* (Lepidoptera: Tortricidae) and *Sabulodes aegrotata* (Lepidoptera: Geometridae) eggs for *Trichogramma platneri* (Hymenoptera: Trichogrammatidae).- *Biological Control*, 18: 79-85.

HUANG K., GORDH G., 1998.- Does *Trichogramma australicum* Girault (Hymenoptera: Trichogrammatidae) use kairomones to recognize eggs of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)?- *Australian Journal of Entomology*, 37: 269-274.

JARJEES E. A., MERRITT D. J., 2003.- Structure of the gut contents of *Trichogramma australicum* Girault (Hymenoptera: Trichogrammatidae) larvae fixed in situ in eggs of its host *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Australian Journal of Entomology*, 42: 203-209.

JARJEES E. A., MERRITT D. J., 2004.- The effect of parasitization by *Trichogramma australicum* on *Helicoverpa armigera* host eggs and embryos.- *Journal of Invertebrate Pathology*, 85: 1-8.

KAINOH Y., HIYORI T., 1982.- Kairomone of the egg-larval parasitoid, *Ascogaster reticulata* Watanabe (Hymenoptera: Braconidae).- *Appied Entomoly and Zoology*, 17 (3): 102-110.

KLOMP H., TEERINK B. J., MA W. C., 1979.- Discrimination between parasitized and unparasitized hosts in the egg parasite *Trichogramma embryophagum* (Hym.: Trichogrammatidae): a matter of learning and forgetting.- *Netherlands Journal of Zoology*, 30: 254-277.

KSENTINI I., JARDAK T., ZEGHAL N., 2014.- Could *Trichogramma* species be provided with old UV-killed *Ephestia kuehniella* eggs during mass rearing programs?- *Bulletin of Insectology*, 67 (2): 213-218.

MAGDARAOG P. M., HARVEY J. A., TANKAKA T., GOLES R., 2012.- Intrinsic competition among solitary and gregarious endoparasitoid wasps and the phenomenon of ‘resource sharing’.- *Ecological Entomology*, 37: 65-74.

MAHMOUD A. M. A., LIM U. T., 2008.- Host discrimination and interspecific competition of *Trissolcus nigripedius* and *Telenomus gifuensis* (Hymenoptera: Scelionidae), sympatric parasitoids of *Dolycoris baccarum* (Heteroptera: Pentatomidae).- *Biological Control*, 45: 337-343.

MAINI S., MOSTI M., 1988.- Relazioni tra *Archips rosanus* (L.) (Lepidoptera, Tortricidae) e *Trichogramma embryophagum* (Htg.) (Hymenoptera, Trichogrammatidae) in ecosistema naturale e coltivato.- *Bollettino dell'Istituto di Entomologia "Guido Grandi" dell'Università degli Studi di Bologna*, 42: 119-129.

MILLS N., 2005.- Selecting effective parasitoid for biological control introductions: codling moth as a case study.- *Biological Control*, 34: 274-282.

MOHAMAD F., MANSOUR M., RAMADAN A., 2015.- Effects of biological and environmental factors on sex ratio in *Ascogaster quadridentata* Wesmael (Hymenoptera: Braconidae), a parasitoid of *Cydia pomonella* L. (Torticidae).- *Journal of Plant Protection Research*, 55: 151-155.

OLSON D. M., 1998.- Oviposition and offspring survival within eggs of European corn borer: discrimination of the host embryo by female *Trichogramma nubilale*.- *Entomologia Experimentalis et Applicata*, 87: 79-84.

PARAISO O., HIGHT S. D., KAIRO M. T. K., BLOEM S., 2013.- Notes on the ovipositional behavior of *Trichogramma fuentesi* (Hymenoptera: Trichogrammatidae), an egg parasitoid of *Cactoblastis cactorum* (Lepidoptera: Pyralidae).- *Florida Entomologist*, 96 (4): 1606-1608.

PEREZ-LACHAUD G., HARDY I. C. W., LACHAUD J. P., 2002.- Insect gladiators: competitive interactions between three species of bethylid wasps attacking the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae).- *Biological Control*, 25: 231-238.

REED-LARSEN D. A., BROWN J. J., 1990.- Embryonic castration of the codling moth *Cydia pomonella* by an endoparasitoid, *Ascogaster quadridentata*.- *Journal of Insect Physiology*, 36 (2): 111-118.

ROSENBERG H. T., 1934.- The biology and distribution in France of the larval parasites of *Cydia pomonella*, L.- *Bulletin of Entomological Research*, 25: 201-256.

RUBERSON J. R., KRING T. J., 1993.- Parasitism of developing eggs by *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae): host age preference and suitability.- *Biological Control*, 3: 39-46.

SANTOS A. M. C., QUICKE D. L. J., 2011.- Large-scale diversity patterns of parasitoid insects.- *Entomological Science*, 14: 371-382.

SCHMIDT J. M., 1994.- Host recognition and acceptance by *Trichogramma*, pp. 165-200. In: *Biological control with egg parasitoids* (WAJNBERG E., HASSAN S. A., Eds).- CAB International, Wallingford, UK.

UKA D., HIRAOKA T., IWABUCHI K., 2006.- Physiological suppression of the larval parasitoid *Glyptapanteles pallipes* by the polyembryonic parasitoid *Copidosoma floridanum*.- *Journal of Insect Physiology*, 52: 1137-1142.

ULYSHEN M. D., DUAN J. J., BAUER L. S., 2010.- Interactions between *Spathius agrili* (Hymenoptera: Braconidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), larval parasitoids of *Agrilus planipennis* (Coleoptera: Buprestidae).- *Biological Control*, 52: 188-193.

VAN ALPHEN J. J. M., VISSER M. E., 1990.- Superparasitism as an adaptive strategy for insect parasitoids.- *Annual Review of Entomology*, 35: 59-79.

VAN LENTEREN J. C., 1981.- Host discrimination by parasitoids, pp. 153-179. In: *Semiochemicals: their role in pest control* (NORDLUND D. A., Ed.).- Wiley and sons, New York, USA.

VAN VELZEN E., PÉREZ-VILA S., ETIENNE R. S., 2016.- The role of within-host competition for coexistence in multiparasitoid-host systems.- *The American Naturalist*, 187: 48-59.

VAVRE F., DE JONG J.H., STOUTHAMER R., 2004.- Cytogenetic mechanism and genetic consequences of thelytoky in the wasp *Trichogramma cacoeciae*.- *Heredity*, 93: 592-596.

VINSON S. B., 1975.- Source of material in the tobacco budworm involved in host recognition by the egg-larval parasitoid, *Chelonus texanus*.- *Annals of the Entomological Society of America*, 68: 381-384.

VINSON S. B., 1998.- The general host selection behavior of parasitoid Hymenoptera and a comparison of initial strategies utlized by larvaphagous and oophagous species.- *Biological Control*, 11: 97-96.

VOLKOFF A. N., DAUMAL J., BARRY P., FRANÇOIS M. C., HAWLITZKY N., ROSSI M. M., 1995.- Development of *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae): time table and evidence for a single larval instar.- *International Journal of Insect Morphology and Embryology*, 24 (4): 459-466.

WANG B., FERRO D. N., WU J., WANG S., 2004.- Temperature-dependent development and oviposition behavior of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae), a potential biological control agent for the European corn borer (Lepidoptera: Crambidae).- *Environmental Entomology*, 33 (4): 787-793.

WANG X. G., BOKONON-GANTA A. H., MESSING R. H., 2008.- Intrinsic inter-specific competition in a guild of tephritid fruit fly parasitoids: effect of co-evolutionary history on competitive superiority.- *Biological Control*, 44: 312-320.

YANG S., DUAN J. J., LELITO J., VAN DRIESCHE R., 2013.- Multiparasitism by *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) and *Spathius agrili* (Hymenoptera: Braconidae): implication for biological control of the emerald ash borer (Coleoptera: Buprestidae).- *Biological Control*, 65: 118-123.

ZANG L. S., LIU T. X., 2007.- Intraguild interactions between an oligophagous predator, *Delphastrus catalinae* (Coleoptera: Coccinellidae), and a parasitoid, *Encarsia sophia* (Hymenoptera: Aphelinidae), of *Bemisia tabaci* (Homoptera: Aleyrodidae).- *Biological Control*, 41: 142-150.

ZAVIEZO T., MILLS N. J., 2001.- The response of *Hyssopus pallidus* to hosts previously parasitized by *Ascogaster quadridentata*: heterospecific discrimination and host quality.- *Ecological Entomology*, 26: 91-99.

ZEHNDER G., GURR G. M., KÜHNE S., WADE M. R., WRATTEN S. D., WYSS E., 2007.- Arthropod pest management in organic crops.- *Annual Review of Entomology*, 52: 57-80.

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Supplemental material

Video: *Ascogaster quadridentata* and *Trichogramma cacoeciae* adults behaviours when put together in a small arena with a fresh *Cydia pomonella* egg.

(Supplemental material available at http://www.bulletinofinsectology.org/suppl/vol72-2019-207-218ksentini-suppl.mp4)