

Potential for combined use of parasitoids and generalist predators for biological control of the key invasive tomato pest *Tuta absoluta*

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Abstract The tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) has recently invaded Mediterranean countries and is a major pest in tomato crops. Trichogrammatid oophagous parasitoids have shown promising potential for controlling the pest before the yield decreases in the greenhouse. In protected tomato crops (greenhouse), mirid predators are commonly used for biological control of whiteflies and they also prey on *T. absoluta*. These predators do not attack *Trichogramma* adults but they may partially decrease the overall impact of parasitoids on *T. absoluta* if intraguild predation (IGP) occurs on parasitized eggs. Under laboratory conditions, we tested if the mirid predator *Macrolophus pygmaeus* shows preference between parasitized and unparasitized *T. absoluta* eggs. We also tested if the predator reduces the number of parasitized eggs on caged tomato plants (microcosms) and assessed the efficacy of natural enemies used alone or together in limiting *T. absoluta* populations. We found that IGP is inflicted on the parasitoid *Trichogramma achaeae* by *M. pygmaeus* and that the risk of IGP depends on the developmental stage of *T. achaeae* inside the egg because

the larva is at risk mostly early in its development (when parasitized egg is still yellow). In addition, we observed that non-consumptive events, likely probing of the predator on parasitized eggs, may induce mortality in parasitoid offspring without actual feeding on parasitized eggs. However, both IGP and non-consumptive events decreased when the predator was not confined with parasitized eggs in small arenas. Despite negative effects of the predator to the parasitoid, results demonstrate that adding *Trichogramma* parasitoids may significantly increase the level of control of the pest over what could be attained when only the mirid predator *M. pygmaeus* is present on tomato. Implications of results are discussed regarding potential of these natural enemies for biological control of *T. absoluta* in greenhouse tomato crops.

Keywords Predator · Parasitoid · Intraguild predation · *Macrolophus pygmaeus* · *Trichogramma* · Preference

Introduction

Invasive species represent a major threat to crops (Suckling and Brockerhoff 2010; Desneux et al. 2011a; Ragsdale et al. 2011) and agricultural pests can reduce yield and increase production costs related to their management (Thomas 1999). The tomato leafminer *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is one of the most devastating pests of tomato in South America (Barrientos et al. 1998). This pest was first detected in 2006 in Spain and it has spread to most of the European and Mediterranean basin countries (Desneux et al. 2010, 2011a); in a few years it has become a key agricultural threat to tomato production worldwide (Desneux et al. 2011a). Plant injury consists of mine-formation within the mesophyll by

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feeding larvae, thus affecting the plant's photosynthetic capacity, resulting in lower fruit yield (Desneux et al. 2010). Appearance of *T. absoluta* has led to extensive insecticide use by tomato growers, potentially causing a multitude of undesired side effects on non-target organisms (Arno and Gabarra 2011; Biondi et al. 2012a, b, and see Desneux et al. 2007 for a thorough review). A comprehensive integrated pest management (IPM) program is needed to keep *T. absoluta* density below the economic injury level on tomato and a key component of such a program is biological control (Desneux et al. 2010).

Among possibilities for controlling the pest, oophagous parasitoids of the family Trichogrammatidae (Hymenoptera) have showed some potential for reducing pest damages in greenhouse grown tomato (Cabello et al. 2009, 2012; Desneux et al. 2010; Trottin-Caudal et al. 2011). Wasps of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) are the principal egg parasitoids of lepidopteran agricultural pests (Smith 1996; Andrade et al. 2011; Pizzol et al. 2010, 2012; Yuan et al. 2012). Most *Trichogramma* spp. are easy to rear, can be released in fields or greenhouses (Tabone et al. 2010, 2012), and various species have been reported as natural enemies of *T. absoluta* in South America and in Europe (Parra and Zucchi 2004; Pratisoli et al. 2005; Desneux et al. 2010; Zappala et al. 2012). Inoculative releases of *Trichogramma* spp. have been used against pests in various agricultural systems (Kuhar et al. 2002; Thomson et al. 2003; Hoffmann et al. 2006; Mills 2010). In Europe, studies are in progress to develop *Trichogramma*-based inoculative and augmentative biological control methods against *T. absoluta* (Cabello et al. 2012; Chailleux et al. 2012; Khanh et al. 2012).

Mirid predators, mainly *Macrolophus pygmaeus* Wagner and *Nesidiocoris tenuis* Reuter, are commonly used against whiteflies in greenhouse tomato crops (Bonato et al. 2006; Nannini et al. 2007; Calvo et al. 2009) and they are also biocontrol agents of *T. absoluta* as they prey on eggs (Urbaneja et al. 2009; Calvo et al. 2012; Bompard et al. 2013). However, *N. tenuis* often prompts insecticide applications at high predator density because of damages it inflicts to both plants and fruits (Calvo et al. 2009; Castane et al. 2011), and *M. pygmaeus* is not totally efficient when used alone against *T. absoluta* (Desneux N, Jaworski CC, unpublished data). The predators may feed on *Trichogramma*-parasitized *T. absoluta* eggs on tomato plants. Such intraguild predation (IGP) may decrease the overall efficacy of parasitism on *T. absoluta* if combining *Trichogramma* wasps and mirid predators. IGP occurs when two species that share a prey resource (and therefore may compete) also engage in trophic interaction with each other (e.g., predation) (Polis et al. 1989; Rosenheim et al. 1995; Holt and Huxel 2007). Identification of such trophic links is of primary importance to the success of pest management programs. Therefore, assessment of IGP is

required before release(s) of *Trichogramma* parasitoids could be recommended in greenhouse grown tomato crops where mirid predators are used as biocontrol agents for IPM purposes.

In this context, we investigated (i) whether *M. pygmaeus* would attack *Trichogramma*-parasitized eggs in a no-choice test, (ii) whether *M. pygmaeus* would show a preference for parasitized eggs (two-choice test), and (iii) what the combined effect of both natural enemy species would be on *T. absoluta* survival (on single plant microcosm). In addition, we assessed the possible impact of non-consumptive events by *M. pygmaeus* on survival of *Trichogramma* pupae inside *T. absoluta* egg. Such events, notably probing, have been reported to increase drastically the mortality rate in probed prey (e.g., adult insect, host embryo developing in egg, parasitoid developing into host, etc.) in case of attack by hemipteran predators (Butler and O'Neil 2006), presumably owing to injection of venom (Edwards 1961; Cohen 1990).

Materials and methods

Biological materials

The plants used in the experiments were 4-week-old tomato plants, *Solanum lycopersicum* L. cv. Marmande (grown in climatic chambers, 24 ± 1 °C, 65 ± 5 % RH, 16L:8D). A *T. absoluta* colony was initiated using greenhouse-collected adults (Alenya, France) (initial number of individuals = 190) 3 months before starting the experiment. The pest was reared on caged tomato plants in climatic chambers (24 ± 1 °C, 65 ± 5 % RH, 16L:8D). We used the egg parasitoid *Trichogramma achaeae* Nagaraja and Nagarkatti as this species is currently available for biological control of *T. absoluta* in Europe and showed highest efficacy on the pest among various *Trichogramma* species tested (Chailleux et al. 2012). The parasitoid was reared on UV-irradiated eggs of a factitious host, *Ephesthia kuehniella* Zeller (25 ± 1 °C, 70 ± 5 % RH, 16L:8D). The parasitoids used for all experiments were 24-h old. *Macrolophus pygmaeus* nymphs were provided by Biotop (Valbonne, France) and kept on potted tomato plants in plastic rearing cages in climatic chambers (23 ± 1 °C, 70 ± 5 % RH, 16L:8D) until they reached the adult stage. UV irradiated *E. kuehniella* eggs were used as supplemental diet. The predators used during the experiments were 48-h-old mated females that had been starved for 24 h prior to the start of the tests.

No-choice assay in glass tube

Individual *M. pygmaeus* were caged in glass tubes (length: 7.5 cm, diameter: 1 cm) with either 12 unparasitized

T. absoluta eggs, 12 yellow *T. achaeae*-parasitized *T. absoluta* eggs, i.e., parasitized for 0–3 days, or 12 black parasitized *T. absoluta* eggs, i.e., parasitized by *T. achaeae* for 4–5 days (these conditions and the method for obtaining the parasitized eggs were based on results of pilot and preliminary experiments, Desneux et al. 2011b; Poncet et al. 2012). For all assays, the parasitized eggs were obtained according to the method of Chailleux et al. (2012). The two *T. achaeae*-parasitized *T. absoluta* egg types (yellow or black) were used because predators could exhibit marked tendency to avoid parasitized hosts in favor of unparasitized hosts (Rouechdi and Voegelé 1981; Ruberson and Kring 1991; Roger et al. 2001) and particularly because the age of the parasitoid within the host (i.e., the egg in case of *Trichogramma* spp.) can influence the degree of preference/avoidance (Hoelmer et al. 1994; Brodeur and Boivin 2004). Eggs were deposited onto a paper card and placed in the tube (one paper card per tube). One tomato stem (2 cm) was also inserted into the tube to provide moisture. There were 12–18 replicates per treatment and mated females remained inside the tube in climatic chambers (25 ± 1 °C, 70 ± 5 % RH, under constant light). After 12 h, the numbers of eaten eggs were recorded. Pilot experiments showed that *T. absoluta* eggs (unparasitized, yellow or black), when consumed by *M. pygmaeus*, are devoid of material and are easily seen as having been attacked. It is therefore easy to distinguish between intact (unparasitized or parasitized) eggs and the remains of eggs previously attacked. At the end of the experiment, unconsumed black eggs were collected for further assessment of possible effect of non-consumptive events by *M. pygmaeus* on the survival of *Trichogramma* pupae inside *T. absoluta* egg, i.e., we assessed adult emergence from black parasitized eggs (see “[Emergence of parasitoids: non-consumptive effects of the predator on parasitized eggs](#)” section below).

Data were subjected to ANOVA and pairwise multiple comparisons were performed using a Tukey’s post hoc test. Datasets were first tested for normality and homogeneity of variance using Kolmogorov–Smirnov D test and Cochran’s test, respectively, and transformed if needed (this test was carried out also for all datasets from other experiments [below], and the SPSS software was used for all analyses).

Choice assay in glass tube

Individual *M. pygmaeus* were caged as above, except that six unparasitized *T. absoluta* eggs were paired with six yellow or six black *T. achaeae*-parasitized *T. absoluta* eggs (for a total of 12 eggs). Thus predators were given a choice of parasitized or unparasitized eggs for 12 h. In addition, six yellow and six black *T. achaeae*-parasitized *T. absoluta* eggs were also paired in order to assess predator’s

preference between early developed versus late developed parasitized eggs. The control group consisted of exposing *M. pygmaeus* to 12 unparasitized eggs per paper card per tube. Individual *M. pygmaeus* were introduced per glass tube with 12–18 replicates per treatment. All other experimental conditions were identical to the no-choice experiment. In the same way as for the *No-choice assay*, black eggs were collected at the end of the experiment for assessing the effect of non-consumptive events by *M. pygmaeus* on *Trichogramma* pupae (see “[Emergence of parasitoids: non-consumptive effects of the predator on parasitized eggs](#)” section below).

Numbers of parasitized eggs preyed (yellow and black) were compared to numbers of unparasitized eggs preyed with a paired *t* test. Total numbers of eggs eaten per situation were compared among groups using ANOVA and pairwise multiple comparisons were performed using least significant differences (LSD) test.

Behavioral assay

We directly observed predator behavior to assess the propensity of *M. pygmaeus* to attack the different types of *T. absoluta* eggs (unparasitized eggs and yellow and black *T. achaeae*-parasitized *T. absoluta* eggs). We placed one egg of a given type onto the bottom of a Petri dish using a fine brush and placed the Petri dish under a binocular microscope. Individual *M. pygmaeus* were introduced into a clear plastic dome (*d*: 1 cm, *h*: 0.65 cm) and the dome was placed over an individual egg (Desneux et al. 2009a). Observations began when the predator first encountered the surface of the Petri dish. Predators were observed for 5 min, or until an attack occurred. We recorded predator behaviors as: “contact” (contact with the egg) and “attack” (mouthparts extended and making contact with the prey [egg], Desneux and O’Neil 2008). Fifteen replicates were carried out per egg type, and observations were done in a randomized fashion.

The proportions of unparasitized eggs encountered and attacked by the predator were compared to those of yellow and black parasitized eggs (*T. achaeae*-parasitized *T. absoluta* eggs) using permuted Fisher’s exact tests.

Microcosm assay

We studied the effect of *T. achaeae* and *M. pygmaeus* on *T. absoluta* populations on tomato plants in microcosm conditions to determine if any interaction occurred between the two natural enemies. Following the design used in previous studies (Desneux et al. 2009b; Mouttet et al. 2011), microcosms were created by placing a clear acetate cylinder over a potted tomato plant (4-week-old plants with four fully expanded leaves were used). Cylinders had a

mesh (350 μm) top and windows were cut and covered with mesh (350 μm) for ventilation. Cylinders were 40 cm high \times 15 cm in diameter and sand was placed on the soil surface. The cylinder was pushed into the sand to ensure a complete seal of the microcosm. All experiments were carried out at a temperature of 25 ± 1 °C, 70 ± 5 % RH and a 16L:8D photoperiod.

To prepare plants for starting the experiment, ten *T. absoluta* mated females were released per plant (microcosm) and removed after 24 h. The number of *T. absoluta* eggs was then adjusted to 100 eggs per plant. Then plants were distributed among four different treatments: (i) Control = no natural enemies released, (ii) 50 *T. achaeae* were released per microcosm (number of *T. achaeae* released was based on the rate recommended by Biotop, France), (iii) one *M. pygmaeus* was released per microcosm, and (iv) 50 *T. achaeae* and one *M. pygmaeus* were released per microcosm, (15–27 replicates per treatment). After 5 days, *T. absoluta* larvae and parasitized eggs (black eggs) in the microcosms were counted. At the end of the experiments, black eggs were counted and collected (for subsequent assessment of emergence of *T. achaeae* offspring, see below).

The numbers of *T. absoluta* larvae were analyzed using a generalized linear model (PROC Genmod, SAS 1999) with “Parasitoid” and “Predator” as factors (a Tukey’s post hoc test was used for pairwise multiple comparisons). The numbers of parasitized eggs were compared using the same statistical method with one single factor “Predator”.

Emergence of parasitoids: non-consumptive effects of the predator on parasitized eggs

We evaluated the possible impact of *M. pygmaeus*, through non-consuming event (e.g., probing, see Butler and O’Neil 2006), on the survival of *Trichogramma* pupae inside *T. absoluta* eggs. We focused our assessment on black parasitized eggs over yellow ones because the latter were shown to be largely preyed on by *M. pygmaeus* (see “Results” section). To compare the success in development of parasitoid offspring in a control situation versus in presence of the predator (i.e., when black eggs were from *No-choice*, *Choice*, and *Microcosm assays*), we prepared batches of *T. absoluta* eggs for parasitism by *T. achaeae* without presence of *M. pygmaeus*, i.e., parasitized control egg batches. All collected black parasitized eggs (from the three assays as well as from the controls) were placed in Petri dishes in a climatic chamber (25 ± 1 °C, 70 ± 5 % RH and a 16L:8D). After 5 days, we counted the adults that emerged.

The proportion of black eggs that yielded parasitoid adults among the assays and controls were fitted to a log-linear model (with factors “assay” and “predator” tested).

The “assay” factor was included in the statistical model to assess how increasing assay complexity, i.e., one egg or two egg types in glass tubes, or one plant in microcosm, may impact occurrence of non-consumptive events on black eggs. For instance, increased complexity in architectural plant characteristics (e.g., plant size, number of nodes and leaves) have been reported to significantly decrease encounter rate between predators and prey (e.g., Coll and Ridway 1995; Rutledge and O’Neil 2005). In addition, proportions of adults emerged from eggs subjected to predator non-consumptive events (from the various assays) were compared with respective control using χ^2 tests.

Results

No-choice and choice assays in glass tube

In a no-choice situation, *M. pygmaeus* fed on *T. achaeae*-parasitized *T. absoluta* eggs at the same rate as unparasitized eggs when all *T. absoluta* eggs were yellow. The predator, however, fed significantly less (about tenfold less) when parasitized eggs were older (i.e., black) (Fig. 1; $F_{2,35} = 40.891$, $p < 0.001$).

When the predators were offered two egg types at the same time (*Choice assay*), the total consumption of eggs per tube was significantly lower in tubes in which black

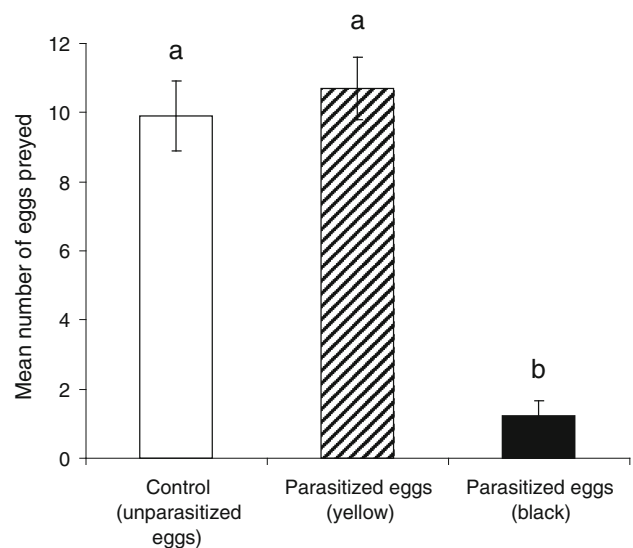


Fig. 1 Mean number (\pm SEM) of *T. absoluta* eggs preyed in the no-choice experiment in glass tubes with one *M. pygmaeus* female exposed to 12 unparasitized *T. absoluta* eggs (control), 12 *T. achaeae*-parasitized *T. absoluta* yellow eggs (i.e., parasitized for 0–3 days), or 12 *T. achaeae*-parasitized *T. absoluta* black eggs (i.e., parasitized for more than 4–5 days). Histograms bearing different letters are significantly different ($p < 0.05$, ANOVA followed by a Tukey’s post hoc test)

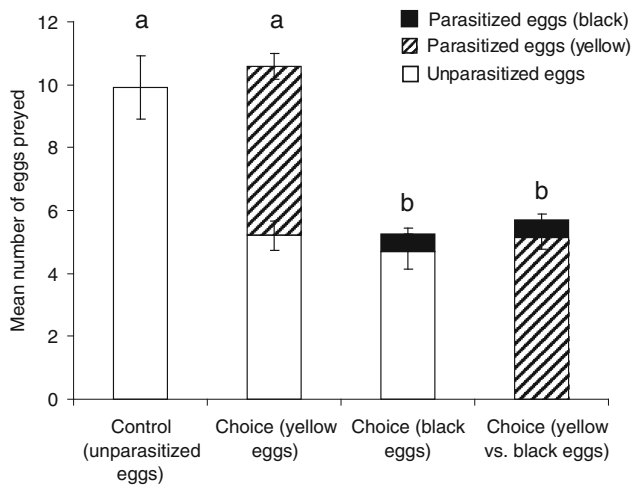


Fig. 2 Mean number (\pm SEM) of *T. absoluta* eggs preyed in the choice experiment in glass tubes with one *M. pygmaeus* female exposed to 12 unparasitized *T. absoluta* eggs (control), or six unparasitized *T. absoluta* eggs and six *T. achaeae*-parasitized *T. absoluta* eggs (yellow or black) placed together, or six *T. achaeae*-parasitized *T. absoluta* yellow eggs and six *T. achaeae*-parasitized *T. absoluta* black eggs placed together. Different letters indicate a significant difference in total consumption of *T. absoluta* eggs ($p < 0.05$, ANOVA followed by a Tukey’s post hoc test)

parasitized eggs were provided (Fig. 2; $F_{3,57} = 14.734$, $p < 0.001$). *Macrolophus pygmaeus* did exhibit a preference for unparasitized over black parasitized eggs ($t = 8.884$, $df = 17$, $p < 0.001$) but did not show any preference when both unparasitized eggs and parasitized eggs were still yellow ($t = -1.382$, $df = 14$, $p = 0.189$). In addition, the predator showed a preference for yellow parasitized eggs over black parasitized eggs (Fig. 2; $t = 13.950$, $df = 12$, $p < 0.001$).

Behavioral assay

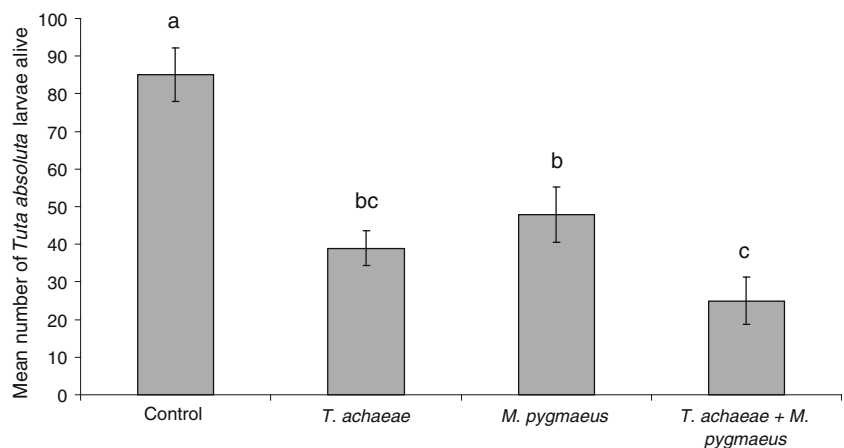
Similar proportions of unparasitized *T. absoluta* eggs, yellow *T. achaeae*-parasitized eggs and black *T. achaeae*-

parasitized eggs were encountered by the predator, 0.93, 0.93, and 0.86, respectively. Parasitism had no effect on the rate at which predators encountered eggs in the observation arenas (permuted Fisher’s exact tests, all $p > 0.05$). However, black parasitized eggs were attacked at a significantly lower rate (0.20) than were unparasitized eggs (0.93, $p = 0.01$), but there was no significant difference between attack rates on yellow parasitized eggs (0.87) and unparasitized eggs ($p = 0.494$).

Microcosm assay

In microcosms, the number of *T. absoluta* larvae at the end of the experiment varied significantly in function of the presence of the parasitoid *T. achaeae* (Fig. 3; significant “Parasitoid” factor: $\chi^2 = 25.99$, $df = 1$, $p < 0.001$) and the presence of the predator *M. pygmaeus* (significant “Predator” factor: $\chi^2 = 13.06$, $df = 1$, $p < 0.001$). Both factors interacted significantly (interaction “Parasitoid” \times “Predator” factors: $\chi^2 = 4.21$, $df = 1$, $p = 0.040$). Therefore, the effect of *T. achaeae* and *M. pygmaeus* on the survival of *T. absoluta* varied differently as a function of the presence of a second natural enemy (either the parasitoid or the predator) in the microcosms. The lowest number of *T. absoluta* larvae was observed when *T. achaeae* and *M. pygmaeus* were released together into microcosms. When the predator *M. pygmaeus* was released alone it reduced *T. absoluta* populations when compared to the control group but less efficiently than when released together with the parasitoid *T. achaeae* (though we did not observe fully additive effects of the two natural enemies together). The number of *T. achaeae*-parasitized eggs varied as function of the presence of the predator (“Predator” factor: $\chi^2 = 4.97$, $df = 1$, $p = 0.026$). Indeed, when both natural enemies were released together, the number of parasitized eggs (black eggs) was two-fold lower (18.20 ± 8.61) than in case the parasitoids had been released alone into the microcosm (35.24 ± 4.43).

Fig. 3 Mean number (\pm SEM) of *T. absoluta* larvae alive per plant after 5 days in microcosm as function of the various treatments: (i) Control (no natural enemy), (ii) *T. achaeae* released, (iii) *M. pygmaeus* released, and (iv) *T. achaeae* + *M. pygmaeus* released together. Histograms bearing different letters are significantly different ($p < 0.05$, generalized linear model followed by a Tukey’s post hoc test)



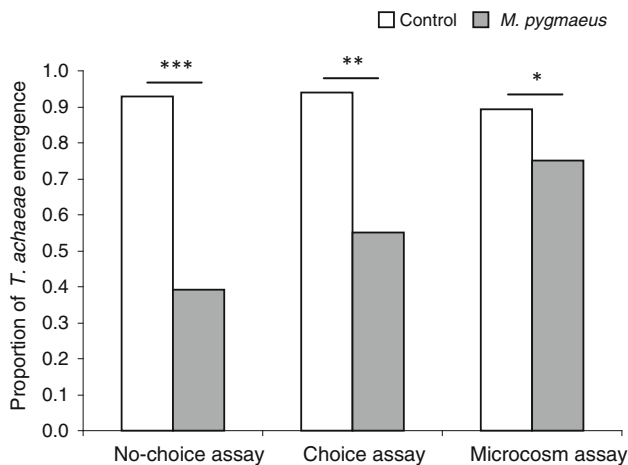


Fig. 4 Percentage of parasitoid emerging from black eggs collected during the *No-choice*, *Choice*, and *Microcosm* assays (as well as from black eggs of respective control groups). *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ (significant difference with the respective control, χ^2 test)

Emergence of parasitoids: non-consumptive effects of the predator on parasitized eggs

The emergence of parasitoids from parasitized eggs varied significantly according to the presence of the predator *M. pygmaeus* (significant “Predator” factor: $\chi^2 = 19.91$, $df = 1$, $p < 0.001$) but not according to the assay considered (Fig. 4; “Assay” factor: $\chi^2 = 4.12$, $df = 2$, $p = 0.128$). However, both factors interacted significantly (interaction “predator” \times “assay” factors: $\chi^2 = 6.71$, $df = 2$, $p = 0.035$), hinting that the effect of the predator on parasitoid emergence depended on the assay considered. When parasitoid emergence was compared with respective control in each assay, we observed significantly fewer parasitoid adults in the assays versus controls (*No-choice* assay: $\chi^2 = 25.04$, $df = 1$, $p < 0.001$, *Choice* assay: $\chi^2 = 10.02$, $df = 1$, $p = 0.002$, *Microcosm* assay: $\chi^2 = 3.89$, $df = 1$, $p = 0.049$). However, the reduction in parasitoid emergence was more important in *No-choice* and *Choice* assays (58 and 41 % reduction, respectively) than in the *Microcosm* assay (15 % reduction) (Fig. 4).

Discussion

Generalist predators are largely acknowledged as providing valuable levels of regulation of pests in crops throughout the world (Symondson et al. 2002; Desneux et al. 2006; Lu et al. 2012). However, development of biological control programs can be compromised through intraguild predation which is actually a common phenomenon in agro-ecosystems (Rosenheim et al. 1995; Müller and Brodeur 2002; Chacon and Heimpel 2010). We found that IGP, as well as

non-consumptive events, did occur on the oophagous parasitoid *T. achaeae* by the generalist predator *M. pygmaeus* under laboratory conditions. The predator can decrease survival of *T. achaeae*'s offspring that are developing in *T. absoluta* eggs because it does feed on parasitized eggs. However, the risk of IGP depends on the developmental stage of *T. achaeae* inside *T. absoluta* parasitized eggs because the larvae are at risk mostly early in their development when parasitized eggs are still yellow (eggs turn black halfway through development of parasitoid). In addition, parasitoid offspring can also suffer mortality when developing in *T. absoluta* eggs through probing behavior exhibited by the predator on parasitized eggs. This behavior is known to increase mortality in prey (Butler and O'Neil 2006) without actual feeding by the predator, probably owing to a toxin injected by hemipteran predators when probing prey (Edwards 1961; Cohen 1990). Despite occurrence of IGP and non-consumptive events, the density of *T. absoluta* larvae at the end of the experiment in microcosms suggested that releasing *T. achaeae* against *T. absoluta* on tomato plants may increase the level of pest suppression achieved over that occurring when only mirid predators are present. Predation of *T. absoluta* eggs by *M. pygmaeus* was consistent with previous studies that reported that *M. pygmaeus* would consume *T. absoluta* eggs under laboratory (Urbaneja et al. 2009) and greenhouse conditions (Bompard et al. 2013). The results of our microcosm assay showed that *M. pygmaeus* does reduce *T. absoluta* population levels on tomato plants.

Predators are known to consume eggs parasitized by oophagous parasitoids (Herrick et al. 2008; Mbata and Shapiro-Ilan 2010) and there are examples of predation on eggs parasitized by *Trichogramma* spp. (Brower and Press 1988; Smith 1996; Kuhar et al. 2002; Philip et al. 2005). By contrast, many studies have reported that egg predators avoid parasitized prey containing parasitoid larvae or pupae (Rouechdi and Voegelé 1981; Ruberson and Kring 1991; Roger et al. 2001). Hemipteran predators, when engaged in IGP with *Trichogramma* spp., are more likely to accept unparasitized host eggs than those that contain pupae of *Trichogramma*, although younger stages of *Trichogramma* appear equally susceptible (Rouechdi and Voegelé 1981; Brower and Press 1988). For instance, we showed that *M. pygmaeus* preferentially attacked unparasitized eggs and parasitized eggs during the early stages of parasitoid larvae development (yellow eggs) compared to black eggs, i.e., when the parasitoid is in the pupal stage during its final development. In addition, behavioral observations showed that the predator would not primarily feed on black eggs, i.e., only 20 % of acceptance at first encounter with black egg versus 87–93 % for yellow eggs. When *Trichogramma* parasitoids reach the prepupa stage, they catalyze the production of melanin which darkens the

parasitized eggs (Pintureau et al. 1999; Knutson 2005). Melanin is a pigment involved in the mechanical strengthening of insects' cuticle and other biological materials (Moses et al. 2006). The presence of melanin might make it harder for predators like hemipterans to pierce parasitized eggs. Melanization alone did not totally prevent feeding by *M. pygmaeus* on these parasitized eggs, but when the parasitoid *T. achaeae* is at late developmental stage (pupae), parasitized eggs (black) are consumed at a lower rate, thus reducing the risk of IGP. Our choice tests confirm that *Trichogramma* parasitoids can be at risk of IGP by *M. pygmaeus* for at least half of their developmental duration (i.e., before pupation) when parasitizing *T. absoluta* on tomato.

However, mortality of *Trichogramma* pupae inside black eggs may still occur through non-consumptive events, i.e., probing which kills parasitoid offspring. The mortality of pupae induced by such non-consumptive behavior was relatively high when the predator was confined in small arenas with black eggs, i.e., *No-choice* and *Choice assays* (Fig. 4). However, behavioral observations suggested that probing on black eggs would occur mostly after several encounters of the predator with black eggs, e.g., probing on black eggs occurred in only 20 % of first encounters with black eggs (vs. in 87–93 % in the case of encounters with yellow eggs), though learning process in the predator may increase (or actually decrease) acceptance rate of these black eggs by *M. pygmaeus*. Encounter rate between hemipteran predators and prey are known to decrease when the complexity in architectural plant characteristics increase (Coll and Ridgway 1995; Rutledge and O'Neil 2005). Consistently with this observation, mortality of parasitoid pupae in black eggs was much lower (barely significant when compared to the control) in the *Microcosm assay* than what was recorded for black eggs from *No-choice* and *Choice assays* (glass tubes), likely due to higher architectural complexity in the *Microcosm assay* reducing encounter rate between the predator and black eggs. In addition, in tomato greenhouse, higher architectural complexity as well as lower pest density may further decrease encounter rate between hemipteran predators and *Trichogramma*-parasitized *T. absoluta* eggs.

We did not observe full additive effects of the two natural enemies on *T. absoluta* survival in the microcosms (Fig. 3). When the predator was present at the time the parasitoids were parasitizing *T. absoluta* eggs, it is likely that the predator both reduced number of eggs available for the parasitoids, and also preyed on eggs recently parasitized by the parasitoid. In case of *Trichogramma* species adapted to *T. absoluta* as host, it could lead to a parasitoid population decrease if host (*T. absoluta* eggs) density becomes very low. By contrast, the omnivorous predator

M. pygmaeus could switch to other food sources, e.g., plant or alternative prey, though the presence of *Trichogramma* parasitoids may still induce a decrease in *T. absoluta* eggs available as food.

This work provided insights into what could be expected to occur in tomato greenhouses. Despite occurrence of IGP (predation on early stage of parasitoid development), a likely competition for resources (*T. absoluta* eggs) between the two natural enemies, and a lack of full additive effects of the two natural enemies on *T. absoluta* survival in microcosm conditions, our results demonstrate that adding *Trichogramma* parasitoids would significantly increase the level of control of the pest over what could be attained when using *M. pygmaeus* alone. Our findings suggest that the presence of mirid predators in greenhouse tomato crops may not interfere significantly with oophagous parasitoids for *T. absoluta* control though population dynamics studies in greenhouse conditions would have to be performed to confirm this result. For instance, increased habitat complexity is known to decrease IGP rates (Finke and Denno 2002; Langellotto and Denno 2006). In addition, plant material may have provided a supplemental food resource that reduced feeding of mirid predators on prey (e.g., Calvo et al. 2009). In our experiment, tomato plants in the microcosms likely (i) decreased encounter rates between *M. pygmaeus* and parasitized eggs, and (ii) served as food and thus reduced occurrence of IGP and non-consumptive events of the predator over parasitized eggs. It could explain why only half of parasitized eggs were consumed in microcosms whereas most of yellow *T. achaeae* parasitized *T. absoluta* eggs were consumed in glass tubes (no-choice and choice assays). Further, we suggest that combining releases of *Trichogramma* parasitoids and *M. pygmaeus* will not strongly interfere with the parasitoids. Although *M. pygmaeus* would feed on parasitized—but still yellow—*T. absoluta* eggs, there was no evidence this degree of IGP impacted *T. absoluta* survival positively (Fig. 3). In addition, juveniles usually represent an important part of *M. pygmaeus* populations in greenhouse tomato crops, and prey consumption by these juveniles is lower than predation by adults (Fauvel et al. 1987), thus lowering the risk of IGP. Efficient integration of both natural enemies into an IPM program might be achieved via adjustment of the frequency of releases according to predator *T. absoluta* and alternative prey (e.g., whitefly) densities in the crop.

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