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Potential for direct interference between natural enemies of *Tuta absoluta* on tomato

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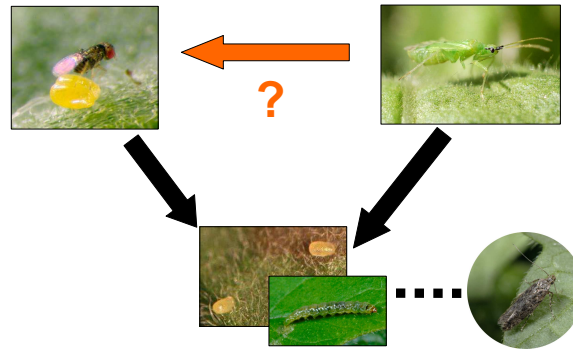
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Introduction

The tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) has recently invaded some Mediterranean countries and is a major pest in both greenhouse and outdoor tomato crops (Desneux et al. 2010). Among possibilities for controlling this pest, Trichogrammatidae oophagous parasitoids have showed promising potential for controlling the pest before yield decreases in greenhouse (Cabello et al. 2009), and one species in particular, *Trichogramma achaeae*, is currently proposed in Spain and France for inundative biological control of *T. absoluta* (Desneux et al. 2010). In greenhouses, Miridae predators (*Macrolophus caliginosus* Wagner and *Nesidiocoris tenuis* Reuter) are also common on tomato and usually used against whiteflies (Arnó et al. 2009). Despite that these predators do not attack *T. achaeae* adults, they may partially decrease overall impact of the parasitoid on *T. absoluta* if intraguild predation (IGP) (i.e. when a natural enemy eats another natural enemy) occurs on *T. achaeae*-parasitized eggs. If IGP occurs, it could reduce parasitoids' offspring and thus the potential additional effect of *T. achaeae* on *T. absoluta*. In instance, combined use of *T. achaeae* and predators in tomato greenhouses suggested efficiency in limiting *T. absoluta* population growth (i.e. better than predators alone).

- In this context, in small arenas in laboratory conditions we studied:
- (1) If the Mirid predator *M. caliginosus* could attack *T. achaeae*-parasitized *T. absoluta* eggs in a non-choice experiment?
 - (2) If the predator shows preference between *T. absoluta* eggs and *T. achaeae*-parasitized *T. absoluta* eggs in a choice experiment?
- Then in laboratory microcosms (encaged tomato plants) we tested:
- (3) If the predator effectively reduces the number of *T. achaeae*-parasitized *T. absoluta* eggs on whole tomato plant?
 - (4) How the predator and the parasitoids impacted the *T. absoluta* population dynamics?



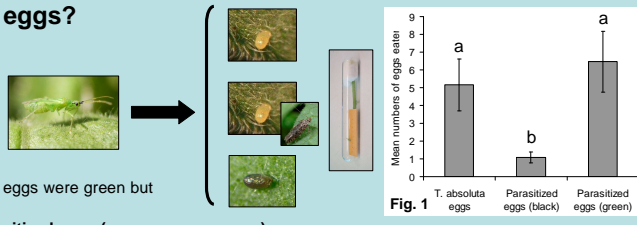
1 - Does *Macrolophus caliginosus* feed on *T. achaeae*-parasitized *T. absoluta* eggs?

Non-choice experiment:

- No choice, glass tubes (length: 7.5cm, diameter: 1cm) with one tomato stem (4cm) and one orange paper card.
- 12 unparasitized *T. absoluta* eggs or 12 green *T. achaeae*-parasitized eggs (parasitized for 0-4 days), or 12 black *T. achaeae*-parasitized eggs (parasitized for more than 4 days) placed per paper card. Individual mated *M. caliginosus* female introduced per glass tube (12-15 replicates per treatment).
- 2 hours exposure, 25±1°C, 70±5% R.H., fluorescent light.
- Measurements: number of eggs remaining on the paper card (used to estimate number of eggs eaten).

Macrolophus caliginosus fed on *T. achaeae*-parasitized *T. absoluta* eggs in a similar way than on unparasitized eggs when eggs were green but significantly less when parasitized eggs were black (Fig. 1; $F_{2,36} = 4.976$, $P = 0.012$).

⇒ *Macrolophus caliginosus* has potential to reduce fitness of *T. achaeae* via intraguild predation when attacking parasitized eggs (more on green eggs)



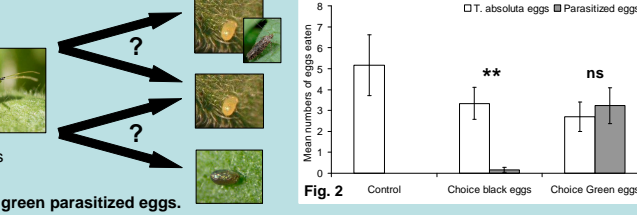
2 - Does *Macrolophus caliginosus* exhibit a preference between parasitized and unparasitized *T. absoluta* eggs?

Dual-choice experiment:

- Glass tubes (length: 7.5cm, diameter: 1cm) with one tomato stem (4cm) and one orange paper card.
- 6 unparasitized *T. absoluta* eggs and 6 *T. achaeae*-parasitized *T. absoluta* eggs (green or black) placed together.
- Control: 12 unparasitized eggs per paper card per tube.
- Individual mated *M. caliginosus* female introduced per glass tube (10-15 replicates per treatment).
- 2 hours exposure, 25±1°C, 70±5% R.H., fluorescent light.
- Measurements: number of eggs (parasitized or unparasitized) eggs remaining on the paper card.

Macrolophus caliginosus does exhibit a preference for unparasitized over black parasitized eggs (Fig. 2, $P < 0.05$) but does not show such preference between unparasitized eggs and green parasitized eggs ($P > 0.05$).

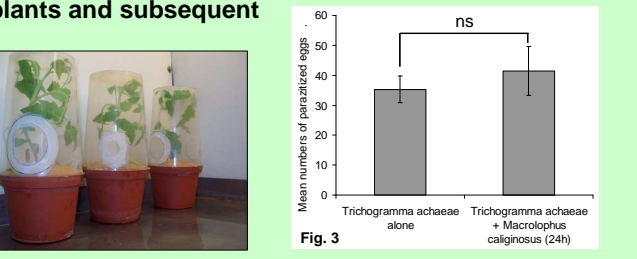
⇒ The predator does not preferentially attack *T. achaeae*-parasitized eggs in tomato greenhouses but attacks largely green parasitized eggs.



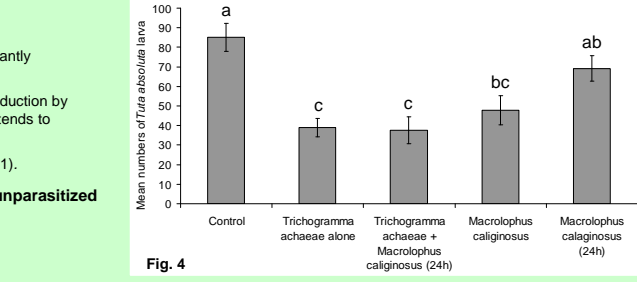
3 and 4 - Potential for IGP on *T. achaeae* by *M. caliginosus* on whole tomato plants and subsequent side effects on control of *T. absoluta*?

Microcosm bioassay:

- Microcosm design (individual tomato plant enclosed), 25°C, 70% RH, fluorescent light.
- 10 *T. absoluta* mated females released per plant and removed after 24 hours.
- Number of *T. absoluta* eggs adjusted to 100 eggs per plant (day 0).
- 5 different treatments (15-27 replicates):
 - Control = no natural enemies released
 - 50 *T. achaeae* released per microcosm (day 0).
 - 1 *M. caliginosus* released per microcosm (day 0).
 - 50 *T. achaeae* (day 0) + 1 *M. caliginosus* (at day 4, remained 24h) released per microcosm.
 - 1 *M. caliginosus* released per microcosm (at day 4, remained 24h) released per microcosm.
- Measurements at day 5: number of parasitized eggs (black eggs), numbers of *T. absoluta* larva alive.



- In microcosms, when *T. achaeae* was allowed to parasitize *T. absoluta* eggs before arrival of *M. caliginosus*, the predator did not significantly induce a decrease in *T. achaeae*-parasitized eggs (black eggs) (Fig. 3) ($F_{1,39} = 0.187$, $P = 0.668$).
 - Both *T. achaeae* alone and *M. caliginosus* alone treatments (released at day 0) induced large decreases in *T. absoluta* survival with a reduction by more than 50% in number of *T. absoluta* larva at the end of the experiment (general ANOVA: $F_{4,105} = 11.548$, $P < 0.0001$). The parasitoids tends to induce a higher reduction in *T. absoluta* survival than *M. caliginosus* (though only marginally significant) (Fig. 4).
 - When *M. caliginosus* was released only for 24h at day 4, it slightly reduced *T. absoluta* survival though only marginally significant ($P < 0.1$).
- ⇒ The predator does not consume largely *T. achaeae* eggs when released for a 24 h period on plants on which both unparasitized *T. absoluta* eggs, green parasitized eggs and black parasitized eggs are present.
- ⇒ There is not, in these conditions, strong direct interference between *T. achaeae* and *M. caliginosus*.
- ⇒ The effects of the two natural enemies on *T. absoluta* survival are not additive in our microcosm conditions.



Conclusions

The predator *M. caliginosus* does attack *T. absoluta* eggs previously parasitized by *T. achaeae* but preferentially when these eggs are still in the early stages of the parasitoid development (i.e. when the eggs are still green). When the parasitized eggs are older (i.e. black), the predator attacks the eggs at a low rate. Our findings hint the importance of integrating the presence of the predators in the tomato crop in greenhouses when using oophagous parasitoids for inundative biological control of *T. absoluta*, notably to ensure a maximum efficacy of the parasitoids against *T. absoluta* both through (1) the inundative release, and (2) subsequent impact of parasitoids' offspring.

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