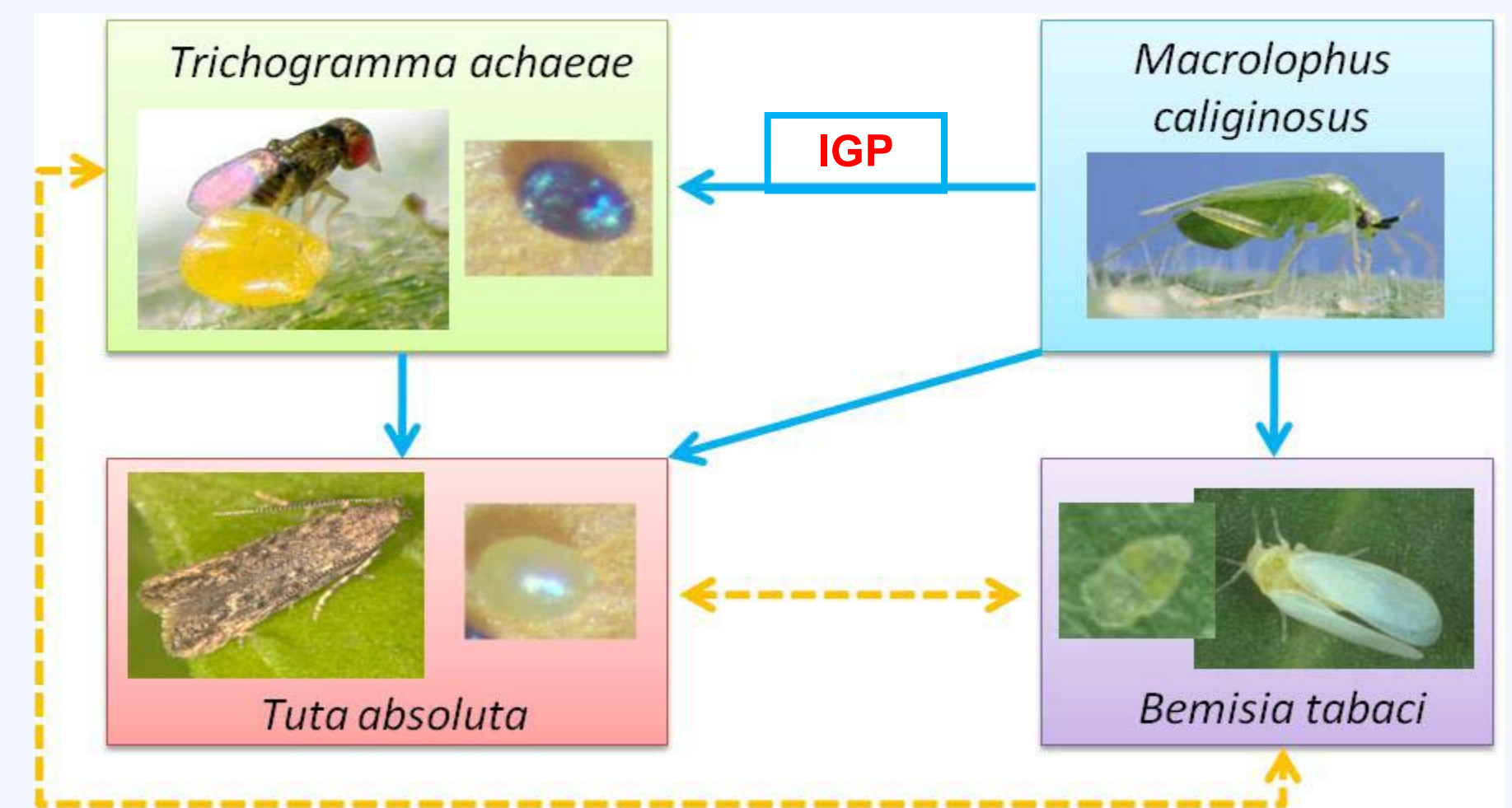


## Introduction

The tomato leaf miner *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) has recently invaded most of Mediterranean countries and is a major pest in greenhouse tomato crops (Desneux et al. 2010). Among possibilities for controlling this pest, the oophagous parasitoid *Trichogramma achaea* Nagaraja & Nagarkatti (Hymenoptera: Trichogrammatidae) has showed promising potential and is already used for inundative biological control (Desneux et al. 2010). In greenhouses, *Miridae* predators (mainly: *Macrolophus pygmaeus* Wagner and *Nesidiocoris tenuis* Reuter) are also common on tomato and usually used against whiteflies (notably *Bemisia tabaci*) (Arnò et al. 2009). *M. pygmaeus* also feed on *T. absoluta* eggs (Urbaneja et al. 2009); thus **intraguild predation (IGP)** (i.e. when a natural enemy eats another natural enemy) with *T. achaea* can occur if the predatory attacks parasitized eggs too. In addition, as the predator attacks both *B. tabaci* and *T. absoluta* on tomato, it may lead to **indirect interactions** (Holt and Lawton, 1994) between the two prey.

Such various interactions may have opposite consequences on growth of pest populations which may differ among time scales as well. These interactions can lead to increased or decreased efficiency in biological control programs. Therefore in this context, we carried out three experiments in small arenas under laboratory conditions to assess:

- (1) the impact of *T. achaea* offspring on *T. absoluta* biocontrol, and thus define a pertinent time scale for our study.
- (2) whether IGP could occur between *M. pygmaeus* and *T. achaea* (choice and no-choice experiments).
- (3.1.) the effect of natural enemies (*M. pygmaeus* and *T. achaea*) on *T. absoluta* population growth.
- (3.2.) the impact of IGP and indirect interactions (with an alternative prey, *B. tabaci*) on efficiency of the natural enemies.



## 1-Impact of *T. achaea* offspring on *T. absoluta*

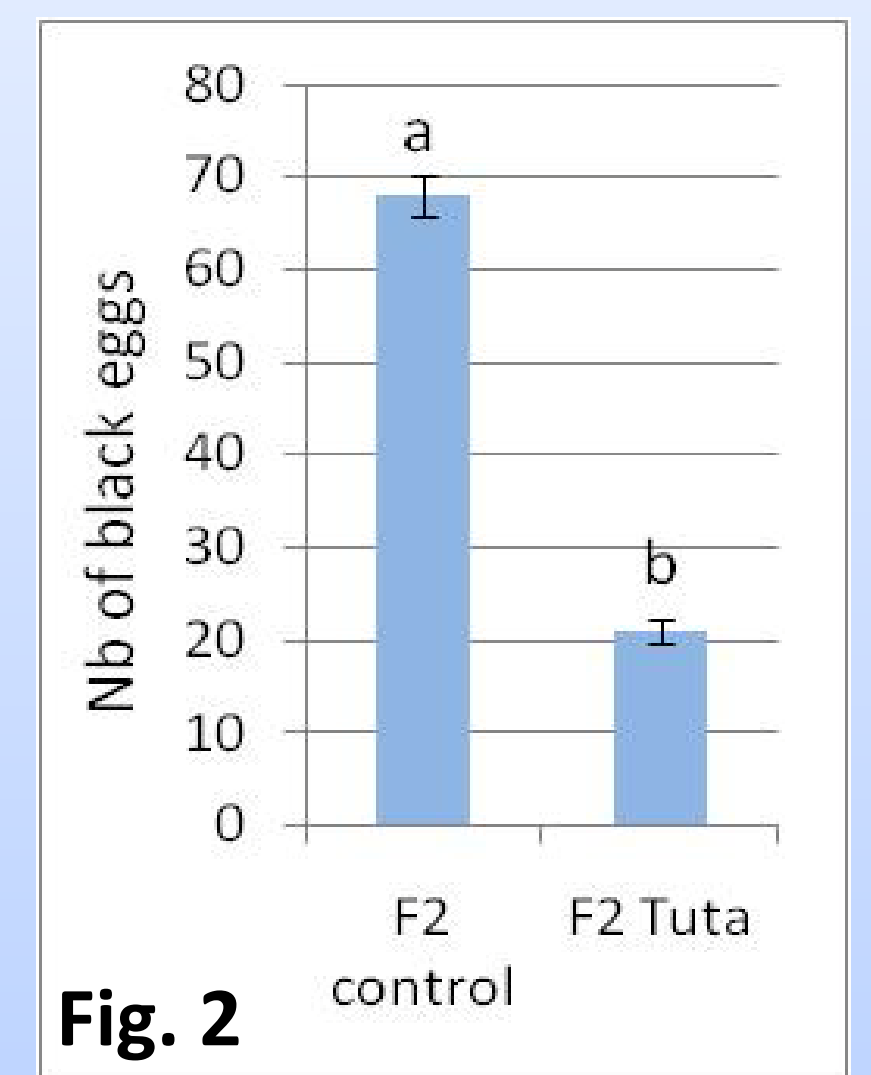
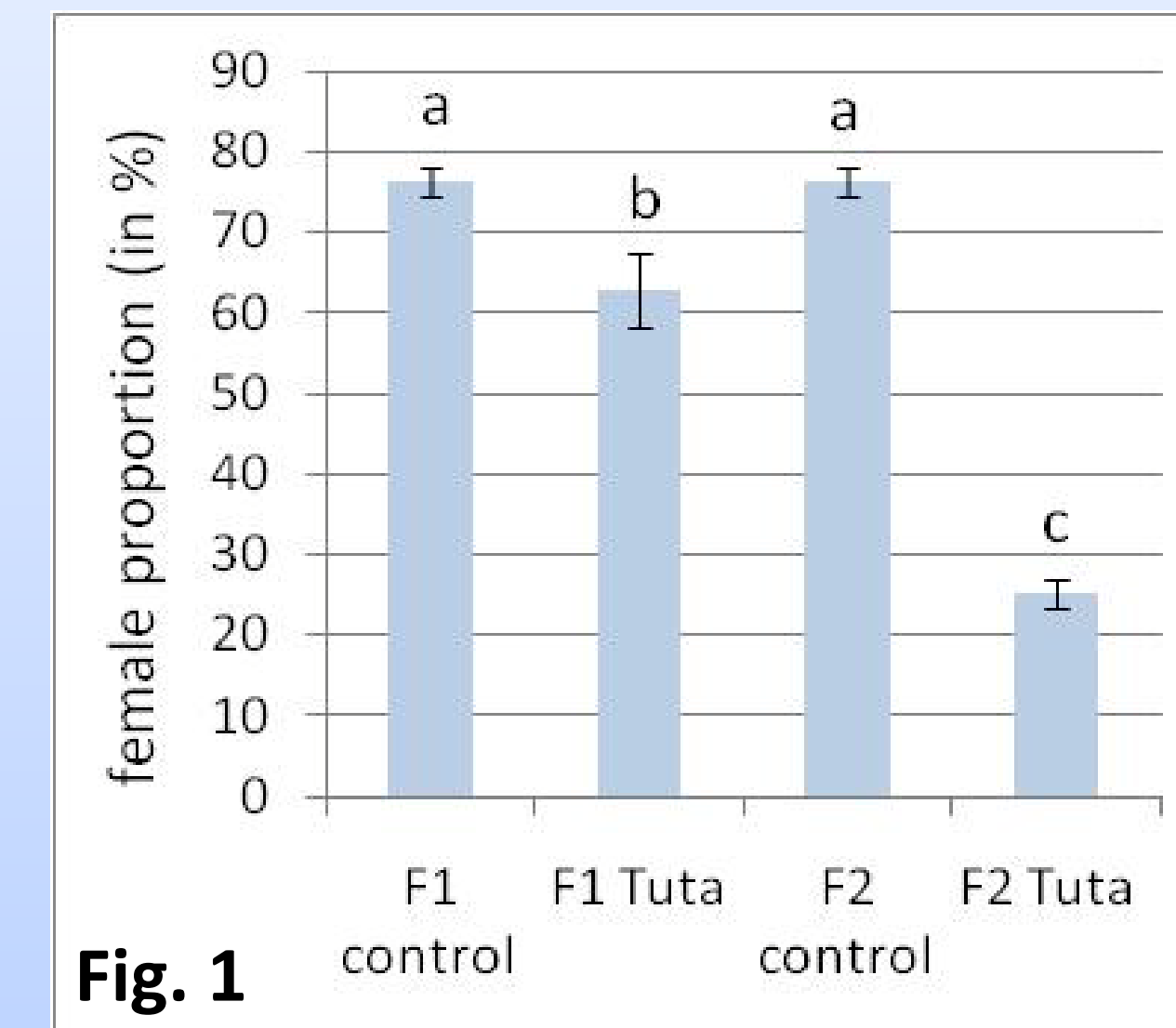
### First step

- Glass tubes (length: 7.5cm, diameter: 1.2cm) with one white paper card, closed by a piece of cotton.
- 12 black parasitized eggs (4 days old) from *T. absoluta* or *Ephesia kuehniella* (host used for rearing) on each paper card (n=20).
- 4 days exposure (until parasitoid emergence), 25±1°C, 70±5% R.H., fluorescent light.
- Parasitoid emerged from *T. absoluta* eggs are named "F1 Tuta"; those from *E. kuehniella* are named "F1 control".
- Measurements: number of adult parasitoids; number of females among them → sex ratio in F1.

### Second step

- Glass tubes (length: 7.5cm, diameter: 1.2cm) with one white sticky paper card, closed by a piece of cotton.
- Eggs of *Ephesia kuehniella* in excess on each paper card.
- 1 female from "F1 Tuta" or from "F1 control" in each glass tube (n=30).
- 7 days exposure with female, 10 days exposure in total (until last parasitized eggs turn black), 25±1°C, 70±5% R.H., fluorescent light.
- Parasitoids emerged from eggs parasitized by "F1 Tuta" females are named "F2 Tuta"; those from eggs parasitized by "F1 control" are named "F2 control".
- Measurements: number of black eggs; number of adult parasitoids; number of females among them → sex ratio in F1.

Two different letters represent two values significantly different ( $P < 0.05$ , Anova and LSD test)



Reduction in proportion of females in "F1 Tuta" and even more in "F2 Tuta" (Fig 1.  $F_{3,99}=138$ ;  $P < 0.001$ ). Results may be biased because all individuals from F1 were subsequently tested on *E. kuehniella* eggs (problem of host switching for "F1 Tuta"?).

Reduction in number of parasitized eggs when females have developed in *T. absoluta* eggs (Fig 2.  $F_{1,39}=128$ ;  $P < 0.001$ ) but such observations don't take into account learning process by parasitoid, which has been shown to be more receptive to chemical stimulus linked to the host in which it developed (Messing & Rabasse 1995; Vet & Dicke 1992).

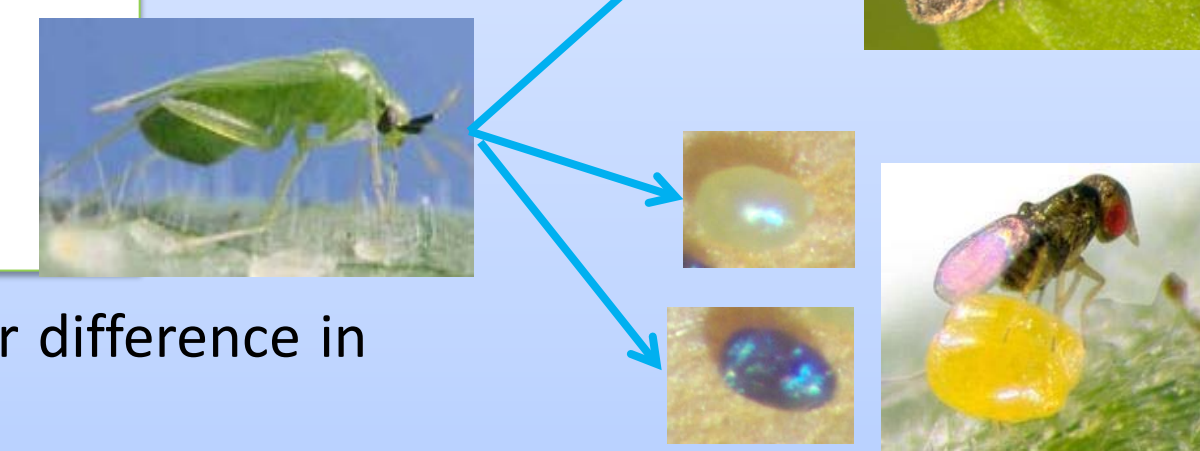
→ Reduction of *T. achaea* fitness when developing in *T. absoluta* eggs.

→ Occurrence of IGP should be assessed at short term study (one generation of insects).

## 2-Potential for IGP : does *M. pygmaeus* attack *T. achaea*-parasitized *T. absoluta* eggs parasitized by *T. achaea*?

### No-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. achaea*-parasitized eggs (parasitized for 0-4 days), or 12 black *T. achaea*-parasitized eggs (parasitized for more than 4 days) placed per paper card. Individual mated *M. pygmaeus* female introduced per glass tube (n=12-15).
- 12 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).



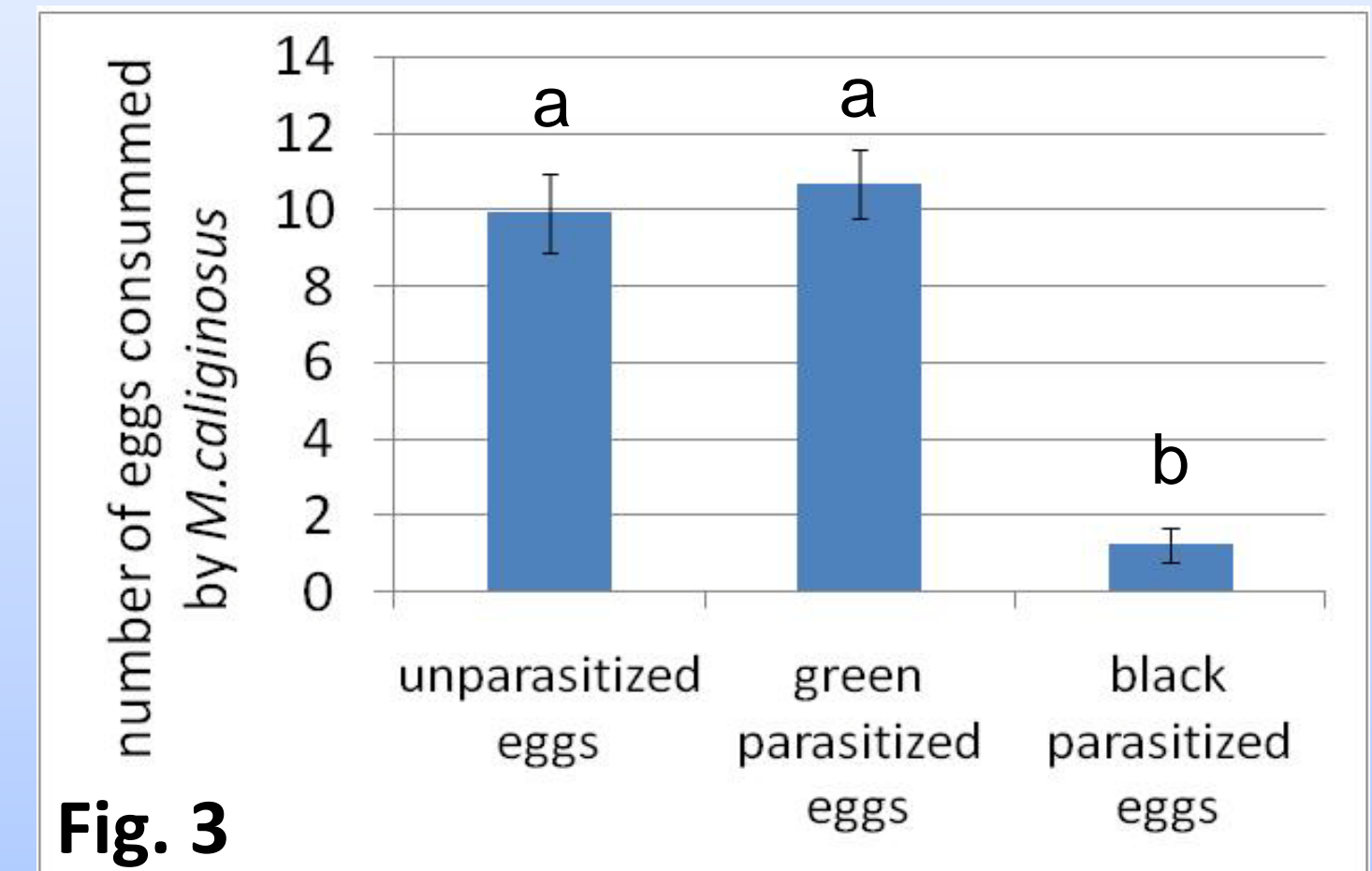
Consumption of parasitized eggs, but *M. pygmaeus* eats more green than black parasitized eggs (Fig. 3  $P < 0.05$ ) (owing to preference or difference in prey handling times).

No preference between green parasitized eggs and unparasitized eggs (Fig. 3  $P > 0.05$ ).

→ Potential for IGP by *M. pygmaeus* on *T. achaea*.

→ Variability of IGP intensity with parasitoid development.

Two different letters represent two values significantly different ( $P < 0.05$  Anova and LSD test)



## 3 - IGP and indirect interactions on whole tomato plants in short-term experiments: side effects on control of *T. absoluta*

### Microcosm bioassay:

- Microcosm design: individual tomato plant enclosed (5-7 leaves), 25°C, 70% RH, fluorescent light.
- 15 *T. absoluta* eggs (12-24 hours old) placed on each plant at day 1.
- 5 different treatments (16 replicates or 32 when using *M. pygmaeus* [16 with males and 16 with females]) → Tab. II (nb of natural enemies = 2-fold dose recommended in biological control strategy [Biotop pers. Com.] for high infestation of *T. absoluta*).
- 5 days exposure 25°C, 70% R.H., fluorescent light.
- Measurements (at day 5): number of *T. absoluta* larvae; number of parasitized eggs (black eggs); number of unparasitized eggs found.

Tab.I	target pest 15 <i>T. absoluta</i> eggs	predator 1 <i>M. caliginosus</i>	parasitoid 30 (± 5) <i>T. achaea</i>	alternativ prey one population of <i>B. tabaci</i>
Control	x			
Tr	x		x	
Ma	x	x		
Tr Ma	x	x	x	
Tr Ma B	x	x	x	x

### 3.1. - Effect of natural enemies on *T. absoluta* control

Significant reduction of *T. absoluta* larvae number by natural enemies (Fig. 4:  $F_{4,132} = 15.50$ ;  $P < 0.001$ ).

### 3.2. - Impact of intraguild predation and indirect interactions on natural enemies efficiency

Natural enemies more efficient when together ([Tr Ma]) than when alone ([Tr] or [Ma]) (Fig. 4:  $F_{4,132} = 15.50$ ;  $P < 0.001$ ).

→ Possibility for using them together in biological control strategy.

But no fully additive effect of two natural enemies on *T. absoluta*.

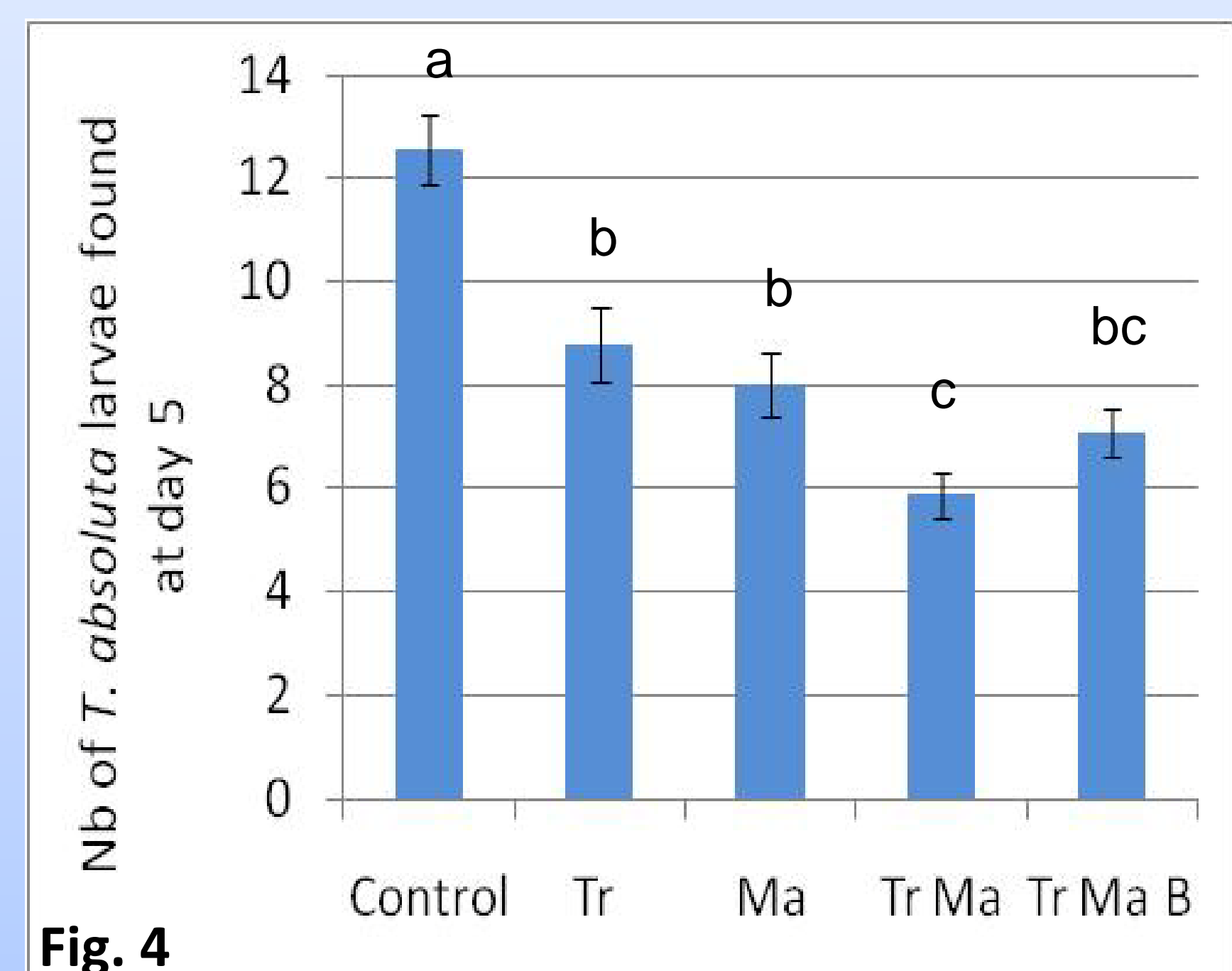
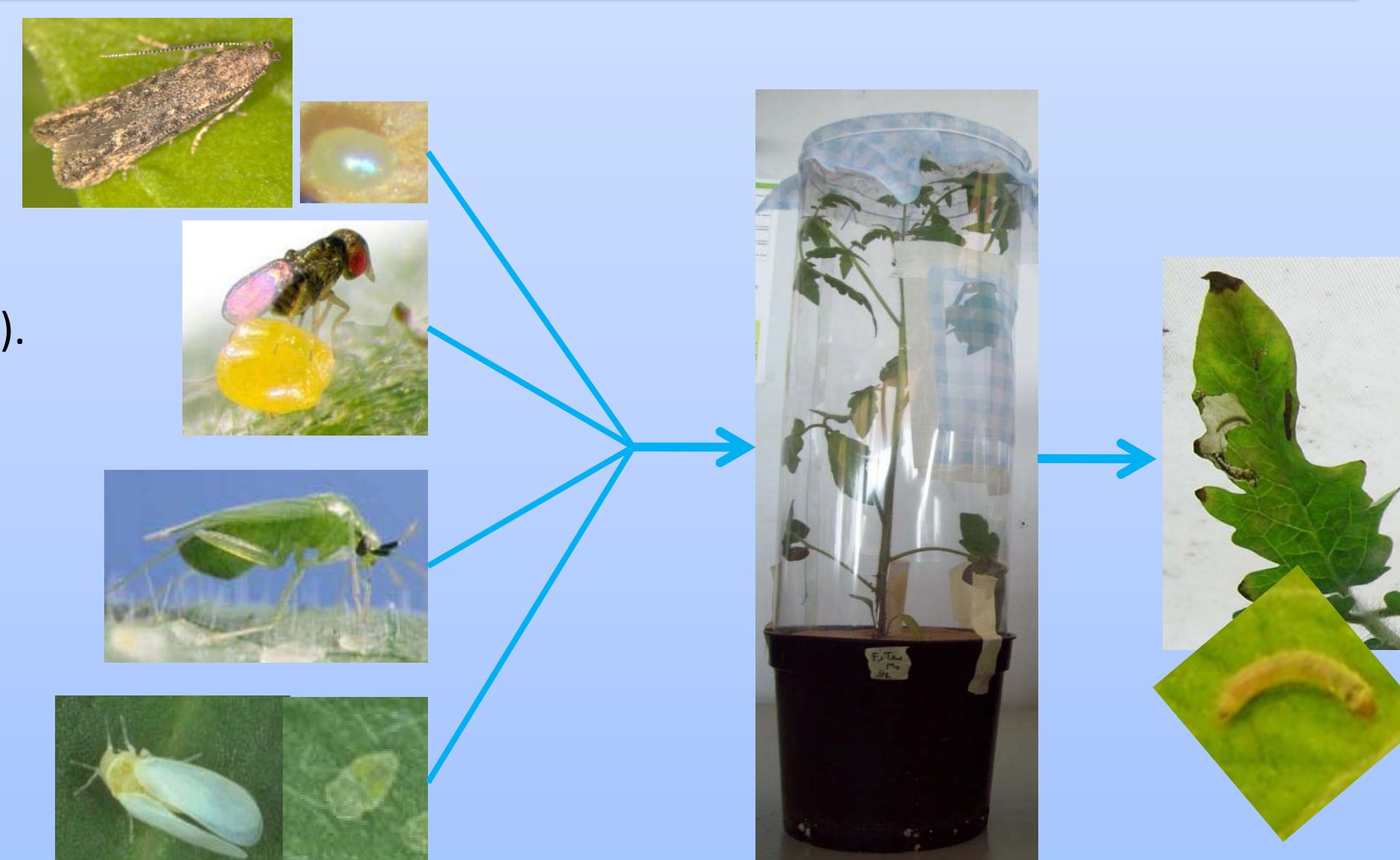
→ IGP by *M. pygmaeus* on *T. achaea*-parasitized *T. absoluta* eggs may weaken their efficiency.

No significant difference in natural enemies efficiency when *B. tabaci* is present (Fig. 4).

→ No significant indirect interaction between *T. absoluta* and *B. tabaci* mediated by *M. pygmaeus* in this case.

No difference in number of larvae remaining between [Tr] and [Tr Ma B].

→ *T. achaea* efficiency is finally not weaken in presence of *M. pygmaeus* and *B. tabaci*.



Two different letters represent two values significantly different ( $P < 0.05$  Anova and LSD test)

## Conclusions

*T. achaea* fitness decrease when developing in *T. absoluta* eggs suggests better efficiency of the parasitoid in inundative biological control strategy. Despite the occurrence of intraguild interactions between *T. achaea* and *M. pygmaeus*, using both natural enemies appears efficient to control *T. absoluta* population growth in short term. At this time scale potential indirect interactions with *B. tabaci* do not weaken significantly the control of *T. absoluta* by predator-parasitoid system. Finally the efficacy of *T. achaea* is not decreased by presence of *M. pygmaeus* and *B. tabaci*, suggesting possibility of using this parasitoid against *T. absoluta* in tomato greenhouses, when *Miridae* are used against whiteflies. Such results need to be confirmed by larger spatial scale experiments, but highlight nonetheless the necessity of considering trophic web interactions when drawing a biological control strategy.

## Acknowledgements

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