PURE

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Collaborative Project
SEVENTH FRAMEWORK PROGRAMME

D9.3
Novel temperature pre-treatments and rearing regimes to increase performance of parasitoids

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Concerned workpackage leader: Dr. Toby Bruce

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PP Restricted to other programme participants (including the Commission Services)	
RE Restricted to a group specified by the consortium (including the Commission Services)	
CO Confidential, only for members of the consortium (including the Commission Services)	

Table of Contents

1. SUMMARY **2**

2. OBJECTIVES **2**

3. MATERIAL AND METHODS **3**

4. RESULTS **4**

5. CONCLUSIONS **9**

1. Summary

The insect *Tuta absoluta* is a serious pest in all the countries were tomato is grown. In protected crops, where the damage can be extremely serious, there are the optimal conditions for combining sustainable tools of control, such as false trail (see WP11) and biological control. This task concentrated on the characterization of insect parasitoids available commercially in order to certify them from a taxonomical and biological point of view and the tests concentrated on *Trichogramma achaeae.* The second part of the task concerns the influence of temperature treatments on the performance of *T. achaeae.* The work has been developed in collaboration with the Department of Bioscience, Aarhus University (AU).

2. Objectives

- Assessment of the best combination of rearing temperature and temperature at oviposition on *T. achaeae* performance
- Assessment of the best host for *Trichogramma achaeae* for its performance as a biocontrol agent of *Tuta absoluta*

3. Material and methods

Trichogramma achaeae was provided by IAS. The strain emerged from eggs of *Ephestia kuehniella* and was tested on *Ephestia kuehniella* eggs. Egg plates of *Ephestia* eggs were offered to *T. achaeae* mated females for 48 h at 25°C and then transferred in climate cambers at 15°C, 25°C or 30°C. Emerging females (<24h) where then singly transferred into glass vials (Ø1.5 cm x 12 cm) with a little drop of honey solution (50%) and moved to receive 1h treatment at the following temperatures: 5°C, 10°C, 15°C, 25°C, 30°C, 35°C, 40°C. Soon after temperature treatment each female was transferred to a Petri dish containing about 200 eggs of *Ephestia kuehniella*. After 48h each female was removed and the eggs transferred at 25°C UR 60% and 18L:6D to assess the rate of parasitization (black eggs). For each combination of rearing temperature and temperature treatment, 15 replicates were considered.

In addition to the bioassay described above, at IPP-CNR it has been created a strain of *T. achaeae* reared on eggs of *Tuta absoluta* (**TA**) to check the importance of the rearing host on the performance of this parasitoid as biocontrol agent of *Tuta absoluta*. The performance of this strain in terms of attack rate on *Tuta absoluta* was compared to those of the **A02** and **A06** strains as provided by IAS.
In these experiments, *T. achaeae* mated and fed females from each strain were offered 20 *T. absoluta* eggs for 2 consecutive days (in total 40 eggs/female/strain).

4. Results

There was a clear effect of both rearing temperatures and temperature treatments on the performance and parasitization rate of *T. achaeae* on *E. kuehniella* eggs.

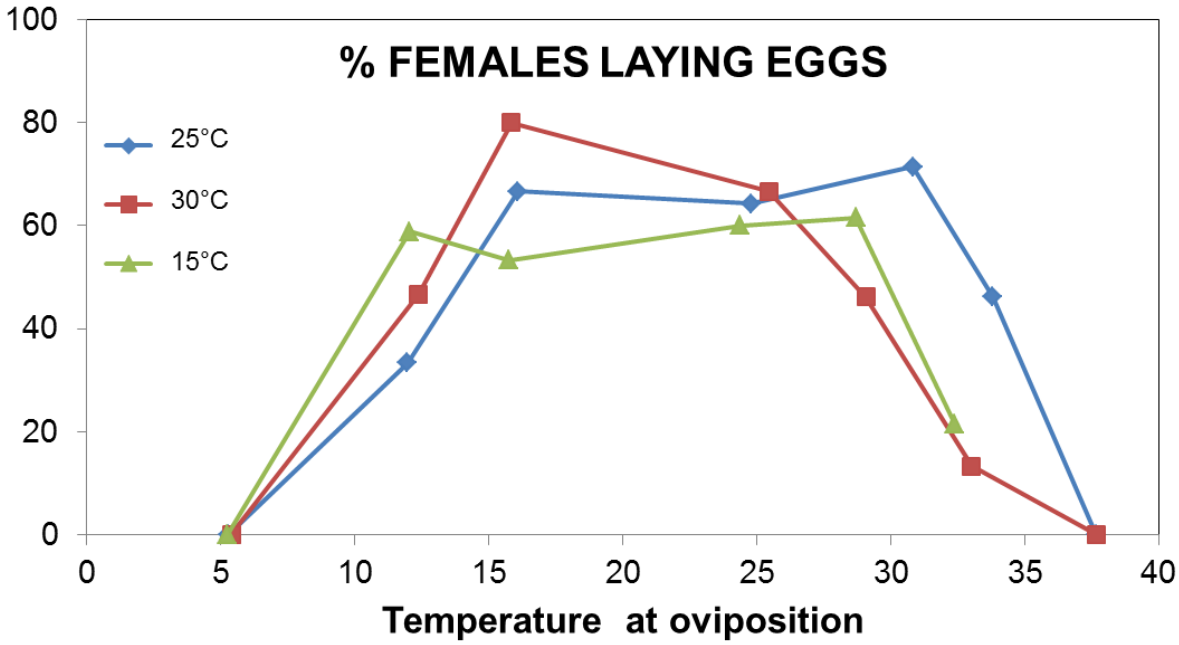

Fig. 1: Percentage of females of *Trichogramma achaeae* reared at three different temperatures (15°C, 25°C, 35°C) and laying eggs at seven different temperatures

Fig. 1 shows that the temperature at oviposition is an important parameter affecting the total number of *Trichogramma* females laying eggs. At 5°C and 40°C, regardless the temperature regime kept during the development, *T. achaeae* females do not lay any egg and, this is important considering the temperatures usually reached in protected tomato crops during summer. Another consideration is that the highest percentage of ovipositing females was recorded for females reared at 30°C and ovipositing at 15°C. In addition, this figure shows that at 25°C (temperature used for the following tests) we have the least difference between the percentage of ovipositing females coming from different rearing temperatures.

Figures 2- 6 show the attack rate (= number of black eggs) as resulted by different combinations of temperatures during the development and temperature treatment. The first consideration is that 1h shock at 5°C results in no oviposition regardless the rearing temperature (data not shown).

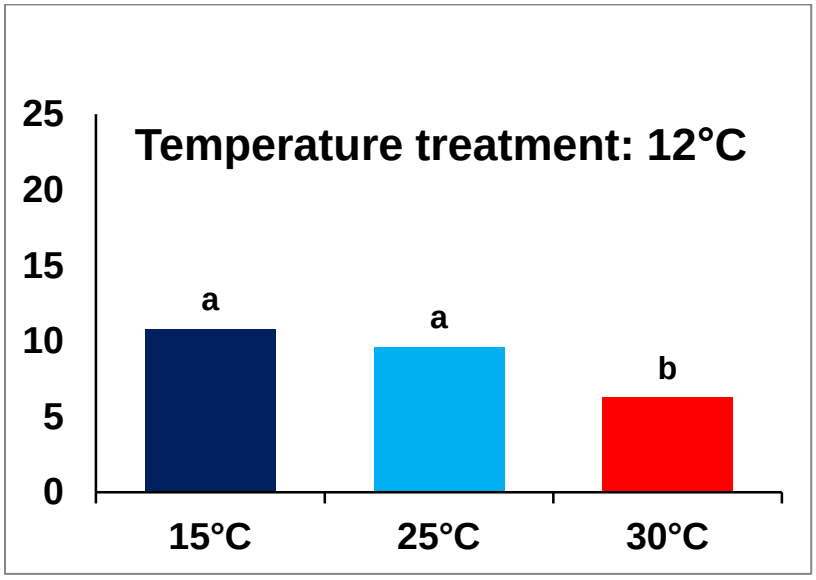

Fig 2: Oviposition rate (black eggs of *Ephestia kühniella*) of *T. achaeae* reared at 3 different temperatures (15°C, 25°C, 30°C) receiving a temperature treatment at 12°C

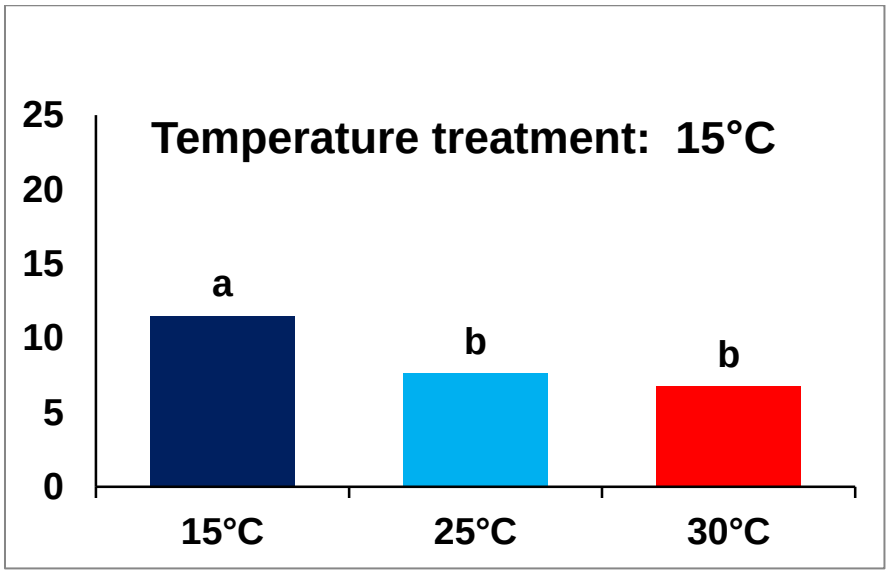

Fig 3: Oviposition rate (black eggs of *Ephestia kühniella*) of *T. achaeae* reared at 3 different temperatures (15°C, 25°C, 30°C) receiving a temperarature treatment at 15°C

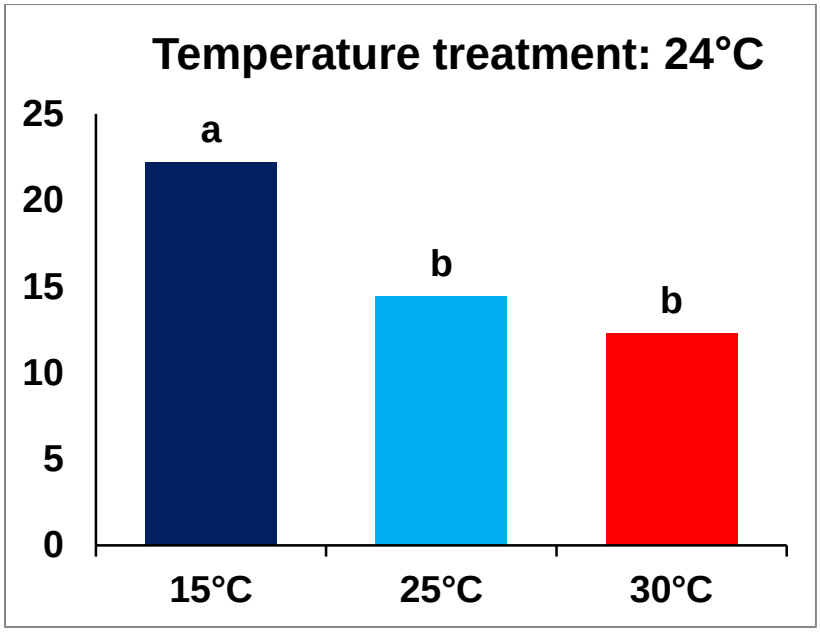

Fig 4: Oviposition rate (black eggs of *Ephestia kühniella*) of *T. achaeae* reared at 3 different temperatures (15°C, 25°C, 30°C) receiving a temperature treatment at 24°C

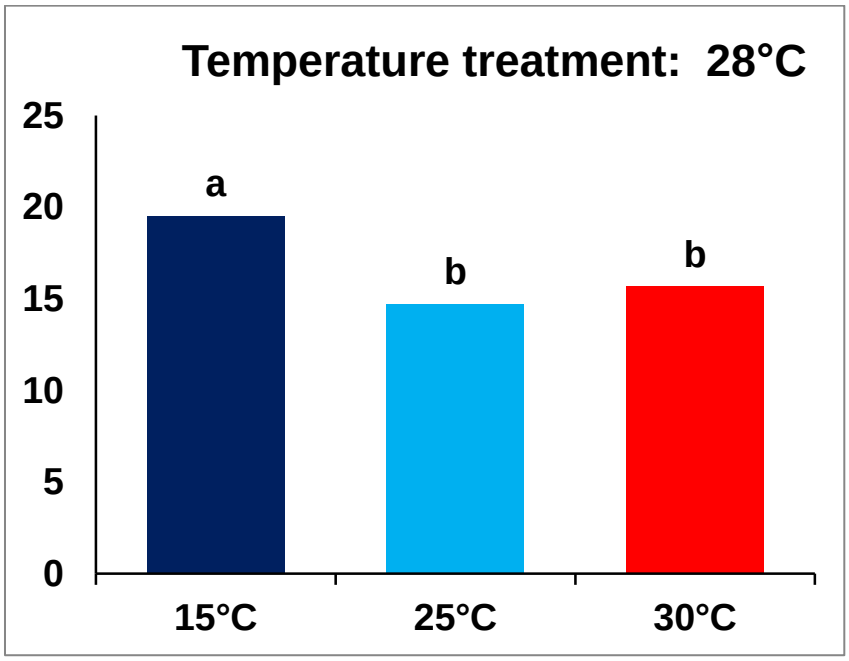

Fig. 5: Oviposition rate (black eggs of *Ephestia kühniella*) of *T. achaeae* reared at 3 different temperatures (15°C, 25°C, 30°C) receiving a temperature treatment at 28°C

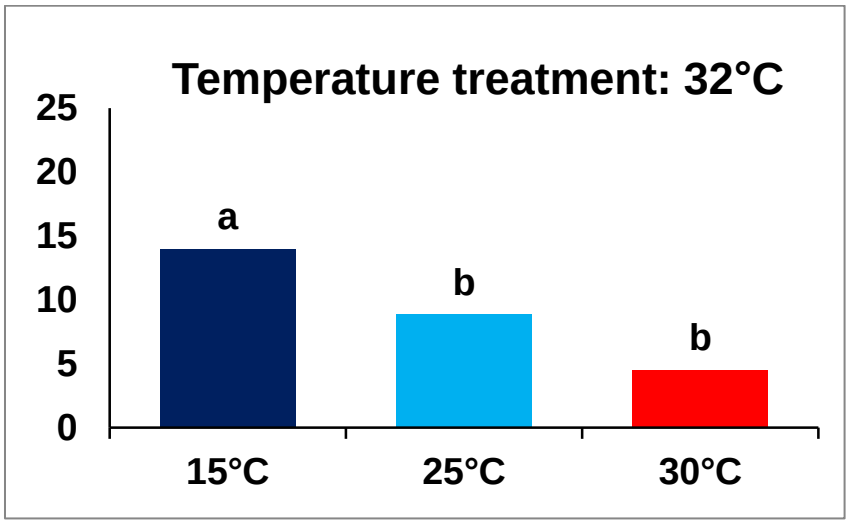

Fig. 6: Oviposition rate (black eggs of *Ephestia kühniella*) of *T. achaeae* reared at 3 different temperatures (15°C, 25°C, 30°C) receiving a temperature treatment at 32°C

The second consideration is that for any temperature treatment, the best performance is recorded for parasitoids reared at 15°C (letters in the figure correspond to statistical differences within each temperature treatment).

If we compare the results obtained by the “strain” of *Trichogramma achaeae* reared at 15°C to assess the best performance in terms of attack rate (Fig. 7), we will find that this occurs after a pre-treatment at 24°C or 28°C.

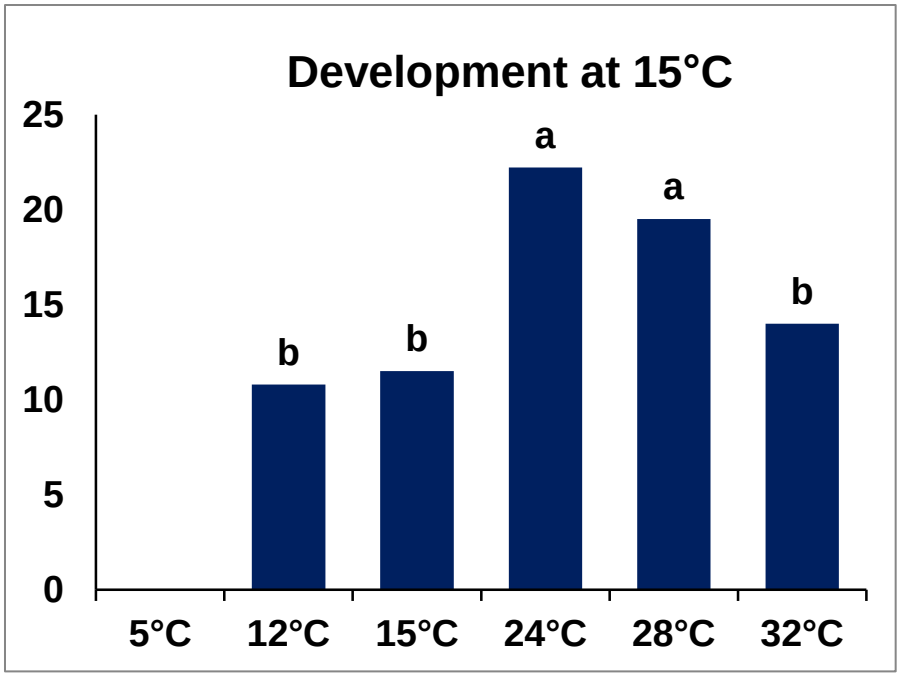

Fig. 7: Oviposition rate (black eggs of *Ephestia kühniella*) of *T. achaeae* reared at 15°C and receiving 6 different temperature treatments (5°C, 12°C, 15°C, 24°C, 28°C, 32°C)

These results were compared with the attack rate of the same strains (A02, A06 and TA) on *T. absoluta* (Fig. 8).

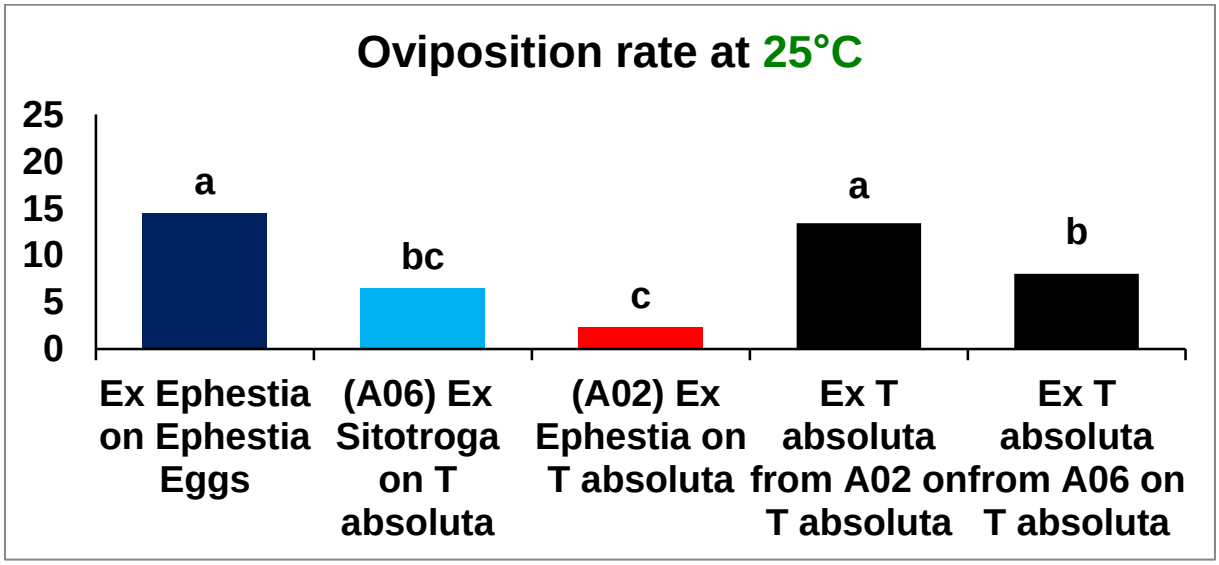

Fig. 8: Oviposition rate (black eggs) of *Trihogramma achaeae* strains with different rearing history

Figure 8 shows the results that highlight the importance of the rearing history (in addition to temperature of development and oviposition thermal pre-treatments) on the performance of *T. achaae* on the target pest *Tuta absoluta*.

There are at least two considerations to draw while examining these results. The first is that the strain A06 (from *S. cerealella*) performs better that the strain A02 (from *E. kuehniella*) when attacking (first generation) *T. absoluta*. However these differences inverted when these strains were reproduced for one generation on *T. absoluta.*

It is also evident that the highest attack rate resulted when the reared host and the target host coincide as it appears by comparing the attack rates of *T. achaeae* from *Ephestia* on *Ephestia* and of *T. achaeae* from *Tuta* on *Tuta*.

5. Conclusions and directions

The indication to improve the performance of *Trichogramma achaeae* as a biocontrol agent of *Tuta absoluta* can be summarized as follows:

1 - Integrative characterization of the biocontrol agent: the combination between morphological and molecular analyses is essential to reduce the possible use of misidentified species.

2 - The best rearing temperature for *T. achaeae* in terms of performances on the target host *Tuta absoluta* is 15°C

3 - Preoviposition temperature shocks (1 h) that improve the oviposition rate of *T. achaeae* should be performed at 24°C and 28°C

4 - At least one generation on f *T. acahaeae* should be reproduced on *T. absoluta* before using it to control the tomato borer.

The results of this study will be published on an ISI journal.