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# Abundance of *Tuta absoluta* (Meyrick, 1917) and its natural enemies on tomato crops in greenhouses of different production modes (Azores, Portugal) (Lepidoptera: Gelechiidae)

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**Abstract**

*Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) is a major pest of tomato (*Solanum lycopersicum* L.) crops produced in the Azorean greenhouses. Despite the major concerns, no systematic study is available to describe population dynamics of *T. absoluta* and its natural enemies. The objective of this study was to compare the abundance of the pest (eggs and larvae) and its native natural enemies, in greenhouses of tomato crops produced under different production modes (biological, traditional and intensive). A sampling program was carried out during 2020-2021 and two production seasons of spring-summer and fall-winter. The abundance of eggs and larvae of *T. absoluta* were higher in tomato crops in intensive production and lower in biological production mode. Infestations by eggs and larvae were higher in spring-summer seasons. The natural enemies recorded were *Macrolophus pygmaeus* (Rambur, 1839) (Hemiptera: Miridae), *Dicyphus cerastii* Wagner, 1951 (Hemiptera: Miridae) and *Trichogramma achaeae* Nagaraja & Nagarkatti, 1960 (Hymenoptera: Trichogrammatidae). The abundance of natural enemies were very low and, virtually, do not occur during the fall-winter seasons. We found no significant difference between the abundance of *M. pygmaeus* amongst tomato crops from different production mode but *D. cerastii* was more abundant on greenhouses of biological production mode. Parasitism rate by *T. achaeae* does not differ between production modes and crop seasons. Considering the major concerns of the growers by the infestation levels of their crops, together with low abundance of native natural enemies, we suggest the use of biological production mode or an inoculative or augmentative strategy to control the pest in intensive and traditional productions modes.
**Keywords:** Lepidoptera, Gelechiidae, *Tuta absoluta*, natural enemies, infestation level, production mode, Azores, Portugal.

**Abundancia de *Tuta absoluta* (Meyrick, 1917) y sus enemigos naturales en cultivos de tomate en invernaderos de diferentes modos de producción (Azores, Portugal) (Lepidoptera: Gelechiidae)**

**Resumen**

*Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) es una de las principales plagas de los cultivos de tomate (*Solanum lycopersicum* L.) producidos en los invernaderos de las Azores. A pesar de las principales preocupaciones por los cultivos, no se dispone de ningún estudio sistemático que describa la dinámica de población de *T. absoluta* y sus enemigos naturales. El objetivo de este estudio fue comparar la abundancia de la plaga (huevos y larvas) y sus enemigos naturales nativos, en invernaderos de cultivos de tomate producidos bajo diferentes modalidades de producción (biológica, tradicional e intensiva). El programa de muestreo se llevó a cabo durante 2020-2021 durante dos temporadas de producción de primavera-verano y otoño-invierno. La abundancia de huevos y larvas de *T. absoluta* fue mayor en cultivos de tomate en producción intensiva y menor en producción biológica. Las infestaciones por huevos y larvas fueron mayores en primavera-verano. Los enemigos naturales registrados

fueron *Macrolophus pygmaeus* (Rambur, 1839) (Hemiptera: Miridae), *Dicyphus cerastii* Wagner, 1951 (Hemiptera: Miridae) y *Trichogramma achaeae* Nagaraja & Nagarkatti, 1960 (Hymenoptera: Trichogrammatidae). La abundancia de enemigos naturales fue muy baja y prácticamente no se presentó durante la temporada otoño-invierno. No encontramos diferencias significativas entre la abundancia de *M. pygmaeus* entre cultivos de tomate de diferente modo de producción, pero *D. cerastii* fue más abundante en invernaderos de modo de producción biológico. La tasa de parasitismo por *T. achaeae* no difiere entre modos de producción y temporadas de cultivo. Considerando las principales preocupaciones de los productores por los niveles de infestación de sus cultivos, junto con la baja abundancia de enemigos naturales nativos, sugerimos el uso del modo de producción biológico o una estrategia inoculativa o aumentativa para controlar la plaga en modos de producción intensivos y tradicionales.
**Palabras clave:** Lepidoptera, Gelechiidae, cultivos de tomate, *Tuta absoluta*, enemigos naturales, nivel de infestación, modo de producción, Azores, Portugal.

**Abundância de *Tuta absoluta* (Meyrick, 1917) e dos seus inimigos naturais em plantações de tomate de estufa de diferentes modos de produção (Açores, Portugal) (Lepidoptera: Gelechiidae)**

**Resumo**

Nos Açores, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) é uma praga-chave da cultura de tomate (*Solanum lycopersicum* L.) produzida em estufa. Apesar dos importantes danos causados à cultura, o facto é que não se conhece qualquer estudo sistemático sobre a dinâmica populacional de *T. absoluta* e dos seus inimigos naturais. O objetivo deste estudo foi comparar a abundância da praga (ovos e larvas) e de seus inimigos naturais em estufas de tomateiro produzidos sob diferentes modos de produção (segundo as orientações do modo de produção biológico, tradicional e intensivo). Foram realizadas amostragens durante os anos de 2020 e 2021 e ao longo de duas épocas de produção, a de primavera-verão e a de outono-inverno. A abundância de ovos e larvas de *T. absoluta* foi maior na plantação de tomate em produção intensiva e menor na plantação conduzida segundo as orientações do modo de produção biológica. As infestações por ovos e larvas foram maiores na estação primavera-verão. Os inimigos naturais observados foram: *Macrolophus pygmaeus* (Rambur, 1839) (Hemiptera: Miridae), *Dicyphus cerastii* Wagner, 1951 (Hemiptera: Miridae) e *Trichogramma achaeae* Nagaraja & Nagarkatti, 1960 (Hymenoptera: Trichogrammatidae). As abundâncias de inimigos naturais foram muito baixas e, virtualmente, não ocorrem durante o outono-inverno. Não encontramos diferença significativa entre a abundância de *M. pygmaeus* entre cultivos de tomate de diferentes modos de produção, mas *D. cerastii* foi mais abundante em estufas conduzidas segundo as orientações do modo de produção biológico. A taxa de parasitismo por *T. achaeae* não difere entre modos de produção e épocas de cultivo. Tendo em conta as preocupações dos produtores com os níveis de infestação nas suas produções, bem como com a baixa abundância de inimigos naturais nativos, sugerimos a utilização do modo de produção biológico ou uma estratégia inoculativa ou aumentativa para o controlo da praga nos modos de produção intensivo e tradicional.
**Palavras-Chave:** Lepidoptera, Gelechiidae, *Tuta absoluta*, inimigos naturais, nível de infestação, modos de produção, Açores, Portugal.

## Introduction

*Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a major pest of tomato crops, causing devastating worldwide economic damage to growers (Biondi et al. 2018). Economic losses inflicted by this species can reach 100% due to attacks on the leaves, flowers, stems and especially on tomato fruits (Chermiti et al. 2009; Balzan & Moonen, 2012; Guedes & Picanço, 2012). This pest was firstly recorded outside of South America (its native range), in Eastern Spain in 2006, and it is now widely distributed in the Mediterranean Basin, (Desneux et al. 2010, 2011). The origin of Mediterranean populations was, probably, from Central Chile near Talca (Guillemaud et al. 2015). Currently, *T. absoluta* distribution area is very wide and includes, North Africa, Middle East, South Asia, sub-Saharan Africa, South Africa and several other countries (Bacci et al. 2021).

In Portugal (mainland), since 2009, *T. absoluta* has been reported in tomato crops produced in greenhouses (Figueiredo et al. 2010; Matos et al. 2012; Payer et al. 2012). In the Azores archipelago (Portugal), the species was accidentally introduced and firstly reported for São Miguel Island, during 2009/2010 (DSA, 2014). Later, in 2014/2015, populations were already distributed in Terceira, Faial

and Pico Islands, infesting tomato crops produced in greenhouses and open fields (Vieira, 2016). More recently it was observed inside of greenhouses of Graciosa, Santa Maria, São Jorge and Flores islands (A. O. Soares and C. Durão, personal observations). The leaf miner is polyphagous and can feed on different plant species, from Solanaceae and Convolvulaceae families, such as eggplant (*Solanum melongena* L.), potato (S*olanum tuberosum* L.), sweet potato (*Ipomea batatas* (L.) Lam.), pepper (*Capsicum annuum* L.), wild tomato (*Lycopersicon hirsutum* Dunal), bittersweet nightshade (*Solanum dulcamara* L.), black nightshade (*Solanum nigrum* L.), common thorn (*Datura stramonium* L.), cape gooseberry (*Physalis peruviana* L.) and tobacco (*Nicotiana tabacum* L.) (Vieira, 2016).

Some parasitoids and predators are important natural enemies of *T. absoluta* (e.g., CABI, 2022; Arnó et al. 2021; Borges et al. 2023). In Mediterranean area, several predators are reported as feeding on *T. absoluta* eggs, but inefficiently against larvae. This seems the case of *Macrolophus pygmaeus* (Rambur, 1839), *Nesidiocoris tenuis* (Reuter, 1895), *Dicyphus errans* (Wolff, 1804) and *Dicyphus bolivari* Lindberg, 1934 (Hemiptera: Miridae) (Arnó et al. 2009; Urbaneja et al. 2009). For Portugal (mainland), *Trichogramma evanescens* Westwood, 1833 (Hymenoptera: Trichogrammatidae) (Payer et al. 2012) and *Diglyphus isaea* (Walker, 1838) (Hymenoptera: Eulophidae) (Payer et al. 2015) appear promising natural enemies. Despite the major concerns for the Azorean tomato crops, no systematic study is available to describe population dynamics of *T. absoluta* and its natural enemies in tomato crops of different production modes (biological, traditional and intensive). However, some casual records detected potentially useful natural enemies, such as the zoophytophagous *M. pygmaeus* and the parasitoid *Trichogramma achaeae* Nagaraja & Nagarkatti, 1969 (Hymenoptera: Trichogrammatidae). Laboratory and field studies using *T. achaeae* have showed promising results (Oliveira et al. 2017), but an effective control of pest populations was not achieved.

This study has the following objectives: i) To compare the seasonal abundance of eggs and larvae of *T. absoluta* in tomato crops of different production modes, ii) to record native natural enemies of *T. absoluta*, iii) to compare the abundance of natural enemies in different tomato crops of production modes and iv) to determine the seasonal abundance parasitism rate on eggs of *T. absoluta* by *T. achaeae* per production mode.

## Material and Methods

### Abundance of *Tuta absoluta* and its natural enemies

The sampling program was carried out from April to December of 2020 and 2021 and took place in greenhouses located in S. Miguel island (Azores, Portugal), from three different production mode: biological (in accordance with the organic production guidelines), traditional (in accordance with integrated pest management guidelines), and intensive (it uses all standard agronomic practices aiming to increase substantially the productivity).

For the parasitoid *T. arhaeae*, fifty tomato leaflets (25 from the upper plant stratum and 25 from the middle plant stratum) were collected every 15 days from 10 randomly tomato plants. The leaflets were bought back to laboratory and observed under a stereomicroscope. The number of eggs, larvae and mines of *T. absoluta*, were recorded and counted. Any observed eggs of *T. absoluta*, along with a small portion of the affected leaf, were individually isolated in a glass tube (0.01 by 0.07 m, each) for a daily observation of the hatching larvae, as well as a determination of the number of species and emerged parasitoids. For the predators, 50 tomato plant leaves randomly selected (25 at the upper plant stratum and 25 at the middle plant stratum) were inspected by direct observation for the presence of adult or nymph mirids in the field. The number of leaflets in each leaf was registered to allow standardize predator density by leaflet.

### Statistical analyses

For *T. absoluta*, were contrasted the effect of production mode (independent variables) on the

abundance of larvae and eggs per leaflet (dependent variables) using general linear models (GLM). Factors were analyzed using one-way ANOVA test for a confidence level of 95%. Pairwise multi comparisons were performed and P values corrected using Bonferroni test. To contrast the abundance of *T. absoluta* larvae and eggs per leaflet per crop season and production mode (dependent variables), we used the Mann-Whitney non-parametric test for 2 samples, for a confidence level of 95%.

For *M. pygmaeus* and *D. cerastii*, we contrasted the effect of production mode during spring-summer season (independent variables) on the abundance of individuals (dependent variables), using general linear model (GLM). Factors were analyzed using one-way ANOVA test for a confidence level of 95%. Pairwise multi comparisons were performed and P values corrected using Bonferroni test.

To contrast parasitism rate on eggs of *T. absoluta* by *T. achaeae* (independent variables) per production mode (dependent variables) we used a general linear model (GLM). Factors were analyzed using one-way ANOVA test for a confidence level of 95%. Pairwise multi comparisons were performed and P values corrected using Bonferroni test. Parasitism rate on eggs of *T. absoluta* by *T. achaeae* (dependent variables) per crop season and production mode (independent variables) we performed using a Mann-Whitney non-parametric test for 2 samples, for a confidence level of 95%.

All the statistical analyses were performed on the SPSS 27.

## Results

### Abundance of *Tuta absoluta* and its natural enemies

In relation to *T. absoluta*, the abundance of eggs ($F_{(3,75)}$ = 4.496, P = 0.014) and larvae ($F_{(3,75)}$ = 6.435, P = 0.003) were significantly higher in tomato crops from intensive production mode and lower in tomato plants from biological and traditional production modes (Figure 1).

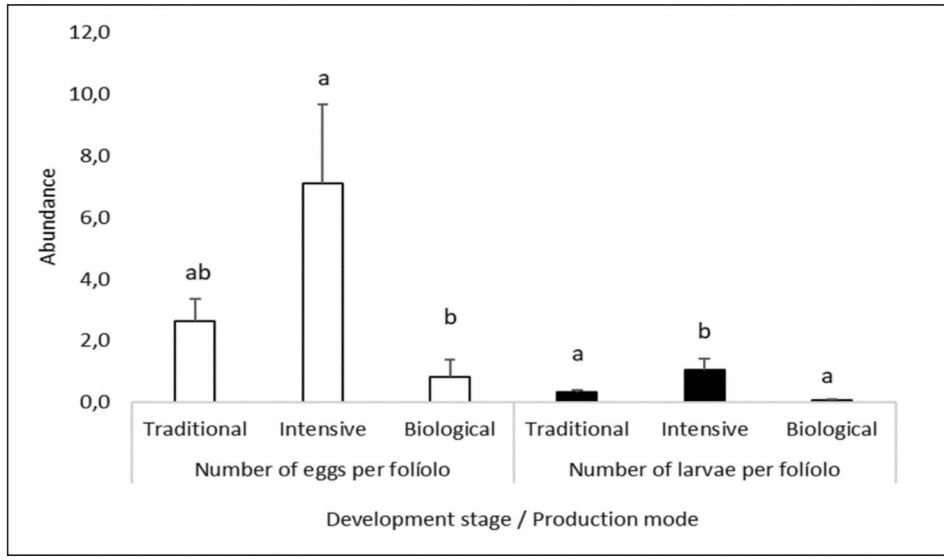


**Figure 1.** Number of eggs and larvae of *T. absoluta* per leaflet, in tomato crops produced under different production modes.

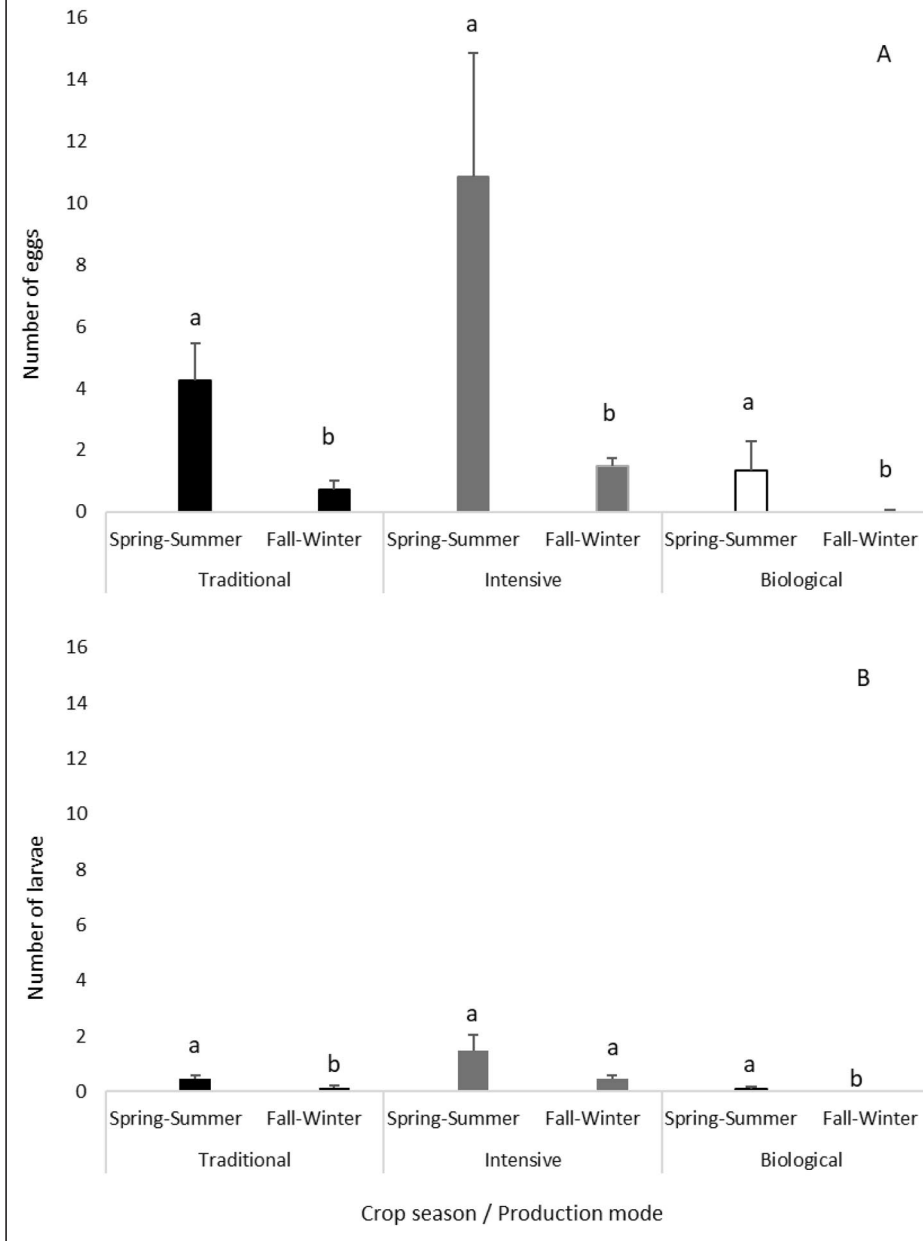


**Figure 2.** Number of eggs (**A**) and larvae (**B**) of *T. absoluta* per foliolo per crop season (spring-summer vs fall-winter) under different tomato crops produced under different production modes.

The infestations of tomato plants by eggs and larvae of *T. absoluta* were significantly higher in spring-summer compared to the fall-winter crop season, in almost all production modes. In terms of the number of eggs per leaflet and crop seasons, significant differences occurred in all production modes, traditional: U = 14.5, P = 0.0001, intensive: U = 35.0, P = 0.026 and biological: U = 46.0, P = 0.039 (Figure 2A). In relation to the number of larvae per leaflet and crop season, significant differences occurred for traditional mode: U = 28.0, P = 0.003 and biological mode: U = 44.0, P = 0.03, but not for intensive mode: U = 51.5, P = 0.196 (Figure 2B).

During the field work, we found that the most abundant natural enemies of *T. absoluta* were the predators *M. pygmaeus* and *Dicyphus cerastii* Wagner (Hemiptera: Miridae) and the parasitoid *T. achaeae*. Concerning to the predators, the abundance was low and, virtually, they do not occur during the fall-winter season. We found no significant difference between the abundance of *M. pygmaeus* between tomato crops from different production modes ($F_{(2,45)}$ = 2.04, P = 0.141) and *D. cerastii* was significantly more abundant on biological production mode ($F_{(2,45)}$ = 4.22, P = 0.021) (Figure 3). Parasitism rate by *T. achaeae* does not significantly differ between production modes ($F_{(3,59)}$ = 1.106, P = 0.338) (Figure 4) and crop seasons: traditional - U(1) = 96.0, P = 0.56, intensive - U(1) = 51.5, P = 0.285 and biological – no parasitism was recorded (Figure 5).

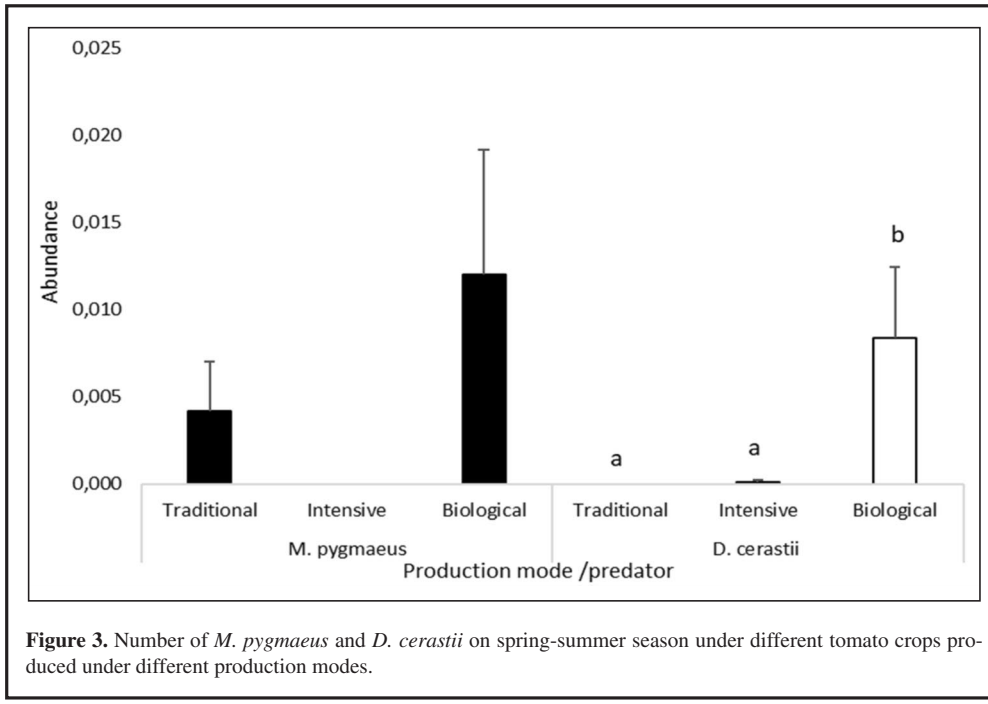


**Figure 3.** Number of *M. pygmaeus* and *D. cerastii* on spring-summer season under different tomato crops produced under different production modes.

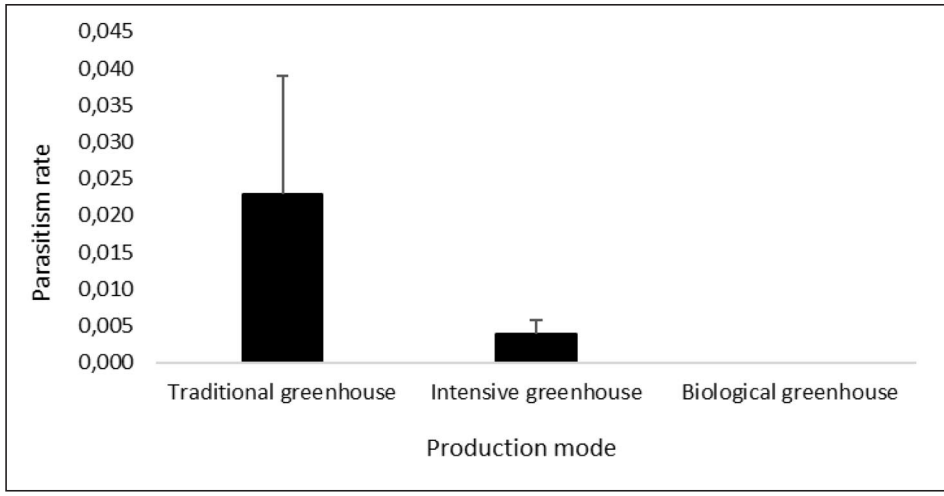


**Figure 4.** Parasitism rate on eggs of *T. absoluta* by *T. achaeae* under different tomato crops produced under different production modes.

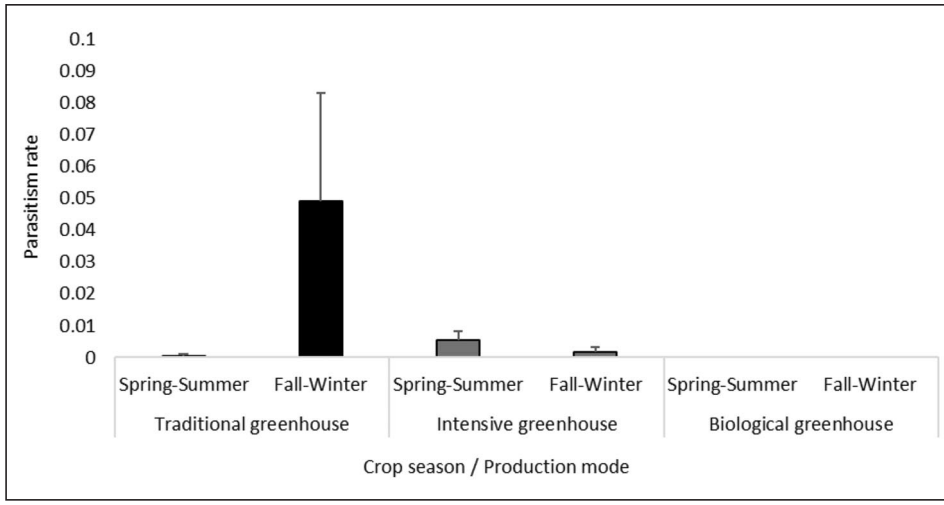


**Figure 5.** Parasitism rate on eggs of *T. absoluta* by *T. achaeae* per crop season under different tomato crops produced under different production modes.

## Discussion

We found that the abundance of eggs and larvae of *T. absoluta* were significantly higher on tomato crops from intensive production mode and lower on tomato plants from biological production mode. Intensive production is characterized by the recurrent use of insecticides against the pest. Despite the positive short-term effect of insecticide on the reductions of mines and injuries caused by the moth larvae, previous study has shown that this strategy of control does not significantly reduce densities below economic threshold, especially in seasons with suitable abiotic conditions for *T. absoluta* (Bacci et al. 2021). Even several applications were found to be inefficient to keep population below the economic threshold (Bacci et al. 2021). Our results may also depict the environmental imbalance caused by the recurrent use of phytopharmaceuticals that originate the appearance of resistance in populations of the pest (Siqueira et al. 2000a; Siqueira et al. 2000b; Lietti et al. 2005; Bielza, 2010). Indeed, *T. absoluta* evolve resistance to conventional insecticides where insecticides are applied, leading to a delay in population dynamics (Silva et al. 2011; Silva et al. 2015; Siqueira et al. 2000a; Siqueira et al. 2000b).

Life cycle of *T. absoluta* develops continuously throughout the year, even at the least favourable time of year, during the fall and the winter seasons. Two reasons might contribute to this. Firstly, due to the absence winter diapause (Urbaneja et al. 2013). Second, *T. absoluta* complete development at temperatures ranging from 15 and 35ºC (Machekano et al. 2018; Bentancourt et al. 1996; Mohamed et al. 2022) and thermal threshold was estimated at 8.1, 7.83, and 11.62ºC, respectively for egg, larva, and pupa (Mohamed et al. 2022). All those thermal conditions are found to occur in the Azores. The abundance of *T. absoluta*, however, differs according to the production season. The infestations of plants by eggs and larvae of *T. absoluta* were significantly higher in spring-summer compared to the fall-winter crop season in almost all production modes, with an exception in relation to the number of larvae per leaflet and crop season in the intensive production mode. The absence of winter diapause coupled with polyphagia, allows the non-stop dispersion and development of *T. absoluta*. Indeed, it can easily disperse between tomato crops and other surrounding cultivations. When cultivation is declining in quality for the pest, individuals may disperse from the site for another more favourable to its establishment (Bacci et al. 2021). In short, population dynamics are governed by an array of local biotic and abiotic factors (Urbaneja et al. 2013).

The most abundant natural enemies of *T. absoluta* were the predators, *M. pygmaeus* and *D. cerastii*, and the oophagus parasitoid *T. achaeae*, previously referred as a promising natural enemies of *T. absoluta* in the Azores (Oliveira et al. 2017) and across other European countries (Chailleux et al. 2012; Polaszek et al. 2012; Urbaneja et al. 2012). Similarly, *D. cerastii* was recorded in tomato crops of Portugal mainland and feeding on several tomato pests, including *T. absoluta*, indicating their potential to control this pest (Abraços-Duarte et al. 2021).

The abundance of the predators was low and, virtually, they do not occur during the fall-winter season, due to the low temperatures observed during this period. According to some authors, the higher feeding and development rates of *M. pygmaeus* is observed at 30ºC (Dionyssios & Dionyssios, 2002; Perdikis et al. 1999), which occur commonly during spring and summer inside the Azorean greenhouses. We found no significant difference between the abundance of *M. pygmaeus* amongst tomato crops from different production modes. *Dyciphus cerastii* was significantly more abundant on biological production mode where no pesticide was used and no disruption of their biological control practice was observed. Indeed, previous studies shows that insecticides may be highly toxic to parasitoids and predators of *T. absoluta* (Moura et al. 2005; Leite et al. 1998).

Parasitism rate by *T. achaeae* does not significantly differ between production modes and crop seasons and was reduced or completely non-existent in the biological production mode probably due to the reduced number of host eggs observed. Similar results were observed by Oliveira et al. (2017).

Despite the potential of *T. achaeae* as biocontrol agent, natural parasitism, however, is low. This fact, according to Chailleux et al. (2012, 2013) maybe due to the poor egg quality for *Trichogramma* offspring development.

Considering the major concerns of the growers by the infestation levels of their crops, together with low abundance of native natural enemies, we suggest the use of biological production mode or an inoculative or augmentative strategy to control the pest in intensive and traditional productions modes.

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