

(RESEARCH ARTICLE)



## Family Trichogrammatidae (Insecta: Hymenoptera) as natural enemies of pest lepidopterans (Insecta: Lepidoptera) for agriculture

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### Abstract

Trichogrammatidae Family contain some of the smallest insects with a total adult length ranging from a paltry fifth of a millimeter to 1.5mm. Insects belonging to the genus *Trichogramma* are micro parasitoid hymenopteran exclusively from eggs of several species of insects, mainly of the order Lepidoptera. Thus, they prevent the host insect reaches the larval stage, a stage that causes damage economics to culture. The objective of this mini-revision is to restore the Family Trichogrammatidae (Insecta: Hymenoptera) as natural enemies of pest lepidopterans (Insecta: Lepidoptera) for agriculture. This review aims to verify the biological characteristics of the Trichogrammatidae Family. In order to achieve the main objective, a qualitative method was used based on research and analysis of theoretical books, theses banks, university dissertations, national and international scientific articles, scientific journals, documents and digital platforms. The verification of the mini review of the Trichogrammatidae Family was carried out from 1995 to 2022.

**Keywords:** Ecological Relationship; Pollination; Parasitoid; Life Cycle; Damage

### 1. Introduction

Worldwide: 800 described species in about 84 genera. Estimated total of 4000 species worldwide. Trichogrammatidae contain some of the smallest insects with a total adult length ranging from a paltry fifth of a millimeter to 1.5mm. Insects belonging to the genus *Trichogramma* are micro parasitoid hymenopteran exclusively from eggs of several species of insects, mainly of the order Lepidoptera. Thus, they prevent the host insect reaches the larval stage, a stage that causes damage economics to culture (Figures 1, 2 and 3) [1,2].



Source: <https://www.artstation.com/artwork/o2Q8kB>

**Figure 1** Specimen of Trichogrammatidae Family

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Figure 2 Trichogrammatidae (Hymenoptera: Chalcidoidea)

Source: <https://www.cabidigitallibrary.org/doi/10.1079/cabicompndium.54673>



Figure 3 Specimen of Trichogrammatidae Family

Source: <https://www.flickr.com/photos/69610519@N08/36533427385>

### 1.1. Description

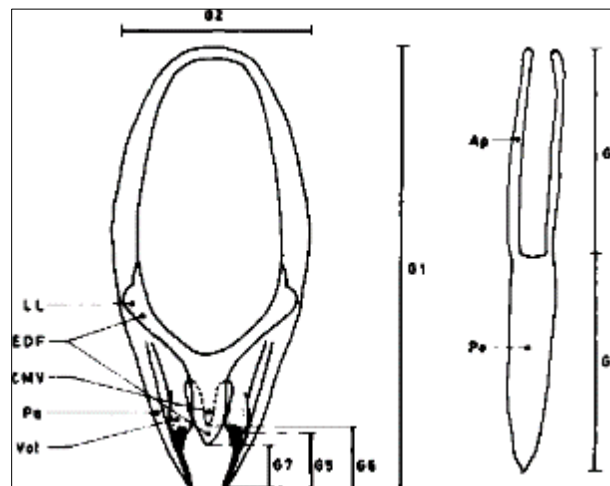
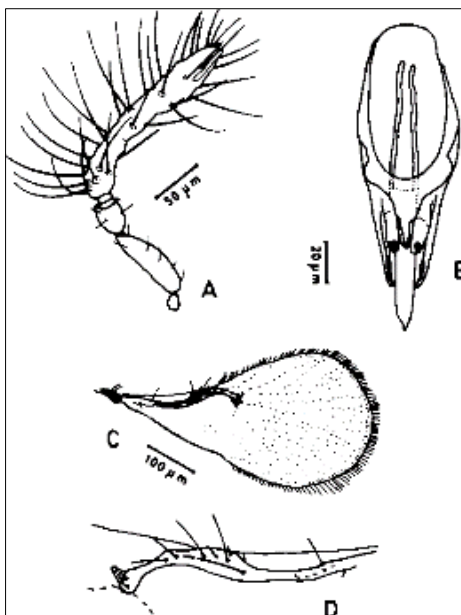


Figure 4 Parts of the genitalia of the male *Trichogramma*: Apodemes (Ap), lateral lobes of the dorsal expansion of the phallobase (LL), mid-ventral carina (CMV), paramere (Pa), penis (Pe), volsella (Vol) and dorsal expansion of the phallobase (DPE) G1 to G7 values considered in the biometric study

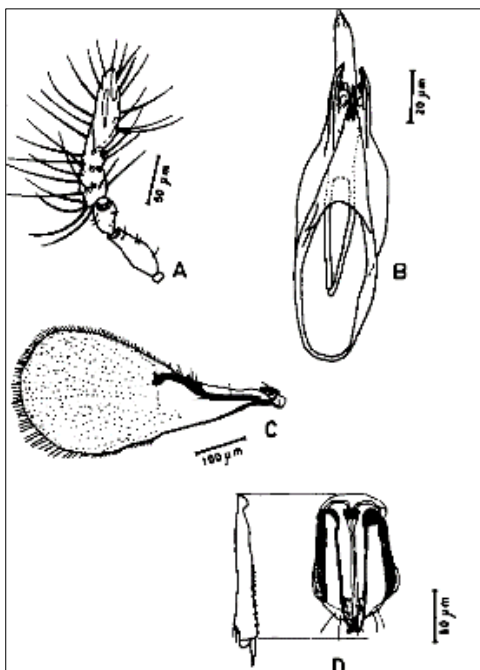
Source: [https://www.semanticscholar.org/paper/Especies-de-Trichogramma-\(HYM.%3A-Trichogrammatidae\)-Garc%C3%ADa/680f7021f6a9e8405bf3cbd03bde234086ccd876](https://www.semanticscholar.org/paper/Especies-de-Trichogramma-(HYM.%3A-Trichogrammatidae)-Garc%C3%ADa/680f7021f6a9e8405bf3cbd03bde234086ccd876)

Diagnostic characters. Tarsus with 3 tarsomeres; metasoma closely linked to the mesosoma, not forming a wasp waist; antenna with 4 to 11 articles, including at least 1 to 3 ringed flagellomeres, club with 1 to 5 flagellomeres; forewing with variable width, from very narrow to wide; membrane with arrows usually partially aligned. They measure from 0.2 to 2.0 mm (Figures 4, 5, 6 and 7).



**Figure 5** *Trichogramma* male: antenna (A), genitalia (B), forewing (C) and detail of wing stigma (D)

Source: [https://www.semanticscholar.org/paper/Especies-de-Trichogramma-\(HYM.%3A-Trichogrammatidae\)-Garc%C3%ADa/680f7021f6a9e8405bf3cbd03bde234086ccd876](https://www.semanticscholar.org/paper/Especies-de-Trichogramma-(HYM.%3A-Trichogrammatidae)-Garc%C3%ADa/680f7021f6a9e8405bf3cbd03bde234086ccd876)



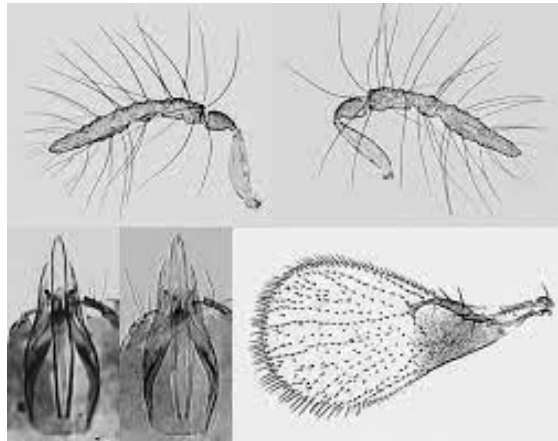
**Figure 6** *Trichogramma*: Male: antenna (A), genitalia (B) and forewing (C). Female genitalia

Source: [https://www.semanticscholar.org/paper/Especies-de-Trichogramma-\(HYM.%3A-Trichogrammatidae\)-Garc%C3%ADa/680f7021f6a9e8405bf3cbd03bde234086ccd876/figure/4](https://www.semanticscholar.org/paper/Especies-de-Trichogramma-(HYM.%3A-Trichogrammatidae)-Garc%C3%ADa/680f7021f6a9e8405bf3cbd03bde234086ccd876/figure/4)



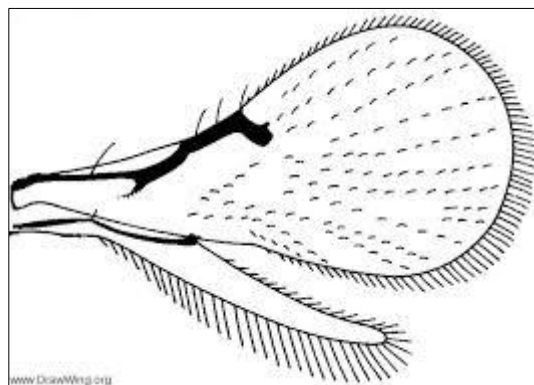
**Figure 7** Trichogrammatidae head and antennae

Source: Copyright Malcolm Storey 2011-2118



**Figure 8** Morphology of *Trichogramma brassicae* Bezdenko, 1968. A, B: Male antennae, inner (A) and outer (B) aspects. C, D: Genital capsule (GC) including aedeagus with focus on dorsal structures (C) and ventral structures (D). E: Fore wing

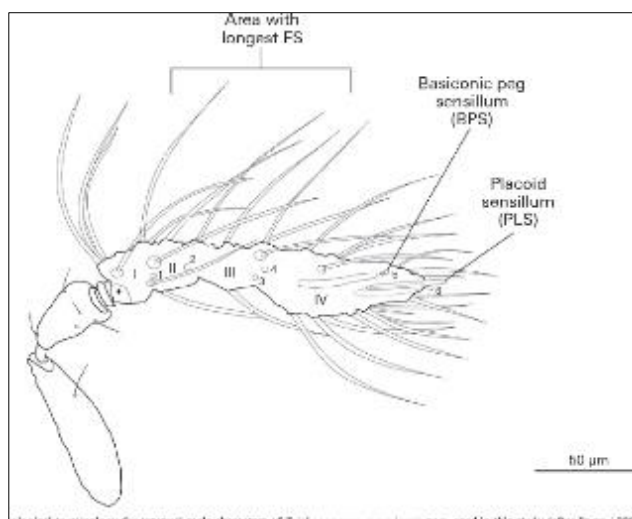
Source: [https://www.researchgate.net/figure/Morphology-of-Trichogramma-brassicae-A-B-Male-antennae-inner-A-and-outer-B\\_fig3\\_258410316](https://www.researchgate.net/figure/Morphology-of-Trichogramma-brassicae-A-B-Male-antennae-inner-A-and-outer-B_fig3_258410316)



**Figure 9** Trichogrammatidae draw wing

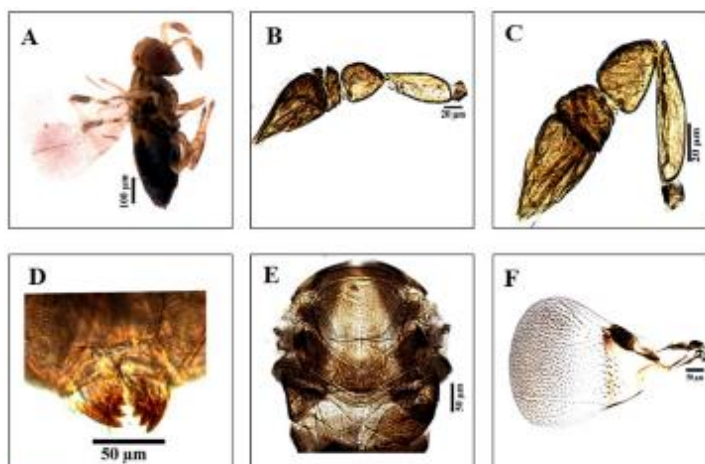
Source: Gothic Catholic

Initially, the identification was carried out by the external morphology of adults, analyzing characters of color, length and density of the bristles on the wing and the length of the bristles on the antenna. The first significant advance in the process occurred from works, where it was possible to verify the importance of the morphological study of the male genitalia (Figures 8, 9, 10 and 11).



**Figure 10** Morphological terminology for antennal male characters of *Trichogramma canariensis* sp. nov. used in this study. Arabic numerals on flagellum refer to “positions 1 – 6 that carry basiconic peg sensilla (BPS); Roman numerals I – IV refer to “sections” of the flagellum

Source: Corpus ID: 56305756

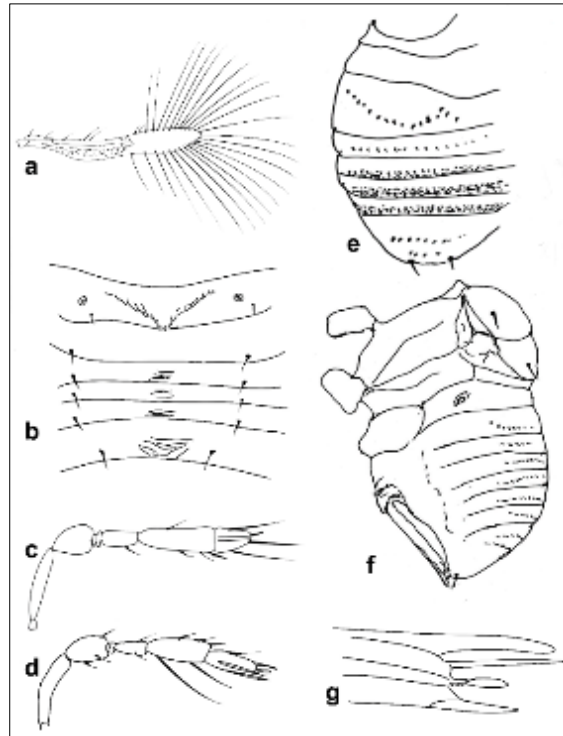


**Figure 11** *Brachygrammatella indica* Viggiani & Hayat. (A) adult ♀; (B) antenna ♀; (C) antenna ♂; (D) mandibles ♀; (E) mesosoma ♀; (F) forewing ♀

**Figure 11** (A) adult ♀; (B) antenna ♀; (C) antenna ♂; (D) mandibles ♀; (E) mesosoma ♀ and (F) forewing ♀

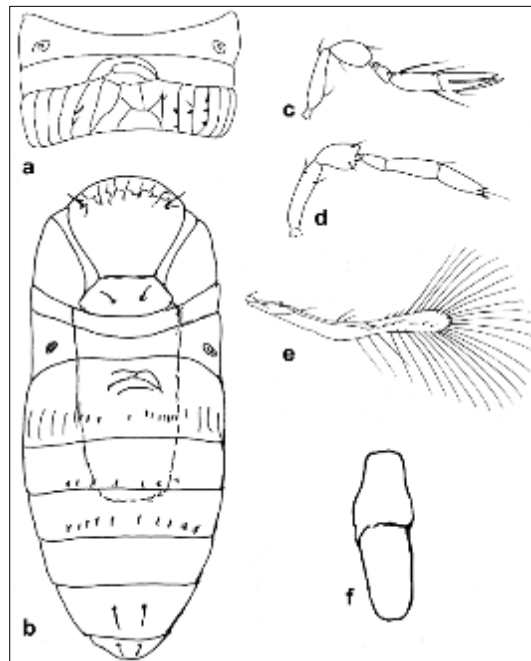
Source: DOI:10.26832/24566632.2019.0401017 and Corpus ID: 91751426

As a result, there was a significant increase in the number of identified species. Currently, species-level identification is mainly based on the morphology of the male's genitalia, with wings, antennae and scutellum helping in this process (Figures 12, 13 and 14) [3,4,5,6,7,8].



**Figure 12** Line drawings (a) female fore wing (Holotype); (b) male propodeum and tergites (Paratype); (c), male antenna (Paratype); (d), female antenna (Holotype); (e) male metasoma (Paratype); (f) female lateral meso- and metasoma (Holotype); (g) female antenna

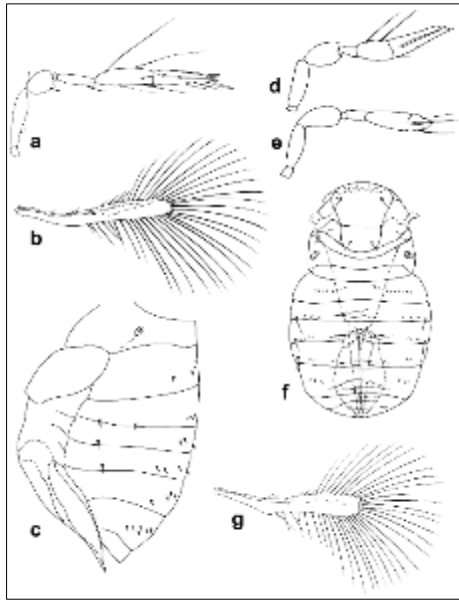
Source: <https://www.mdpi.com/2075-4450/13/6/561>



**Figure 13** Line drawings: (a) female fore wing (Holotype); (b) male propodeum and tergites (Paratype); (c) male antenna (Paratype); (d) female antenna (Holotype); (e) male metasoma (Paratype); (f), female lateral meso- and metasoma (Holotype); (g), female antenna, detail (Holotype)

Source: <https://www.mdpi.com/2075-4450/13/6/561>





**Figure 14** Line drawings: (a) female antenna (Paratype); (b) female fore wing (Paratype); (c) female propodeum and metasoma, lateral view (Paratype); (d) female antenna (Neotype); (e) male antenna (non-type); (f), female dorsal meso- and metasoma (Neotype); (g), female fore wing (Neotype)

Source: <https://www.mdpi.com/2075-4450/13/6/561>

## 1.2. Biology

Endoparasitoids from eggs of various insect orders. Parasitoids of eggs of other insects most commonly Hemiptera, but Coleoptera and Lepidoptera eggs are also parasitized (Figure 15).



**Figure 15** Female *Trichogramma dendrolimi* Matsumura, 1926 on egg of armyworm (Lepidoptera: Noctuidae)

Source: <https://en.wikipedia.org/wiki/Trichogramma>

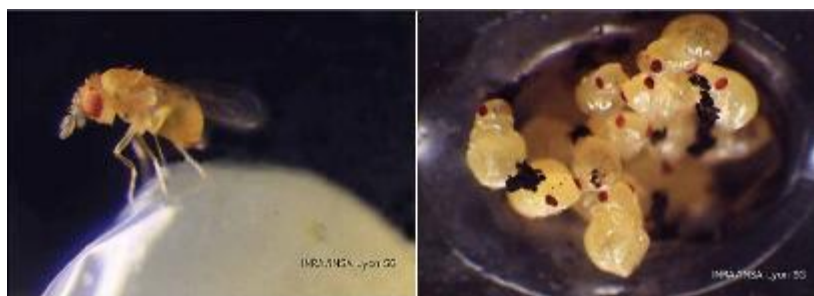
Species of the genus *Trichogramma* spp. are highlighted due to the ease of rearing them in alternative hosts, in addition to their aggressiveness in the parasitism of eggs of different insect pests (Figure 16).



**Figure 16** *Trichogramma* on eggs of agricultural pests. A) *Diatraea saccharalis* (Fabricius, 1794) B) *Heliothis virescens* (Fabricius, 1781); C) *Spodoptera frugiperda* (Smith, 1797); D) *Tuta absoluta* Meyrick, 1917 (Lepdoptera)

Source: [https://www.researchgate.net/figure/Trichogramma-on-eggs-of-agricultural-pests-A-D-saccharalis-B-H-virescens-C-S\\_fig1\\_250030136](https://www.researchgate.net/figure/Trichogramma-on-eggs-of-agricultural-pests-A-D-saccharalis-B-H-virescens-C-S_fig1_250030136)

The female lays her eggs inside her host's egg. Within a few hours, its larva is born and feeds on the contents of the host's egg. The entire parasitoid cycle takes place inside the pest egg. From this, the adult wasp emerges, which immediately begins the process of searching for a new posture to continue the propagation of the species (Figure 17).



**Figure 17** *Trichogramma* female laying eggs inside an artificial host egg (left); *Trichogramma* pupae grown in an artificial host egg (right)

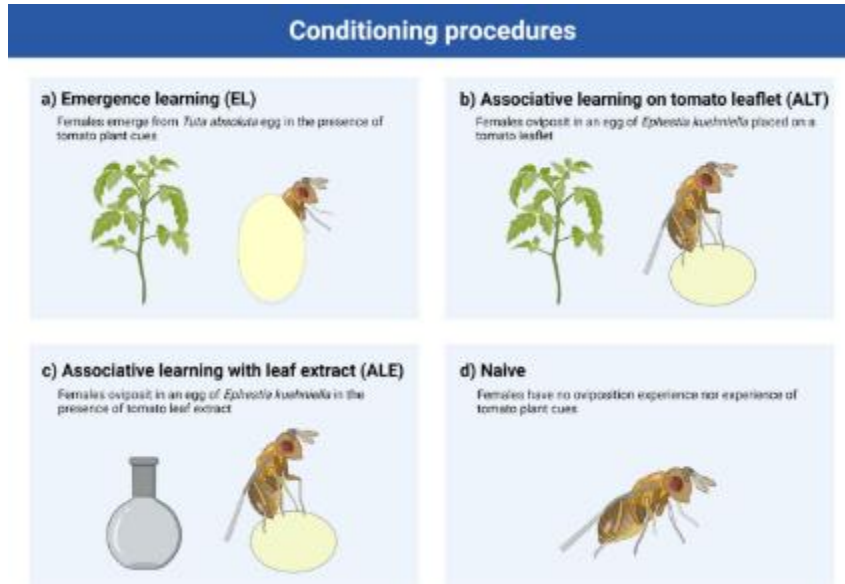
Source: Photos: INRA/INSA de Lyon, Simon Grenier. UGA1390018

Within the family Trichogrammatidae the genus *Trichogramma* presents a greater number, reaching about 235 described species. These results are achieved due to the discovery of methods developed to facilitate the identification of these insects (Figure 18).

However, owing to of the size and of some cryptic species other tools have helped in the process. For example, there are morphometry, reproductive data and molecular studies, therefore, the integrated use of these tools is key in the identification and knowledge of the faunal diversity of parasitoids (Figure 19).

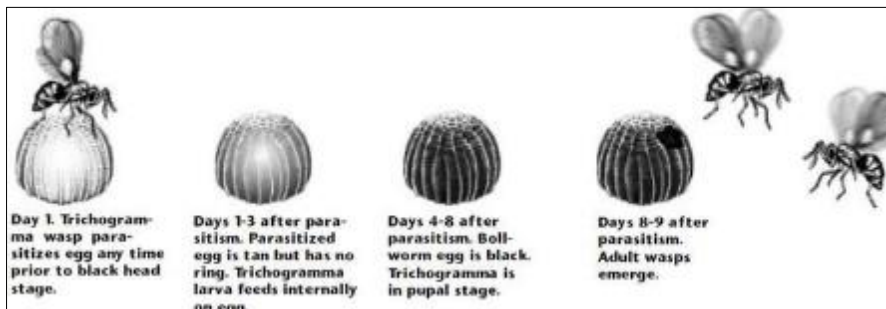
Correct identification of *Trichogramma* spp. it is directly linked to the success of mass breeding, its use in biological pest control and the conservation and maintenance of fauna. In Brazil, there are 29 species of *Trichogramma* found in agroecosystems and other modified habitats (Figure 20).



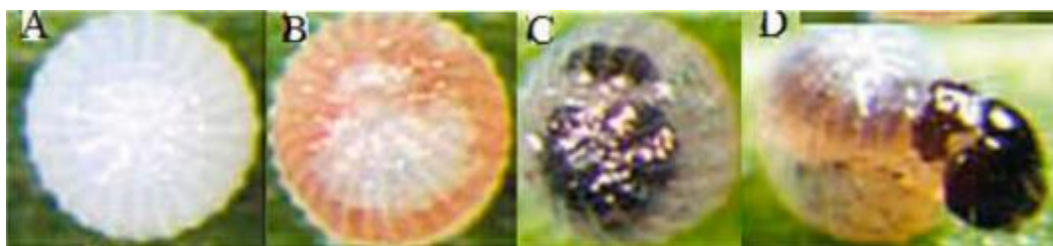


**Figure 18** *Trichogramma* spp. females were allowed to gain experience with tomato and host cues before the experiments. Different conditioning procedures were used. a) Emergence learning (EL): parasitoids were reared for one generation on *Tuta absoluta* Meyrick, 1917 eggs on tomato leaflets. b) Associative learning on tomato leaflet (ALT): female wasps experienced oviposition on *Ephestia kuehniella* Zeller, 1879 eggs placed on a tomato leaflet. c) Associative learning with a tomato leaf extract (ALE): female wasps experienced oviposition on *E. kuehniella* eggs placed on a filter paper with tomato leaf extract. d) Naive: control female wasps were reared on *E. kuehniella* eggs without any exposure to *T. absoluta* or tomato cues

Source: This figure was created with BioRender.com



**Figure 19** Life cycle of the parasitoid moth egg, *Trichogramma* developing on the bollworm egg (*Helicoverpa armigera*) (Hübner, 1808) egg



**Figure 20** Light micrographs of development of the pod borer eggs (unparasitized), *Helicoverpa armigera* (Hübner, 1808) showing the embryonic developmental sequences: (A) newly laid egg (yellowish-white); (B) 2 days old egg (tan egg); (C) egg before hatching or black head stage (larva visible) and (D) hatching stage (larva)

Source: <https://www.intechopen.com/chapters/67152>

Among these species, some have economic importance, such as *T. pretiosum*, which has been associated with several crops of agricultural interest, hosting several species of insect pests [8,9,10,11,12].

### 1.3. Hosts and parasitism

Several species of *Trichogramma* have already been described in association with different pests. Specifically in corn, the species *Trichogramma pretiosum* Riley, 1879, egg control of Lepidoptera species such as *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) fall armyworm, *Helicoverpa zea* (Boddie, 1850) (Lepidoptera, Noctuidae) earworm and *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera, Pyralidae) sugarcane borer by *Trichogramma atopovirilia* Oatman & Platner, 1983 *Trichogramma galloi* (Zucchi, 1988) have been the most common (Figures 21, 22 and 23).



**Figure 21** *Trichogramma pretiosum* Riley, 1879, egg control of Lepidoptera species such as *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae)

Source: [https://www.researchgate.net/figure/Trichogramma-pretiosum-females-parasite-Spodoptera-frugiperda-a-and-Spodoptera-litura\\_fig2\\_335159538](https://www.researchgate.net/figure/Trichogramma-pretiosum-females-parasite-Spodoptera-frugiperda-a-and-Spodoptera-litura_fig2_335159538)



**Figure 22** *Trichogramma pretiosum* Riley, 1879, egg control of *Helicoverpa zea* (Boddie, 1850) (Lepidoptera, Noctuidae)

Source: [https://www.researchgate.net/figure/Figura-3-Trichogramma-pretiosum-parasitando-ovo-de-lagarta-Fonte\\_fig3\\_306569900](https://www.researchgate.net/figure/Figura-3-Trichogramma-pretiosum-parasitando-ovo-de-lagarta-Fonte_fig3_306569900)



**Figure 23** *Trichogramma galloi* (Zucchi, 1988) earworm and *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera, Pyralidae)

Source: <https://pdfs.semanticscholar.org/ea6b/0f9d8f36a77856a82525f7dfd466aae4b96b.pdf>

Several cases of successfully identified with *Trichogramma* sp. (Hymenoptera: Trichogrammatidae), such as *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) for the control of different pest lepidopteran species in crops of agricultural importance (Figure 24) [10,11,12,13,14].

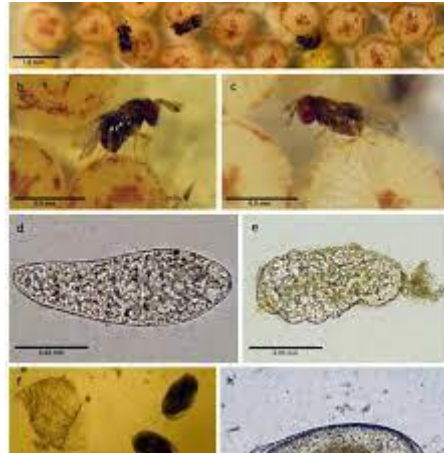


**Figure 24** *Trichogramma galloi* (Zucchi, 1988) control of *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera, Pyralidae)

Source: <https://www.facebook.com/KoppertBrasil/photos/o-parasitoide-trichogramma-galloi-emergindo-dos-ovos-da-broca-da-cana-diatraea-s/2446759768697889/>

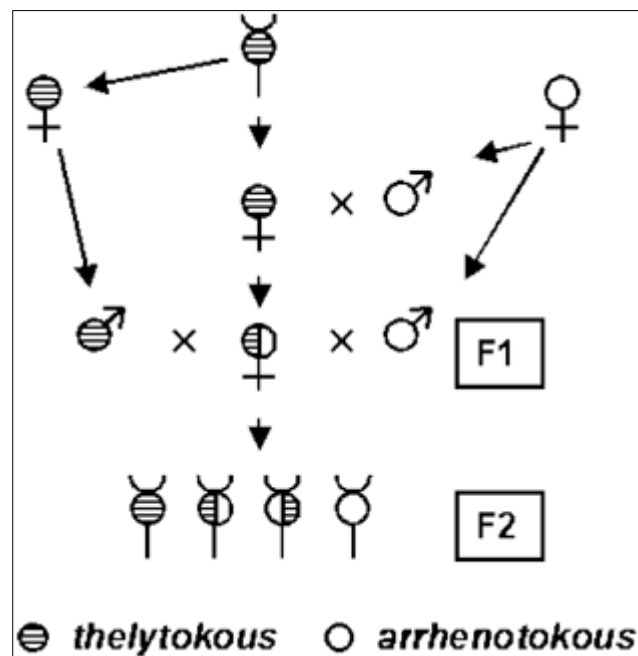
#### 1.4. Life cycle

*Trichogramma* species are holometabolic, their development is completed by the passage of the egg, larva, prepupa and pupa. Since the outstanding feature of parasitism is perceived from the development of the pre-pupal stage, in which the host egg becomes darkened due to the presence of urate salts accumulated in the integument of the insect Adults are free-living and the most common mode of reproduction in *Trichogramma* is arrhenotoky, where fertilized eggs produce females' diploids and unfertilized eggs produce haploid males (Figures 25A and 25B).



**Figure 25A** Biology of *Trichogramma chagres* sp. nov. strain L21: (a-c) female adult(s) parasitizing egg (s) of *Mamestra brassicae* (Linnaeus, 1758); (d) freshly laid wasp egg; (e) newly hatched larva; (f-h) mature larva: (f-g) two mature larvae and chorion of consumed host egg in direct (f) and reflected (g) light, (h) habitus of mature larva with pulsing mid gut full of host egg yolk

Source: [https://www.researchgate.net/figure/Biology-of-Trichogramma-chagres-sp-nov-strain-L21-a-c-female-adults-parasitizing\\_fig1\\_329648893](https://www.researchgate.net/figure/Biology-of-Trichogramma-chagres-sp-nov-strain-L21-a-c-female-adults-parasitizing_fig1_329648893)

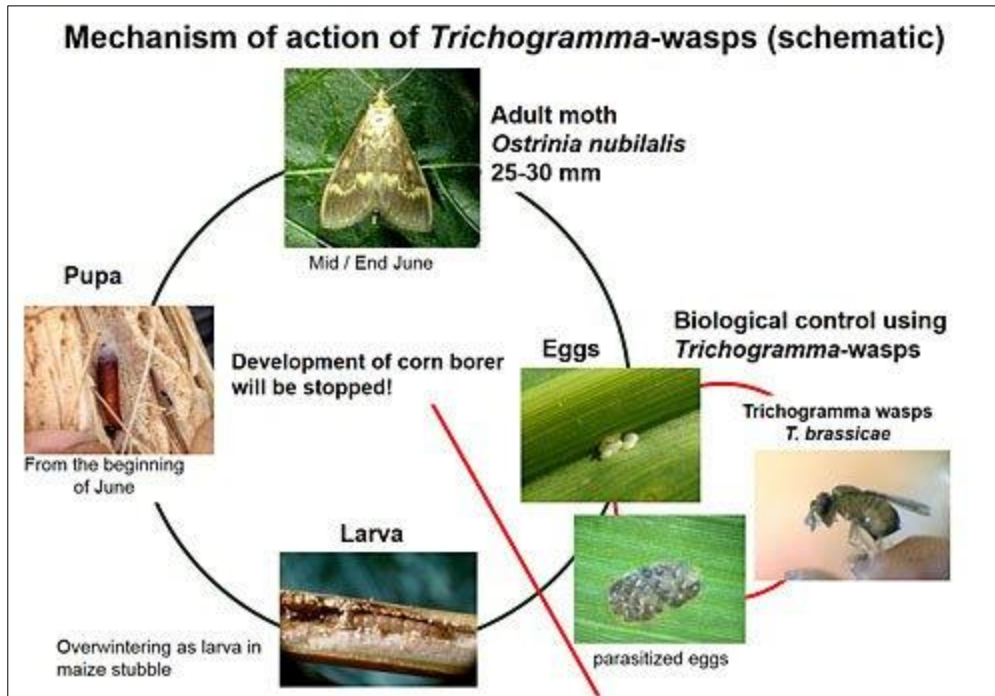


**Figure 25B** Double backcross of two honeybees. Types of parthenogenesis (thelytokous and arrhenotokous) ((Hymenoptera))

Source: <https://www.nature.com/articles/6800654>

Thelytoky is also a mode of reproduction, however less common, in which eggs fertilized and unfertilized produce diploid females. two ways to thelitochia exist in *Trichogramma*, the reversible one associated with infections microbial) and non-reversible. Oviposition and sex ratio of parasitoids are variable. The quantity varies as a function of the quality and volume of the host's egg and the sex ratio is influenced by temperature, humidity, age of the female of the *Wolbachia* and by the host (Figure 26).



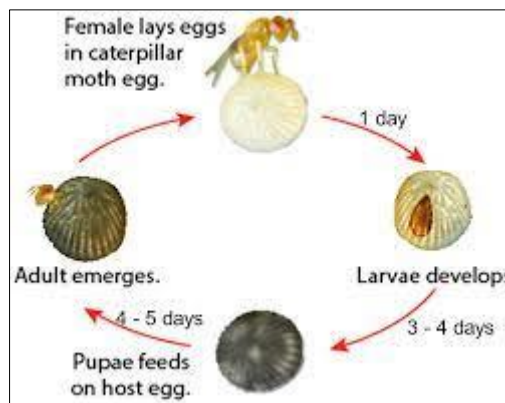


**Figure 26** *Trichogramma* life cycle

Source: <https://www.amwnuetzlinge.de/en/application-areas/trichogramma-parasitic-wasps-2/>

Note: There are two types of parthenogenesis: the one in which unfertilized females produce only females thelytokal parthenogenesis or the arrhenotoco, in which the unfertilized eggs originate only males, as occurs with the production of drones by queen bees. It is found in several groups of Hymenoptera, including Apidae, Aphelinidae, Cynipidae, Formicidae, Ichneumonidae, and Tenthredinidae. It can be induced in Hymenoptera by *Wolbachia* bacteria.

Therefore, the quality of the host is the main factor influencing sex ratio. The development of *Trichogramma* is influenced by factors abiotic factors such as temperature, humidity and light. Temperature has a direct effect on duration of the cycle, of parasitism (Figures 27, 28 and 29) [14,15,16,17,18,19].



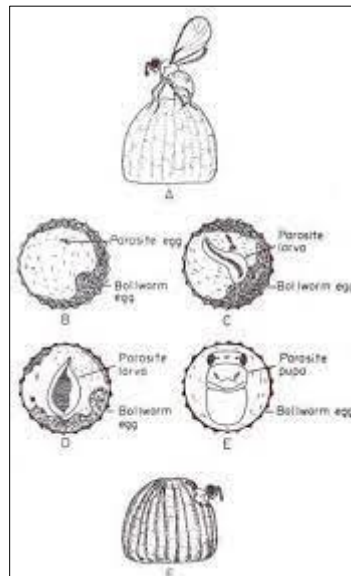
**Figure 27** Life cycle of *Trichogramma* spp.

Source: [https://www.researchgate.net/figure/Life-cycle-of-Trichogramma-spps\\_fig15\\_335978434](https://www.researchgate.net/figure/Life-cycle-of-Trichogramma-spps_fig15_335978434)



**Figure 28** Life cycle: Adults are approximately 1/25 inch (1 mm) or less the size of a period at the end of a sentence. They often have wing hairs (setae) arranged in rows. Their body is relatively compact and the antennae are short. *Trichogramma* species are difficult to identify due to their minute size and generally uniform morphological features

Source: [http://ipm.ucanr.edu/natural-enemies/trichogramma\\_spp.html](http://ipm.ucanr.edu/natural-enemies/trichogramma_spp.html)



**Figure 29** *Trichogramma* life cycle: A) female ovipositing a moth egg; B) parasite egg inside host egg; C) developing larvae; E) pupa; F) adult wasp emerging from host egg

Source: From van den Bosch & Hagen, 1966

### 1.5. Taxonomy

The family has a cosmopolitan distribution, with about 1000 species and 90 genera. In the Neotropical region there are about 40 genera.

**Some genus:** *Adelogramma*, *Adryas*, *Brachistagrapha*, *Brachyia*, *Burksiella*, *Centrobiopsis*, *Ceratogramma*, *Chaetostrichella*, *Eteroligosita*, *Eutrichogramma*, *Haeckeliania*, *Hayatia*, *Hispidophila*, *Ittysella*, *Japania*, *Kyuwia*,

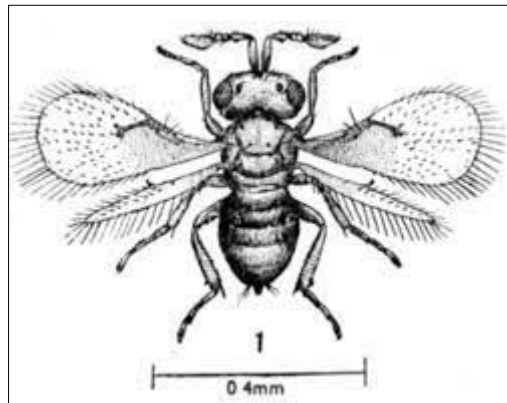


*Lathromeroidea, Lathromeromyia, Monorthochaeta, Neobrachista, Ophioneurus, Pachamama, Paracentrobia, Paraittys, Sinepalpigamma, Soikiella, Thanatogramma, Tumidifemur, Ufens, Ufensia, Urogramma, Viggianiella, Xiphogramma and Zelogramma* (Figures 30, 31, 32, 33, 34, 35, 36, 37 and 38) [20,21,22,23,24,25,26,27].



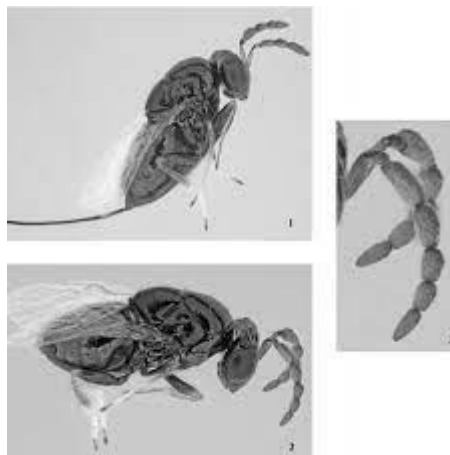
**Figure 30** Genus *Kyuwia* Pinto & George, 2004

Source: Photographs © Simon van Noort (Iziko Museums of South Africa)



**Figure 31** *Trichogrammatoidea* Girault, 1911

Source: <https://www.tandfonline.com/doi/abs/10.1080/00305316.1978.10432534?journalCode=toin20>



**Figure 32** *Poropoea* Foerster, 1851

Source: [https://www.researchgate.net/figure/Figures-1-3-Poropoea-bella-sp-nov-1-body-lateral-female-2-body-lateral-male-3\\_fig3\\_271568656](https://www.researchgate.net/figure/Figures-1-3-Poropoea-bella-sp-nov-1-body-lateral-female-2-body-lateral-male-3_fig3_271568656)



**Figure 33** *Trichogramma* Westwood, 1833

Source: By Christopher Taylor at May 14, 2013



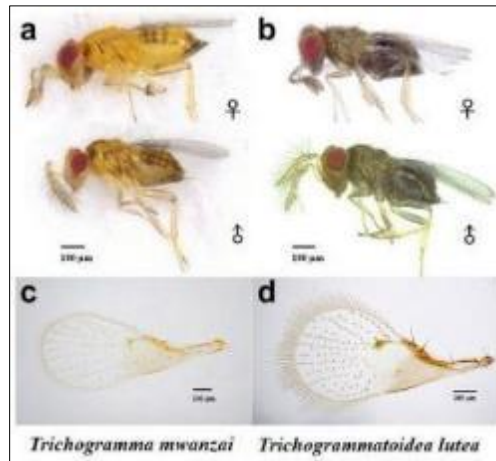
**Figure 34** *Trichogramma galloi* (Zucchi, 1988)

Source: <https://vittia.com.br/produto/galoi-vit/>



**Figure 35** *Trichogramma pretiosum* Riley, 1879

Source: [https://www.researchgate.net/figure/Figura-3-Trichogramma-pretiosum-parasitando-ovo-de-lagarta-Fonte\\_fig3\\_306569900](https://www.researchgate.net/figure/Figura-3-Trichogramma-pretiosum-parasitando-ovo-de-lagarta-Fonte_fig3_306569900)

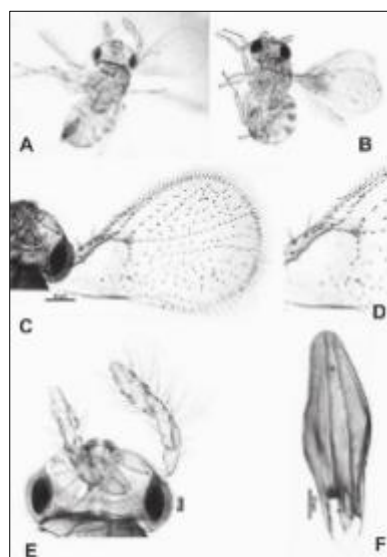


**Figure 36** Comparison of adult morphological characters of two trichogrammatid species. ((a): male and female of *Trichogramma mwanzai* Schulten & Feijen, 1982; (b): male and female of *Trichogrammatoidea lutea* Girault, 1911; (c): forewing of *T. mwanzai*; (d): forewing of *T. lutea*).



**Figure 37** *Trichogrammatoidea cryptophlebiae* Nagaraja, 1979

Source: Photographs © Simon van Noort (Iziko Museums of South Africa)



**Figure 38** *Trichogramma yousufi* sp. nov. (Hymenoptera: Trichogrammatidae) adult male (A), adult female (B), fore wing (C), RS1 (D), head with antennae (E), and male genitalia (F)

## 1.6. Objective

The objective of this mini-revision is to restore the Family Trichogrammatidae (Insecta: Hymenoptera) as natural enemies of pest lepidopterans (Insecta: Lepidoptera) for agriculture.

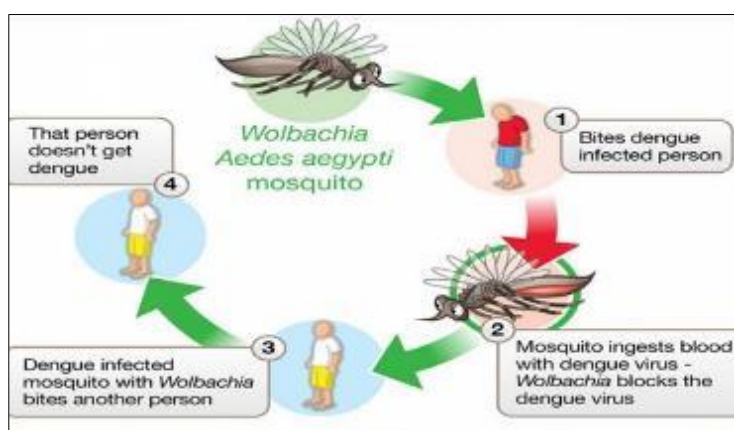
## 2. Methods

In order to achieve the main objective, a qualitative method was used based on research and analysis of theoretical books, theses banks, university dissertations, national and international scientific articles, scientific journals, documents and digital platforms. The verification of the mini review of the Trichogrammatidae Family was carried out from 1995 to 2022.

## 3. Selection of items used

### 3.1. Study 1

*Wolbachia* is the most important microorganism associated with modulation of determination sex in insects. In addition to manipulating the reproductive process, this bacterium can also influence several biological parameters, resulting in relevant alterations in the aptitude of their hosts. However, the effect of this symbiont on fitness of the host is dependent on the genetic composition of the host and on the *Wolbachia* lineage associated (Figure 39).

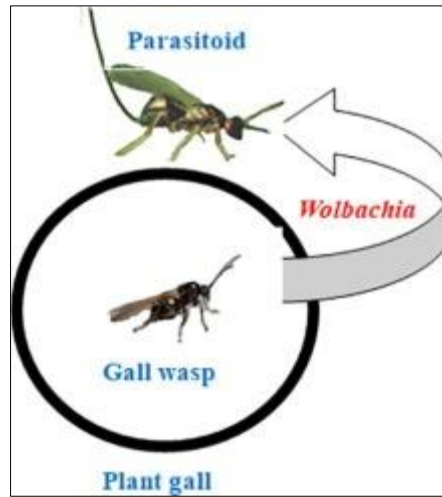


**Figure 39** The Health Ministry will resume this month the fieldwork to release '*Aedes aegypti*' mosquitoes that carry natural '*Wolbachia*' bacteria to control dengue. The programme was suspended for nearly four months because of the COVID-19 pandemic

Source: <https://www.dailynews.lk/2021/02/13/local/241381/wolbachia-control-dengue>

*Wolbachia* too can influence the relationship of its host with other trophic levels, affecting metabolism of iron, its ability to respond to pathogens and to locate hosts. The interaction of this symbiote with its hosts can still influence their resistance to different sources of stress, such as temperature and pesticides. In cases where the relationship *Wolbachia*-host symbiosis approaches mutualism, this bacterium can become essential to ensure the production of eggs and the normal growth and development of their hosts.

Manipulation of the reproductive process is seen by the expression of one of the phenotypes induced in the host due to its association with *Wolbachia*, such as thelytoky, 16 male feminization, male death, or cytoplasmic incompatibility (Figure 40).



**Figure 40** *Wolbachia* are endosymbiotic bacteria that are widely present in nematodes and arthropods and sometimes have a significant impact on the evolution, ecology, and biology of their hosts. Given that parasitoids will kill their hosts, *Wolbachia* may be horizontally transferred from gall wasps to their parasitoid

Source: Gao-Zhi Z, et al. *Wolbachia* infection in six species of gall wasps and their parasitoids. *Journal of Asia-Pacific Entomology*. 2021; 24(1): 21-25

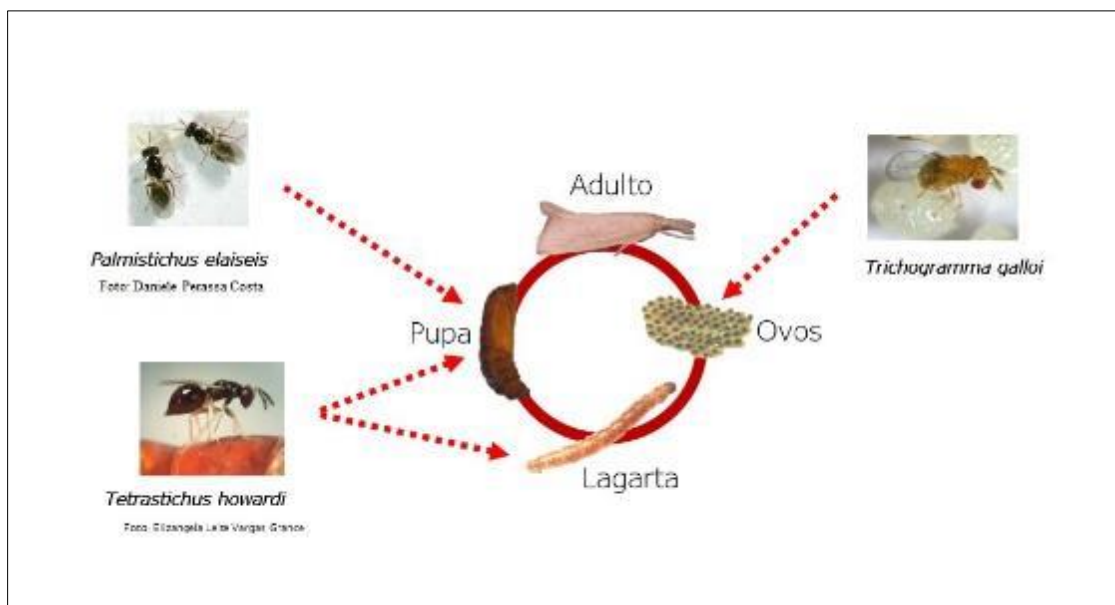
Thelytoky is common in Hymenoptera, among which the parasitoids of *Trichogramma* eggs. These parasitoids are the most used biological control agents in insect pest control, mainly Lepidoptera. In Brazil, *Trichogramma galloi* Zucchi, 1988 (Hymenoptera: Trichogrammatidae) stands out as the main parasitoid of eggs of *Diatraea saccharalis* (Fabr., 1794) (Lepidoptera: Crambidae), one of the most important pests of the sugarcane crop, being, today, used in control programs biological system of this pest in about 300 thousand hectares (Figure 41).



**Figure 41** Parasitoid penetrating its ovipositor in the larva containing eggs and *Wolbachia*

Source: Valmir Antônio Costa. Centro Experimental Central. Instituto Biológico de Campinas, Brazil

The parasitoid-symbionts interaction has been investigated in several associations, many of them involving *Trichogramma*. However, the relationship *T. galloi* - *Wolbachia* is completely unknown (Figure 42).



**Figure 42** Sugarcane borer cycle: *Diatraea saccharalis* (Fabr., 1794) (Lepidoptera: Crambidae): Adulto=adult, Ovos= eggs, lagarta= larvae, pupa=pupa. Parasitoids= *Tetrastichus howardi* (Olliff, 1893) (Hymenoptera: Eulophidae), *Palmistichus elaeisis* (Delvare e LaSalle, 1993) (Hymenoptera Eulophidae) and *Trichogramma galloi* Zucchi, 1988 (Hymenoptera: Trichogrammatidae)

Source: <http://www.canaonline.com.br/conteudo/as-vantagens-do-parasitoide-tetrastichus-howardi-em-comparacao-a-cotesia-flavipes-no-controle-da-broca-da-cana.html>

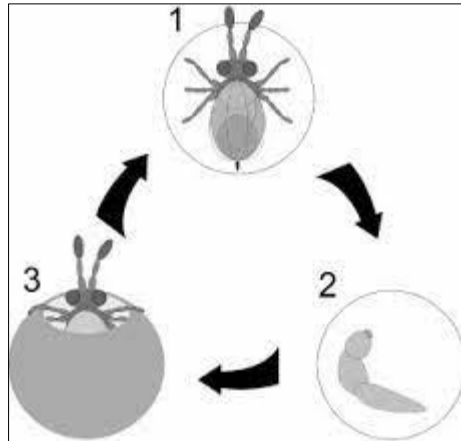
Thus, given the known effects of *Wolbachia* on the biology of their hosts, which may be beneficial or harmful, and the importance of this parasitoid of eggs in the biological control of *D. saccharalis*, this work aims to identify the symbiosis relationship established in the *T. galloi* - *Wolbachia* interaction, verifying the occurrence of infections in natural populations; ii) the effect of symbiote elimination on biological fitness of the parasitoid and iii) the host's tolerance to heat stress (Figures 43 and 44).



**Figure 43** *Tetrastichus* parasitizing the sugarcane borer *Diatraea saccharalis* (Fabr., 1794) (Lepidoptera: Crambidae)

Source: <http://www.canaonline.com.br/conteudo/as-vantagens-do-parasitoide-tetrastichus-howardi-em-comparacao-a-cotesia-flavipes-no-controle-da-broca-da-cana.html>

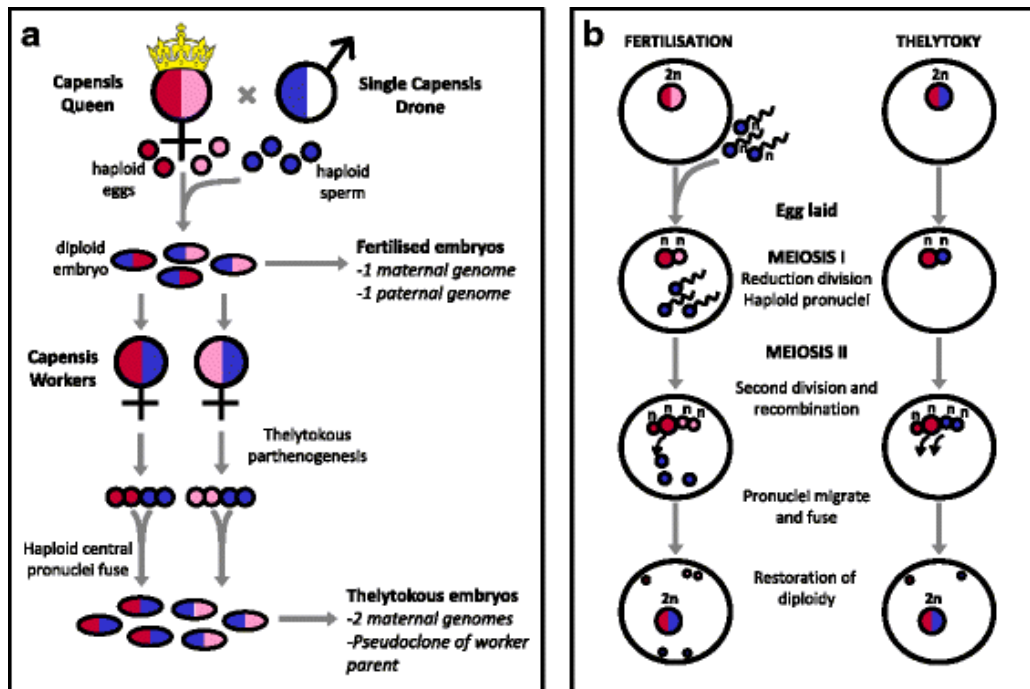




**Figure 44** Typical life cycle of *Trichogramma* spp. egg-parasitoids: 1) adult female parasitizes host egg; 2) *Trichogramma* larva develops, consuming pest embryo; 3) *Trichogramma* pupates and an adult emerges

Source: <https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/trichogramma>

These studies will allow to verify if the association *T. galloi* – *Wolbachia* results in a neutral, beneficial or harmful to the natural enemy, which could direct the lineage selection process infected with the parasitoid for use in biological control programs (Figure 45).



**Figure 45** A *Capensis* queen was inseminated with sperm from a single *Capensis* drone, to produce fertilized embryos. A subset of embryos were collected to produce the fertilized embryo methylome, while the remaining embryos were left to emerge to adult workers. When the queen was removed, the daughter workers began to produce diploid eggs thelytokously and these embryos were used to produce the thelytokous embryo methylome. Early meiotic divisions in fertilisation and thelytoky follow the same process. The first meiotic division occurs after oviposition in a newly laid egg. If the egg is fertilized there will be 3–7 spermatozoa, one of which becomes the male pronucleus. During thelytokous reproduction, the egg is unfertilized and two maternal pronuclei fuse to form a diploid nucleus

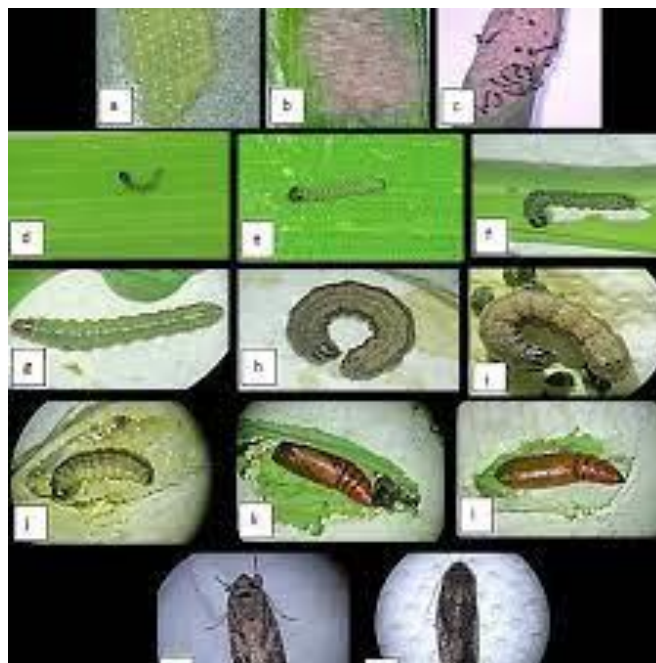
Source: Verma S, Ruttner F. Cytological analysis of the thelytokous parthenogenesis in the cape honeybee (*Apis mellifera capensis* Escholtz). *Apidologie*. 1983;14(1): 41–57.

The use of the thelytocal lineages of *Trichogramma* would benefit the mass production process of these natural enemies, given the reduction in production costs [28,29,30,31,32,33,34].

### 3.2. Study 2

Natural occurrence and competition of *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) egg parasitoids in field

*Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae), known as fall armyworm, is one of the main pests of corn in Brazil. Its management is dependent on insecticides. However, egg parasitoids can contribute to the control of this species. These parasitoids are microwasps (< 1mm) that lay eggs inside the eggs of *S. frugiperda*, killing the pest in its embryonic stage, before it harms the culture (Figure 46).



**Figure 46** Developmental stages of *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae): Eggs laid on a wax paper (a), on corn leaf (b), on stalk of corn starting to hatch, (c); first instar larva on a corn leaf (d); second instar larva (e); third instar larva (f); fourth instar larva (g); fifth instar larva (h); sixth instar larva (i); pre-pupa (j); male (k) female (l) pupae; male (m) and female (n) adults

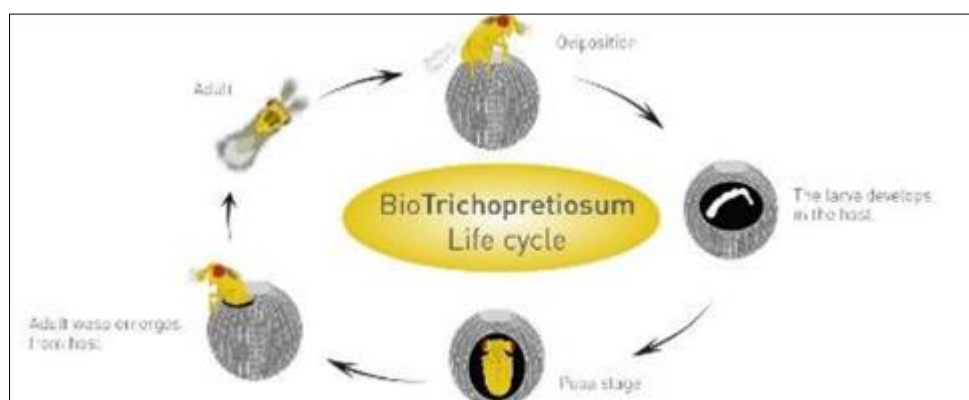
Source: [https://www.researchgate.net/figure/Developmental-stages-of-Spodoptera-frugiperda-Eggs-laid-on-a-wax-paper-a-on-corn-leaf\\_fig1\\_346000914](https://www.researchgate.net/figure/Developmental-stages-of-Spodoptera-frugiperda-Eggs-laid-on-a-wax-paper-a-on-corn-leaf_fig1_346000914)

Among the parasitoids with the greatest potential for control, *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae), *Trichogramma atopovirilia* Oatman. & Platner, 1983 (Hymenoptera: Trichogrammatidae) and *Telenomus remus* Nixon, 1937 (Hymenoptera: Scelionidae) out. Both *T. pretiosum* as *T. atopovirilia* have been observed on *S. frugiperda* eggs naturally in the field, without releasing laboratory-produced parasitoids (Figure 47).



**Figure 47** Posture of *Spodoptera frugiperda* Smith, 1797 (Lepidoptera: Noctuidae). *Spodoptera frugiperda* eggs parasitized by parasitoids of different species and families: *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae), *Trichogramma atopovirilia* Oatman. & Platner., 1983 (Hymenoptera: Trichogrammatidae) and *Telenomus remus* Nixon, 1937 (Hymenoptera: Scelionidae). This type of indirect competition is known as exploitation competition

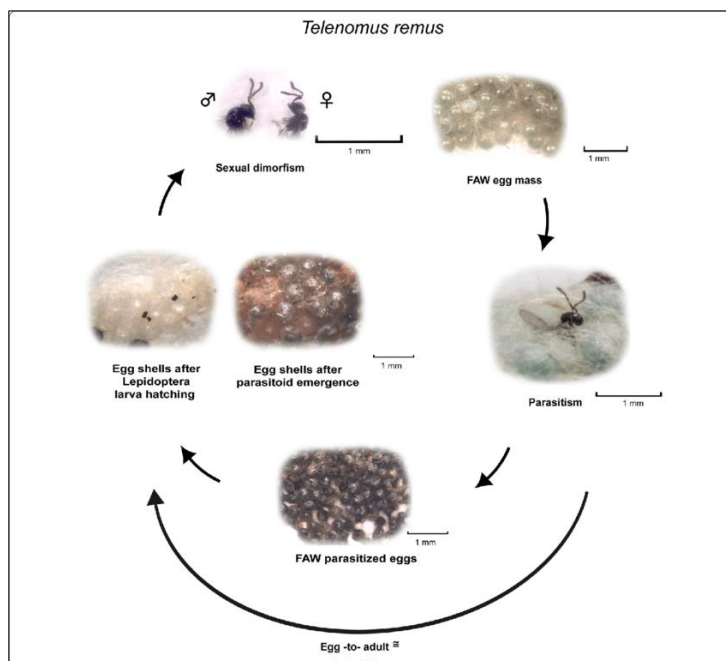
This demonstrates that, with natural populations and given the ideal conditions for the development of these species, they can thrive in the field and thus contribute to biological control. On the other hand, the same does not seem to be true for *Tr. remus*, since the natural occurrence of this species in the field has been rarely reported. Imagine, then, our surprise when we found, under natural field conditions, *S. frugiperda* postures parasitized not only by *Te. remus*, but also by *Tr. atopovirilia* and *Tr. Pretiosum* (Figure 48).



**Figure 48** Life cycle *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) in the host *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae)

Source: <https://www.biobee.com/solutions/biotrichopretiosum/>

The fact was recorded in Piracicaba-SP, in a corn area without artificial application of parasitoids. Fifty-two postures of *S. frugiperda* were hung on corn plants. Two days later, the eggs were incubated in the laboratory, where the parasitoids emerged. *Telenomus remus* emerged from all 52 postures, while *T. pretiosum* emerged from 19, and 3 posture *T. atopovirilia*. One of the postures was parasitized by the three species of wasps simultaneously, an event that had never been recorded before (Figure 49).



**Figure 49** Life cycle *Telenomus remus* Nixon, 1937 (Hymenoptera: Scelionidae) in the host *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae)

Source: [https://www.researchgate.net/figure/Telenomus-remus-life-cycle-on-FAW-eggs\\_fig1\\_357871606](https://www.researchgate.net/figure/Telenomus-remus-life-cycle-on-FAW-eggs_fig1_357871606)

### 3.2.1. Interference competition

Is characterized by defensive/aggressive behavior among the birds. Interference competition occurs when different wasp species are in a posture of *S. frugiperda*, and this encounter results in behavioral changes that affect parasitism. In addition, competition for interference can also occur between immature forms of wasps.

For example, in our studies, we observed that a single *S. frugiperda* egg can be parasitized by all possible combinations of *Tr. pretiosum*, *Tr. atopovirilia* and *Te. remus*, that is, the young forms of the parasitoids face competition for interference inside the pest egg.

However, when *Tr. pretiosum* and *Tr. atopovirilia* parasitize the same egg within an interval of up to 6 hours, both species survive to adulthood in between 30 and 40% of cases. In other cases, only one species survives. Competitions for exploration and interference can affect the population dynamics of parasitoids, with consequences for the biological control of fall armyworm [35,36,37,38].

### 3.3. Study 3

The cultivation of sugarcane is of great economic importance for some countries in the Americas, especially for Brazil, which stands out as one of the world's leading producers of sugar and cane alcohol. In the Northeast, the sugarcane culture comprises the Zona da Forest in the States of Paraíba, Pernambuco, Alagoas and Sergipe to the Bahian Recôncavo. In the state of Alagoas, the sugarcane production reaches an area of 450 thousand hectares, favored by the agroclimatic conditions (Figure 50).

Despite the ease of adaptation to Brazil's climate, the culture faces a series of phytosanitary problems, such as the incidence of insect pests, such as genus *Diatraea* (Lepidoptera), which reduce the productivity, resulting in economic loss for the producers (Figure 51).

Among the different pests that attack the crop, the sugarcane borer, *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae), is considered the most important, due to its wide geographic distribution and high economic losses, with direct losses (agricultural productivity reduction) and indirect (losses in the quality of the raw material), and treatment costs, estimated at around 1 billion dollars annually (Figures 52, 53 and 54).



Source: IBGE

**Figure 50** Brazil regions map



**Figure 51** Northeast region map

Source: IBGE



**Figure 52** Larva of *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera, Pyralidae) parasitizing sugarcane *Saccharum officinarum* L. (Poaceae)

Source: Photo: Marina Torres





**Figure 53** Pupa of *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera, Pyralidae) parasitizing sugarcane *Saccharum officinarum* L. (Poaceae)

Source: <https://docplayer.com.br/63537297-Parasitismo-e-desenvolvimento-de-tetrastichus-howardi-hymenoptera-eulophidae-em-lagarta-e-pupa-de-diatraea-saccharalis-lepidoptera-crambidae.html>



**Figure 54** Adult of *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera, Pyralidae) parasitizing sugarcane *Saccharum officinarum* L. (Poaceae)

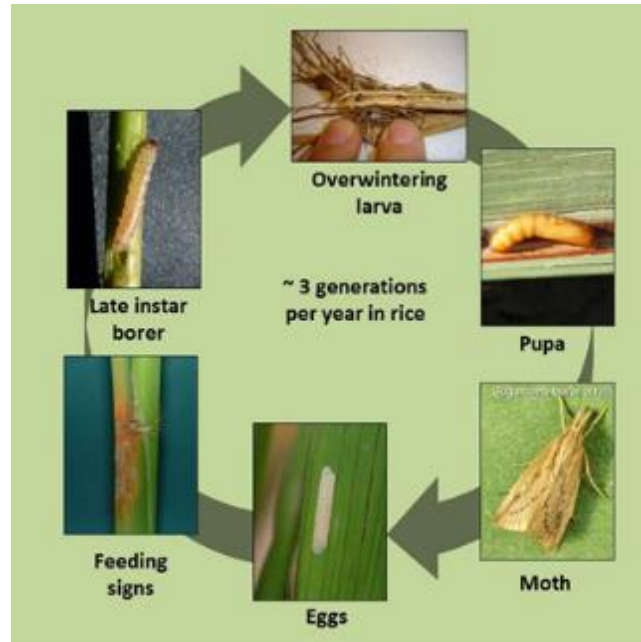
Source: [https://www.agrolink.com.br/problemas/broca-do-colmo\\_375.html](https://www.agrolink.com.br/problemas/broca-do-colmo_375.html)

These numbers show us the relevance of this history and the adoption of MIP in the sugarcane crop. After mating, the females lay on the leaves of the sugarcane, usually located on the tops. The eggs are laid superimposed in the shape of “fish scales” and after an average incubation period of 6 days, the caterpillars hatch. The duration of the larval period is about 40 to 60 days (Figure 55).

The pupal stage lasts from 9 to 14 days. The total cycle of the pest is about 60 to 90 days, varying according to the temperature, and can originate from 4 to 5 generations per year. Adults have an average lifespan of 5 to 7 days. The greatest development of the pest is observed in periods of high temperature and rainfall, and greater damage is observed at the beginning of plant development and in sugarcane fields with lower numbers of cuts (Figure 56).

The most typical symptoms of direct damage by the pest are the holes caused by the feeding of the borer caterpillars in the internodes of the cane stalks, and the galleries formed inside these, giving rise to the common name of the pest “sugar cane borer”. Through the openings caused by the feeding of the caterpillars, fungi can penetrate that cause red rot and the consequent reduction of the quality of the raw material (Figure 57).





**Figure 55** Life cycle of *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera, Pyralidae)

Source> <https://www.lsuagcenter.com/topics/crops/rice/insects/presentations/5-sugarcane-borer>



**Figure 56** Damage of *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera, Pyralidae)

Source: <https://www.lsuagcenter.com/topics/crops/rice/insects/presentations/5-sugarcane-borer>



**Figure 57** Gurdaspur borer (from Left)- Gurdaspur pupa, Larva, infected internode, tunnel made by Gurdaspur borer, and dry tops

Source: Courtesy: Dr Faqir Gul, SCRI - 2010

Currently, the most efficient control of *Diatraea* spp. has been the biological one using the larval endoparasitoid *Cotesia flavipes* (Cameron, 1891) (Hymenoptera: Braconidae). However, the key factor in the growth of the sugarcane borer population is the egg stage, which presents an abundance of parasitoid species, especially those of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) (Figure 58).

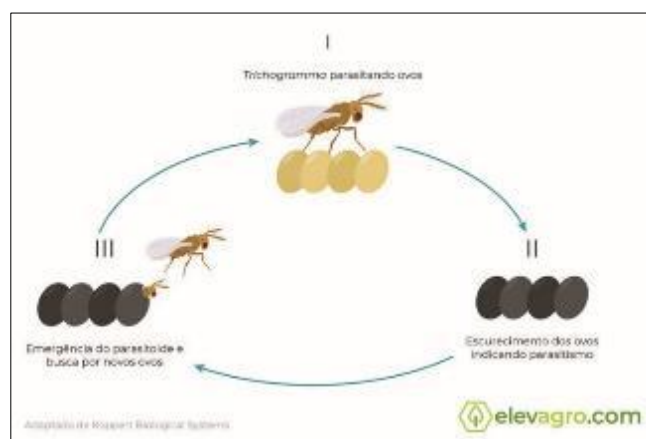


**Figure 58** Sugarcane Stem borer moth, larva, and damage

Source: <http://agropedia.iitk.ac.in/content/early-shoot-borer-sugarcane>

Some species of *Trichogramma* have been used in flood releases in several countries to control pests of agricultural significance. Although they have a preference for Lepidoptera eggs, they also parasitize about 200 species belonging to more than 70 families of Insecta and concerning eight orders of agricultural importance.

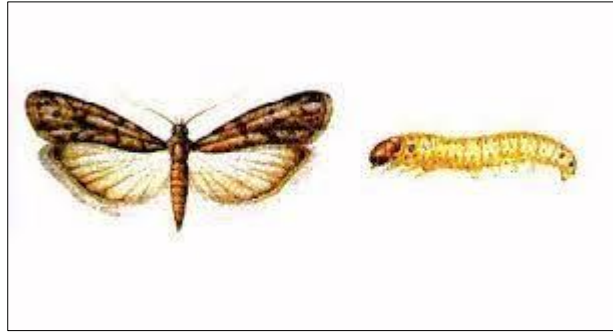
Climatic factors are the direct and indirect causes of the fluctuations and population dynamics of the borer. They modify the duration of the biological cycle and their reproductive capacity and, mainly, exert pressure on their various natural enemies, thus creating imbalances in the interrelationships between the phytophagous organism and the host (Figure 59).



**Figure 59** *Trichogramma* spp. in biological pest control

Source: <https://elevagro.com/conteudos/materiais-tecnicos/trichogramma-spp-no-controle-biologico-de-pragas>

Among the physical factors, temperature has the greatest influence on fecundity, duration of the developmental cycle, sex ratio, viability and longevity of parasitoids. The biological cycle of two species of *Trichogramma* reared on eggs of *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) and *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae), can be affected by several factors. That parasitism by *Trichogramma galloi* Zucchi, 1988 (Hymenoptera: Trichogrammatidae) was higher when kept constantly on *D. saccharalis* eggs than when kept on *A. kuehniella* eggs (Figure 60) [39,40,41].



**Figure 60** *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) flour moth

Source: [https://www.agrolink.com.br/problemas/traca-da-farinha\\_1914.html](https://www.agrolink.com.br/problemas/traca-da-farinha_1914.html)

### 3.4. Study 4

The objective of this work was to evaluate the rate of parasitism, viability, sex ratio, number of egg parasitoids, pre-imaginal development and longevity of males and females of *Trichogramma galloi* Zucchi, 1988 (Hymenoptera: Trichogrammatidae), multiplied in *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera: Crambidae) eggs, aiming at their mass rearing and subsequent field release (Figures 61 and 62).



**Figure 61** *Trichogramma* creation in laboratory

<https://revistadeagronegocios.com.br/biocompany-e-o-controle-biologico-de-pragas-por-trichogramma-pretiosum/>



**Figure 62** Emergence of parasitoids from their host in laboratory

Source: <http://www.usp.br/agen/?p=198497>

The *T. galloi* species was collected in sugarcane fields in the region. The parasitoids were multiplied in eggs of *D. saccharalis* and kept in 17 cm x glass containers 10.5 cm, sealed by perforated laminated PVC film with entomological pin, for aeration. For food of the adults of *T. galloi*, a droplet of honey was deposited on the inner surface of the breeding vessels. Individuals from this creation were used in the experiments (Figure 63).



**Figure 63** Adults present in life flasks ready to be released

Source: <https://revistapesquisa.fapesp.br/inseto-contra-inseto/>

The first day *D. saccharalis* eggs showed a mean parasitism of  $79.3 \pm 5.2\%$  and those of second day  $55.2 \pm 2.3\%$ . There was a significant difference in percentage of parasitism, when considering the age eggs, showing that the parasitoid has a preference for younger eggs (Figure 64) [42,43,44].



**Figure 64** *Trichogramma* release in the field and its monitoring

Source: <https://assistconsult.com.br/produtos/produto-1>

The average duration of the egg-adult period for *T. galloi* reared on *D. saccharalis* eggs was  $9.46 \pm 0.7$  days. The viability of the parasitized eggs was 78.1%. The average number of *T. galloi* adults emerged per egg of *D. saccharalis* was  $2.29 \pm 0.43$ . The sex ratio of *T. galloi* was 0.64 (1.55 females for 1.0 male) at  $26 \pm 2^\circ\text{C}$ . Longevity for males and females was on average  $3.26 \pm 0.12$  days for insects without food and  $6.36 \pm 0.19$  days for those who were fed.

### 3.5. Study 5

One of the successful agents introduced in Brazil in the 1990s is *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae), a generalist microwasp, created in a laboratory responsible for regulating the population of pests in the field, such as the pests of *Oxydia* spp. (eucalyptus crop) *Alabama argillacea* (Hübner, 1818) (Lepidoptera: Noctuidae) (cotton crop), *Tuta absoluta* (Meyrick, 1917). (Lepidoptera: Gelechiidae) (tomato crop), *Plutella xylostella* (Linnaeus, 1758). (Lepidoptera: Plutellidae) (cruciferous crop) *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae). (Corn crop), *Bonagota cranaodes* (Meyrick, 1937) (Lepidoptera: Tortricidae) (apple crop), *Diatraea* spp. (sugar cane crop) and other typical pests of attacks on vegetable crops.

### 3.5.1. Biofactory

Biofactory is a biological laboratory whose mission is to create and maintain a biological agent capable of controlling pests in the most diverse agricultural crops. This tiny biological agent depends on the body of another insect to complete its life cycle. The wasp therefore uses the egg of the *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) moth to reproduce (Figure 65).



**Figure 65** Creation: Eggs of *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) are offered to females of *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) in the laboratory

Source: <https://revistadeagronegocios.com.br/biocompany-e-o-controle-biologico-de-pragas-por-trichogramma-pretiosum/>

As *Anagasta* eggs are laid, popularly known as “Flour Moth”, part of these eggs are removed and offered to *T. pretiosum*, and part are kept intact, in order to ensure the continuity of *A. kuehniella*. The eggs offered to the parasitoid are then taken to a chapel, exposed to ultraviolet light, to be made unviable. Subsequently, the eggs are placed in checkered cards, placed in glass jars, containing adult females of *T. pretiosum*, eager to lay their eggs in the host’s eggs, in order to guarantee the development of their progeny. As the parasitism occurs, and this is noticeable by the darkening of the host’s eggs, the cards are then ready to be applied in the field (Figures 66, 67, 68 and 69).



**Figure 66** Creation of *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae)

Source: <https://revistadeagronegocios.com.br/biocompany-e-o-controle-biologico-de-pragas-por-trichogramma-pretiosum/>



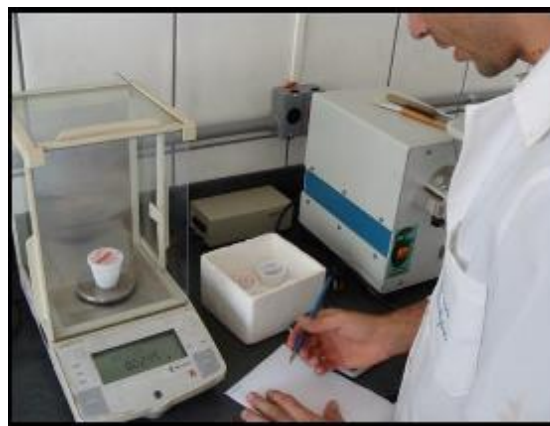


**Figure 67** Creation of *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae)

Source: <https://revistadeagronegocios.com.br/biocompany-e-o-controle-biologico-de-pragas-por-trichogramma-pretiosum/>



**Figure 68** Manipulation to obtain adult parasitoids of *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae)



**Figure 69** Obtaining and weighing the parasitoid eggs to verify how many individuals will be placed on the cards

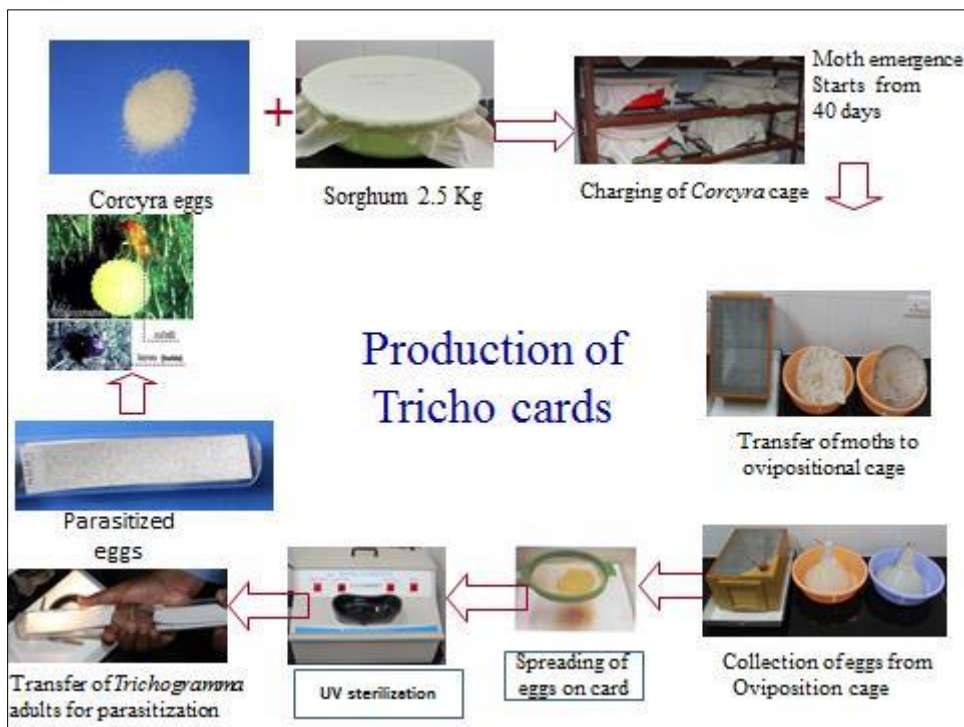
Source: <https://revistadeagronegocios.com.br/biocompany-e-o-controle-biologico-de-pragas-por-trichogramma-pretiosum/>

Finally, it is important to point out that the use of biological agents in agricultural crops requires discipline and, above all, “elimination of the use of non-selective chemical products” in crops. Rural producers increasingly work with prevention, using biological pest control in association or not with other pest management methods, ensuring in the end a quality product, without pesticides, healthier and at a reduced cost (Figures 70 and 71) [45,46,47,48,49,50,51,52].



**Figure 70** Cards are placed in the field for the release of adults

Source: <https://greenmethods.com/trichogramma/>

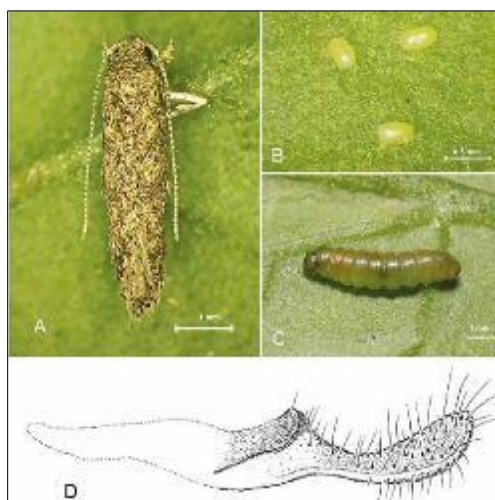


**Figure 71** *Trichogramma* cards: Stages for release of two parasitoids in the field

Source: <https://niphm.gov.in/mothercultures.html>

### 3.6. Study 6

The tomato *Lycopersicon esculentum*, Mill. (Solanaceae), cultivated in all the Brazilian regions, is a culture considered high risk, due to infestation by various pests, both in crops destined for consumption in nature, and for industry (Figure 72).



**Figure 72** *Tuta absoluta* (Meyrick, 1917): A – adult; B – eggs; C – fifth instar larvae; D – detail of the male genital armature, left valve

Source: [https://www.researchgate.net/figure/Tuta-absoluta-Meyrick-1917-A-adult-B-eggs-C-fifth-instar-larvae-D-detail\\_fig2\\_268432892](https://www.researchgate.net/figure/Tuta-absoluta-Meyrick-1917-A-adult-B-eggs-C-fifth-instar-larvae-D-detail_fig2_268432892)

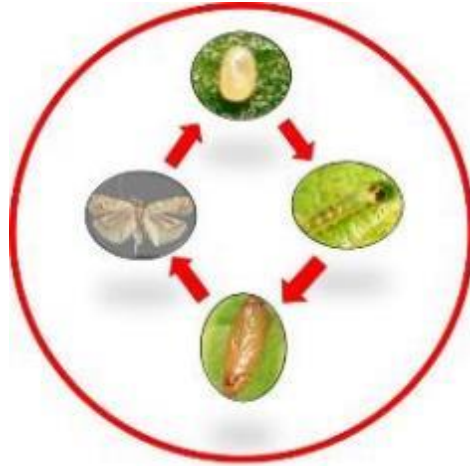
The pragues that cause prejudice to culture, to trace-do-tomateiro, *Tuta absoluta* (Meyrick, 1917). (Lepidoptera: Gelechiidae), deserves highlight, because its occurrence is registered in all national territory, always in high infestation. It generally occurs during the entire culture cycle, damaging all parts of the plant except the roots (Figure 73).



**Figure 73** Different insects affecting tomato. A: The whitefly; B: Adult thrips; C: Large variegated cutworm larva; D: Tomato Sphinx; E: Adult of *Tuta absoluta* (Meyrick, 1917). (Lepidoptera: Gelechiidae). F: Symptoms of *T. absoluta* on tomato leaves

Source: Corpus ID: 165865056

The control of *T. absoluta* by means of chemical products has often not promoted satisfactory reductions, allowing an increase in the population of the pest and its damage. Alternative measures for its management have been investigated, and biological control has shown to be promising, mainly through the release of parasitoids of the genus *Trichogramma* (Hym.: Trichogrammatidae), a natural enemy with wide distribution and highly specialized and efficient (Figures 74, 75, 76, 77 and 78).



**Figure 74** The duration of the *Tuta absoluta* life cycle in different temperatures: 14°C will take approx. 76 days, 20°C will take approx. 24 days and 27°C will take approx. 24 days



**Figure 75** American pinworm *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae)] famous as ooji fly in Kannada and uji in Telugu is one of the major and important pest of tomato. *Tuta absoluta* has become the serious pest with its highly damaging nature in all the stages of its life cycle. The crop losses are high as 60 to 100% due the infestation by *T. absoluta*

Source: Reddy KS Senior Agronomist, BigHaa



**Figure 76** *Trichogramma achaeae* Nagaraja and Nagarkatti, 1970 (Hymenoptera: Trichogrammatidae) adult female (native entomophagous) parasitizing eggs of *Tuta absoluta* (Meyrick, 1917) (exotic species)

Source: [https://www.researchgate.net/figure/T-achaeae-adult-female-native-entomophagous-parasitizing-eggs-of-Tuta-absoluta-exotic\\_fig1\\_258516987](https://www.researchgate.net/figure/T-achaeae-adult-female-native-entomophagous-parasitizing-eggs-of-Tuta-absoluta-exotic_fig1_258516987)





**Figure 77** *Trichogramma achaeae* Nagaraja and Nagarkatti, 1970 (Hymenoptera: Trichogrammatidae) adult female (native entomophagous) parasitizing eggs of *Tuta absoluta* (Meyrick, 1917) (exotic species)

Source: <https://www.mfrural.com.br/detalhe/142816/controlo-biologico-trichogramma>



**Figure 78** Biological control is carried out by pest predators. That is, insects that feed on *Tuta absoluta* (Meyrick, 1917) are released into the field, and these predators destroy the tomato moth. *Tuta absoluta* predators, which are commercially available and widely used, are the leaf parasites *Nesidiocoris tenuis* (Reuter, 1895) (Hemiptera: Miridae), *Podisus* (Heteroptera: Pentatomidae), and *Macrolophus pygmaeus* (Rambur 1839) (Hemiptera: Miridae). In addition, *Trichogramma* and *Bacillus thuringiensis* (Bt) bacteria can be used to destroy older plant

Source: [https://harvesso.com/about\\_tuta\\_absoluta.html](https://harvesso.com/about_tuta_absoluta.html)

The success of biological control programs using egg parasitoids of the genus *Trichogramma* basically depends on research aimed at evaluating their search capacity. Some authors report that this behavior can be influenced by several factors, such as: host, climatic conditions, number of insects to be released, pest density, species and lineage of the



parasitoid to be used, time and number of releases, distribution method as well as plant phenology (Figure 79) [53,54,55].



**Figure 79** *Trichogramma* spp. undergo complete metamorphosis. The adult wasp lays an egg within a recently laid host egg, and as the wasp larva develops, it eats the host embryo, causing the egg to turn black. Because their life cycle from egg to adult is about 7 to 10 days, these parasites have many more generations than their hosts, and their populations can increase rapidly. *Trichogramma* turns the eggs of some caterpillar species black. This is the best way to detect parasitization by *Trichogramma*

Source: [http://ipm.ucanr.edu/natural-enemies/trichogramma\\_spp.html](http://ipm.ucanr.edu/natural-enemies/trichogramma_spp.html)

### 3.7. Study 7

The objective of this work was to estimate the ideal number of *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) Riley to be released in the field to control the tomato leafminer *Tuta absoluta* (Meyrick, 1917). (Lepidoptera: Gelechiidae) (Figure 80).

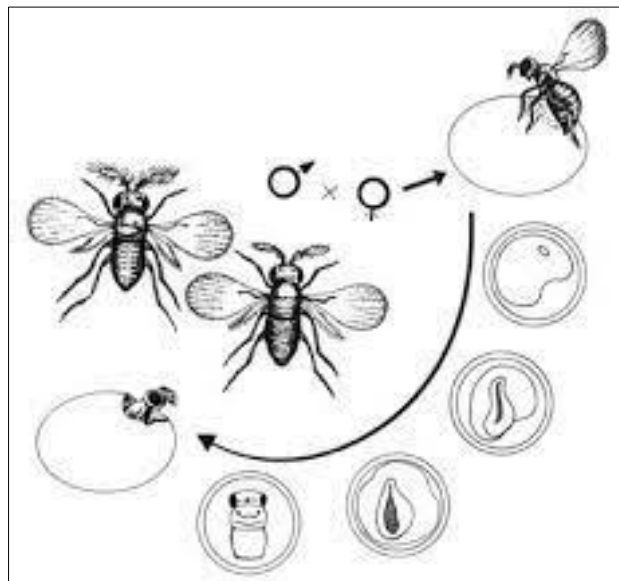


**Figure 80** *Trichogramma pretiosum* Riley, 1879 parasitizing *Tuta absoluta* eggs (Meyrick, 1917)

Source: <https://www.koppert.com.br/pretiobug/>

The experiment was implemented in a greenhouse, where, 60 days after transplanting, tomato plants were infested with 200 eggs of the alternative host *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) (Zeller).

By analyzing the percentage of parasitism (*T. pretiosum*) it was verified that, in the upper third of the plant, by the percentage of parasitism analysis, there was a direct relationship between parasitism and the density of the parasitoid released, in the range of 1 to 16 individuals per egg, being found at the peak, 73% of the parasitized eggs (Figure 81).



**Figure 81** Life cycle diagram of the egg parasitoid (A) *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae). The Adult female laying tomato moth eggs. (B) *T. pretiosum* egg is laid inside the tomato moth egg. C and D Larval development of *T. pretiosum* in the host. Adult parasitoid emerging from tomato moth egg

Source: [https://ainfo.cnptia.embrapa.br/digital/bitstream/CNPH-2009/31470/1/ct\\_36.pdf](https://ainfo.cnptia.embrapa.br/digital/bitstream/CNPH-2009/31470/1/ct_36.pdf)

By analyzing the percentage of parasitism (*T. pretiosum*) it was verified that, in the upper third of the plant, by the percentage of parasitism analysis, there was a direct relationship between parasitism and the density of the parasitoid released, in the range of 1 to 16 individuals per egg, being found at the peak, 73% of the parasitized eggs.

Similar to what happened in the other thirds of plant, the maximum parasitism in the lower third was reached in the proportion of 16 parasitoids per egg, reaching 81.7%. According to the results, the proportion of 16 parasitoids per egg of the pest is the closest to the ideal for release in commercial staked tomato plantations, in the management of the *T. absoluta* moth (Figure 82).



**Figure 82** *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) is a major destructive pest of tomato worldwide

Source: <https://www.greenlife.co.ke/tuta-absoluta/>

The recommendation of 16 parasitoids to be released per egg becomes ideal because it is the point at which maximum efficiency was obtained from the parasitoid. From this point on, there is a tendency to reduce the efficiency of the parasitoid, due to the lower probability of an individual finding non-parasitized eggs, and superparasitism may occur in many cases (Figure 83) [56,57].



**Figure 83** Damage caused by tomato leafminer of *Tuta absoluta* (Meyrick, 1917). (Lepidoptera: Gelechiidae)

Source: <https://www.greenlife.co.ke/tuta-absoluta/>

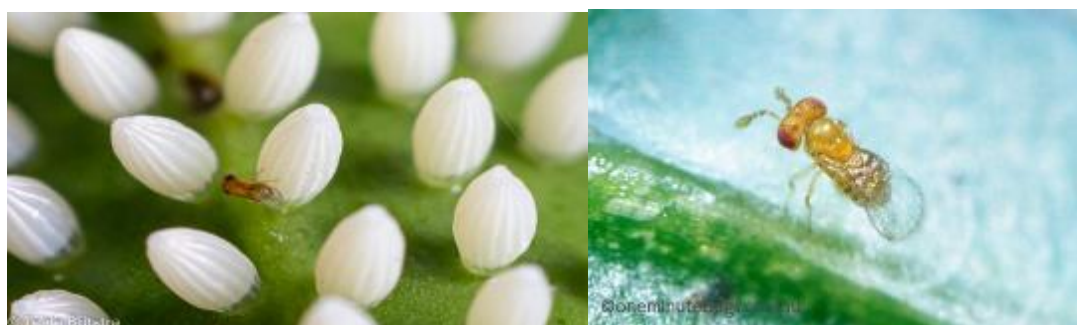
### 3.8. Study 8

Among the various representatives, *Trichogramma* is one of the most studied and used in the world, since the beginning of the last century, being used in 20 million hectares in different parts of the world. There are more than 200 described species of *Trichogramma*, 26 of them reported in Brazil (Figure 84).



**Figure 84** *Trichogramma minutum* Riley 1871 a North American species found in natural areas, annual crops, and orchards. In the United States, it is used as a biological control agent primarily in orchards

Source: <https://arthropod.uark.edu/minute-egg-wasp/>



**Figure 85** *Trichogramma* parasitizing *Helicoverpa* spp. (Lepdoptera)

Source: Photo 67426637, (c) Teale Britstra and <https://oneminutebugs.com.au/i-love-parasitoids/>

One of the great advantages of this parasitoid lies in the fact that by destroying the embryo, it prevents the pest from progressing. In addition, to be multiplied in the laboratory for release in the field, it can be created in alternative hosts, in this case, moths, easily created in the laboratory (Figures 85 and 86).



**Figure 86** *Trichogramma* are considered to be the most important natural enemies of the budworm (*Helicoverpa* spp.) our worst caterpillar pest

Source: <https://oneminutebugs.com.au/i-love-parasitoids/>

Studies show that *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera, Pyralidae) is the most efficient moth, as it is richer in nutrients, for the commercialized Brazilian species, that is, *Trichogramma galloi* Zucchi, 1988 (Hymenoptera, Trichogrammatidae), *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) and *Trichogramma atopovirilia* Oatman. & Platner, 1983 (Hymenoptera: Trichogrammatidae) (Figure 87).



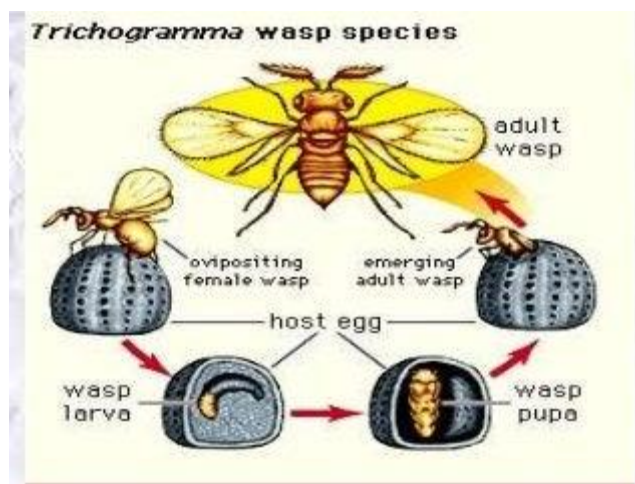
**Figure 87** Egg, larva, pupa of *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera, Pyralidae)

Source: [https://www.agric.wa.gov.au/sites/all/modules/custom/seed\\_tools/pestweb/907904924.html](https://www.agric.wa.gov.au/sites/all/modules/custom/seed_tools/pestweb/907904924.html) and [https://en.wikipedia.org/wiki/Mediterranean\\_flour\\_moth](https://en.wikipedia.org/wiki/Mediterranean_flour_moth)

There are companies in the world that produce about 40 kg of moth eggs per day, as they are used to create the parasitoid *Trichogramma* and various predators. It is worth noting that 1g of *A. kuehniella* moth eggs contains 36,000 eggs.

It is a very specialized insect that is attracted to volatile substances in the scales of lepidopteran pests when laying. These volatiles that favor *Trichogramma* and which are called kairomones have even been identified as tricosan (Figure 88).





**Figure 88** *Trichogramma* life cycle

Source: <https://www.slideshare.net/prajshi123/seminar-trichogramma-a-living-insecticide>

A female *Trichogramma* lays from 70 to 120 eggs, with 1, 2, 3 wasps or sometimes a large number of parasitoids emerging from each parasitized egg, depending on the size of the host egg.

Currently, there are companies marketing *Trichogramma*, which is released in a flooding form and which, therefore, has a faster action, more similar to the agrochemicals that farmers are more familiar with. This is called applied or augmentative biological control (Figure 89).



**Figure 89** Main pests of *Glycine max* (L.) Merr. (Fabaceae) soybean: False-bearing caterpillar *Chrysodeixis includens* (Walker, 1858) (Lepidoptera: Noctuidae); Soybean caterpillar *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae); Apple caterpillar *Chloridea* (= *Heliothis*) *virescens* (F., 1781) (Lepidoptera: Noctuidae) *virescens*); Apple cat; *Helicoverpa armigera* (Hübner, 1809) (Lepidoptera: Noctuidae)

Source: <https://blog.aegro.com.br/inseticidas/>

After 30 years of studies in Brazil, *Trichogramma* is already a reality in our country, being released in 500,000ha of sugarcane for the control of the sugarcane borer, *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera, Pyralidae), in addition to *T. pretiosum*, which is released in soybean to control *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae), *Chrysodeixis includens* (Walker, 1858) (Lepidoptera: Noctuidae), and *Helicoverpa armigera* (Hübner, 1809)



(Lepidoptera: Noctuidae), with other examples in tomato, to control *Tuta absoluta* (Meyrick, 1917). (Lepidoptera: Gelechiidae), in addition to being used in common bean, cotton and corn *Helicoverpa zea* (Boddie, 1850) (Lepidoptera, Noctuidae). It is effective in open fields, in organic farming or in a greenhouse.

### 3.9. Study 9

#### 3.9.1. Agents registered in Brazil and *Trichogramma* spp. in biological pest control

The literature mentions that there are more than 230 species of the genus *Trichogramma*, with the capacity to parasitize more than 200 different species of insects. However, in Brazil only two species are available to the producer. The table below shows which pests these species are registered for (Table 1).

**Table 1** Agents registered in Brazil or Appointing an Agent (Sales Representative) in Brazil

<i>Trichogramma galloi</i> Zucchi, 1988 (Hymenoptera, Trichogrammatidae)	<i>Trichogramma pretiosum</i> Riley ,1879 (Hymenoptera: Trichogrammatidae)
<i>Diatraea saccharalis</i> (Fabricius, 1794) (Lepidoptera, Pyralidae).	<i>Tuta absoluta</i> (Meyrick, 1917) (Lepidoptera: Gelechiidae)
	<i>Helicoverpa zea</i> (Boddie, 1850) (Lepidoptera, Noctuidae)
	<i>Spodoptera frugiperda</i> (Smith, 1797) (Lepidoptera: Noctuidae)
	<i>Anticarsia gemmatalis</i> Hübner, 1818 (Lepidoptera: Noctuidae)
	<i>Pseudoplusia includens</i> (Walker, 1857). (Lepidoptera: Noctuidae)

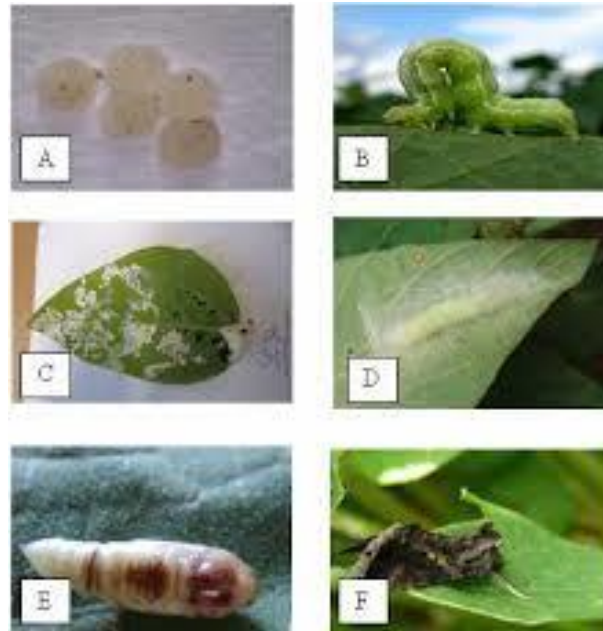
Source: Nora DD. *Trichogramma* spp. in biological pest control. 2022. All rights reserved to Elevagro: Porto Alegre; <https://elevagro.com/> Porto Alegre. Parra JRP Consolidated technology: *Trichogramma* in the management of lepidopterans. Great Cultures. Cultivar Magazine 190. 2015

#### 3.9.2. How and when to use it?

As mentioned, *Trichogramma galloi* Zucchi, 1988 (Hymenoptera, Trichogrammatidae) has already been used in large areas of sugarcane and *Trichogramma pretiosum* Riley ,1879 (Hymenoptera: Trichogrammatidae) also in large areas of soybean, corn, cotton, to control falsa-medideira *Chrysodeixis (=Pseudoplusia) includens* (Walker, 1858) (Lepidoptera: Noctuidae), soy caterpillar, *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae) and old world caterpillar *Helicoverpa armigera* (Hübner, 1815) (Lepidoptera: Noctuidae), the equivalent to more than 250,000 ha, being no longer used due to the non-availability of the biological input.

In other countries, Biological Control, in general, is more used, but mainly in more restricted areas, that is, greenhouses. Our Agriculture is peculiar, as there are large areas, sometimes 30,000 ha, 50,000 ha, 100,000ha, of a crop exploited by a single farmer.

Evidently, in this case, there are problems in surveying the pest, which must be sampled at the beginning of the culture and after the detection of the eggs, the parasitoids must be released (Figure 90) [58].



**Figure 90** *Chrysodeixis includens* (Walker, [1858] (Lepidoptera: Noctuidae) in egg (A), larva (B), damage done (C), pre-pupal cocoon (D), pupa (E) and moth (F) stage

Photos D, E and F: Regiane Cristina Oliveira de Freitas Bueno and <https://ainfo.cnptia.embrapa.br/digital/bitstream/item/163883/1/Andrade-Karine-Me-2014.pdf>

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#### 4. Conclusion

The genus *Trichogramma* is of great interest, mainly because it is used to control of pests of economic importance of the order Lepidoptera, in addition to being easily bred in laboratory. *Trichogramma* species are associated with 42 hosts on the South American continent, being *Trichogramma exiguum* Pinto & Platner (Hymenoptera: Trichogrammatidae) one of which have a greater number, parasitizing eggs of twelve species, associated with seven cultures.

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