Research, Society and Development, v. 14, n. 11, e183141150055, 2025
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v14i11.50055

Reproductive biology of *Tetrastichus howardi* (Hymenoptera: Eulophidae) fed transgenic maize pollen

Biologia reprodutiva de *Tetrastichus howardi* (Hymenoptera: Eulophidae) alimentado com pólen de milho transgênico

Biología reproductiva de *Tetrastichus howardi* (Hymenoptera: Eulophidae) alimentado con polen de maíz transgénico

Received: 11/06/2025 | Revised: 11/19/2025 | Accepted: 11/20/2025 | Published: 11/22/2025

Maria Freire De Sousa[1]
Universidade Federal da Grande Dourados, Brazil
E-mail: marialuz1920@hotmail.com
Marcos Gino Fernandes[1]
ORCID: https://orcid.org/0000-0003-4377-5562
Universidade Federal da Grande Dourados, Brazil
E-mail: marcosfernandes@ufgd.edu.br
Fabricio Fagundes Pereira[1]
ORCID: https://orcid.org/0000-0003-1638-7409
Universidade Federal da Grande Dourados, Brazil
E-mail: fabriciofagundes@ufgd.edu.br
Thiago Alexandre Mota[1]
ORCID: https://orcid.org/0000-0001-8011-5972
Universidade Federal da Grande Dourados, Brazil
E-mail: thiamota@hotmail.com
Elias Soares Gomes[2]
ORCID: https://orcid.org/0000-0002-7401-3352
Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso do Sul, Brazil
E-mail: elias.gomes@ifms.edu.br
Eduardo Neves Costa[3]
ORCID: https://orcid.org/0000-0001-9837-9570
Universidade Estadual Paulista "Júlio de Mesquita Filho", Brazil
E-mail: costa_ne@yahoo.com.br
Leonardo Rego Sant'Anna[3]
ORCID: https://orcid.org/0009-0007-0580-5763
Universidade Estadual Paulista "Júlio de Mesquita Filho", Brazil
E-mail: leonardo.santanna@unesp.br

Abstract
Hymenopteran parasitoids are important natural enemies of pests and are used in biological control programs due to their specificity and ability to suppress pest abundance. However, the consumption of transgenic plant pollen may affect the biology of these natural enemies. The purpose of this research was to evaluate the effects of Bt corn pollen on certain biological characteristics of the parasitoid *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae). The biological characteristics used to evaluate the effects of transgenic pollen were: parasitism (%), parasitoid emergence (%), development time (egg to adult, d), number of females emerging from hosts, total number of parasitoids (males and females) emerging from hosts, sex ratio, number of offspring per female, and offspring longevity (d). The study was conducted for 4 generations of the parasitoid. The results of this study suggest that the consumption of pollen did not significantly affect the biological characteristics of the parasitoid evaluated for 4 generations. Also, only a few parameters of the insect's biology varied over the 4 generations of the study, but, according to the scientific literature, this biological variation observed in our study is natural for this species.
Keywords: Biologic control; Parasitoid parameters; Transgenic cultivar; *Tetrastichus howardi*; Bt pollen.

[1] Universidade Federal da Grande Dourados (UFGD), Faculdade de Ciências Biológicas e Ambientais, (FCBA), Dourados, MS, Brazil.
[2] Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso do Sul (IFMS), Dourados, MS, Brazil.
[3] Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agronômicas (FCA), Câmpus de Botucatu, Brazil.

Resumo
Os himenópteros parasitoides são importantes inimigos naturais de pragas e são utilizados em programas de controle biológico devido à sua especificidade e capacidade de suprimir a abundância de pragas. No entanto, o consumo de pólen de plantas transgênicas pode afetar a biologia desses inimigos naturais. O objetivo desta pesquisa foi avaliar os efeitos do pólen de milho Bt sobre determinadas características biológicas do parasitoide *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae). As características biológicas utilizadas para avaliar os efeitos do pólen transgênico foram: parasitismo (%), emergência de parasitoides (%), tempo de desenvolvimento (ovo a adulto, dias), número de fêmeas emergidas dos hospedeiros, número total de parasitoides (machos e fêmeas) emergidos dos hospedeiros, razão sexual, número de descendentes por fêmea e longevidade da prole (dias). O estudo foi conduzido por quatro gerações do parasitoide. Os resultados deste estudo sugerem que o consumo de pólen não afetou significativamente as características biológicas do parasitoide avaliadas ao longo de quatro gerações. Além disso, apenas alguns parâmetros da biologia do inseto variaram entre as gerações, mas, de acordo com a literatura científica, essa variação biológica observada em nosso estudo é natural para esta espécie.
Palavras-chave: Controle biológico; Parâmetros de parasitoides; Cultivar transgênica; *Tetrastichus howardi*; Pólen Bt.

Resumen
Los himenópteros parasitoides son importantes enemigos naturales de las plagas y se utilizan en programas de control biológico debido a su especificidad y capacidad para suprimir la abundancia de plagas. Sin embargo, el consumo de polen de plantas transgénicas puede afectar la biología de estos enemigos naturales. El propósito de esta investigación fue evaluar los efectos del polen de maíz Bt sobre ciertas características biológicas del parasitoide *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae). Las características biológicas utilizadas para evaluar los efectos del polen transgénico fueron: parasitismo (%), emergencia de parasitoides (%), tiempo de desarrollo (huevo a adulto, días), número de hembras emergidas de los hospedadores, número total de parasitoides (machos y hembras) emergidos de los hospedadores, proporción sexual, número de descendientes por hembra y longevidad de la descendencia (días). El estudio se realizó durante cuatro generaciones del parasitoide. Los resultados de este estudio sugieren que el consumo de polen no afectó significativamente las características biológicas del parasitoide evaluadas durante cuatro generaciones. Además, solo algunos parámetros de la biología del insecto variaron entre las generaciones, pero, según la literatura científica, esta variación biológica observada en nuestro estudio es natural para esta especie.
Palabras clave: Control biológico; Parámetros de parasitoides; Cultivar transgénico; *Tetrastichus howardi*, Polen Bt.

1. Introduction

Maize (*Zea mays* L.; Poaceae) is one of the world's most important agricultural crops because it is used extensively, for both human and animal nutrition (Bruyn et al., 2024). Brazil is the world's third largest maize producer, with a cultivated area of nearly 16 million hectares and a production of over 81 million tons (Conab, 2025; IBGE, 2025). However, several factors can compromise its production, including the incidence of pests, which can cause production losses and, therefore, have a great economic impact.

Diatraea saccharalis (Fabricius) (Lepidoptera: Crambidae) is one such pest. Newly emerged *D. saccharalis* caterpillars feed on maize leaves and eventually penetrate the stalk's interior, building galleries and obstructing the transport of the vascular tissue (Souza et al., 2025). Because larval development takes place inside the plant, the use of chemical control is inadequate for managing the pest, and biological control (especially the use of parasitoids) is of great importance.

Hymenopteran parasitoids are important natural enemies of pest insects and have been used in large-scale biological control programs, owing to their specificity and ease of cultivation (Cabello et al., 2024). *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae), for example, is a gregarious endoparasitoid with potential to be used for controlling Lepidopteran pests (Bermúdez, 2023) (especially because it has the capacity to parasitize its natural host (*D. saccharalis*) at 2 developmental stages (caterpillar and pupa; Oliveira et al., 2024). However, the effects of genetically modified crops, including maize, on beneficial insects such as *T. howardi* remain largely unknown.

In transgenic Bt crops, the Bt protein is expressed in all plant tissues, including pollen, where the concentration of the toxin is extremely variable (Lohn et al., 2023). Thus, non-target organisms that interact with the plant's pollen may suffer toxic effects. For example, a mathematical model produced by (Holst et al., 2013) demonstrated that caterpillars of *Inachis io* (L.) (Lepidoptera: Nymphalidae) are at a risk of exposure to the toxin in the Bt maize (MON 810). However, the effect of Bt maize on parasitoids could be direct or indirect effects. Direct effects occur via feeding on plant tissues that express the Bt protein, such as pollen (Bueno et al., 2024), whereas indirect effects are associated with the quality of the insect host, which could influence the biology of parasitoids, including both development time and emergence rate (Souza et al., 2023). Sometimes, Bt toxin may negatively affect the parasitism, emergence, female weight, and the feeding of different species of parasitoids (Zhang et al., 2025).

Therefore, it is desirable to better understand the interaction of parasitoids such as *T. howardi* with transgenic Bt crops. Because pollen is often used by insects as a nutritional resource, and we know that the transgenic protein can also be expressed in the pollen, the objective of the present study was to evaluate the effects of Bt maize pollen on the biological characteristics of adult females of *T. howardi*.

2. Methodology

An experimental, laboratory-based, quantitative study was conducted (Pereira et al., 2018) using descriptive statistics with mean and standard deviation values (Shitsuka et al., 2014) and statistical analysis (Vieira, 2021).

Insect Origin and Cultivation

This study was conducted at the Laboratory of Insect Cultivation of the Faculty of Biological and Environmental Sciences of the Federal University of Grande Dourados, Brazil, using pupae of the host *D. saccharalis* and the parasitoid *T. howardi*, both insects reared in laboratory for several generations.

Viable *D. saccharalis* eggs were placed in glass flasks that contained an artificial diet modified of Hensley & Hammond (1968) containing soybean meal, wheat germ, vitamins and minerals and the caterpillars were kept in the flasks until the last instar. After that, the caterpillars were individually transferred to Petri dishes that contained artificial diet and were kept there until pupal development (Parra, 2007).

After emergence, *T. howardi* adults were kept in glass tubes that were sealed with cotton and contained a drop of pure honey. Additional generations were produced by exposing 24- to 48-h-old *D. saccharalis* to parasitism by *T. howardi* females for 24 h at 25 ± 2°C, 70 ± 10% relative humidity, and 14 h of light, in a climate-control chamber (Vargas et al., 2011) and then rearing them on artificial diet.

Pollen Source

The maize pollen used in the present study was harvested from both Bt transgenic maize (MON 89034), which expressed the Cry1A105 and Cry2Ab2 proteins, and conventional non-Bt maize (DKB177) at the Experimental Farm of the Federal University of Grande Dourados. The crop area comprised 0.5 hectares and was cultivated using a conventional cultivation system, without the use of insecticides during the pollen harvesting period.

The pollen was harvested by carefully placing paper bags over the tassels of plants during anthesis, stapling the bags closed, and removing the bags after 48 h. After collection, the pollen was sieved, transferred to plastic containers, and stored in a refrigerator at 4 °C, using methods adapted from Wang et al. (2007).

Bioassays

Three treatments (diets) were provided in this study: pollen from Bt maize: (20 mg pollen suspended in a 10% honey solution), pollen from non-Bt maize (20 mg pollen suspended in a 10% honey solution), and a 10% honey solution (control), based on methods adapted from Wang et al. (2007).

Fifty *D. saccharalis* pupae aged 24 to 48-h-old were placed in glass tubes and exposed to parasitism by a single *T. howardi* female for 24 h, from each treatment. After this period, each *T. howardi* female was removed, and the pupae were retained in their original glass tubes in a climate-control chamber at 25 ± 1ºC, 60 ± 10% relative humidity, and 14 h of light, until the emergence of *T. howardi* adults. This procedure was repeated four times (4 generations).

The biological characteristics used to evaluate the effects of transgenic pollen for 4 generations were: parasitism (%), parasitoid emergence from hosts (%), development time (egg to adult, d), number of females emerging from hosts, total number of parasitoids (males and females) emerging from hosts, sex ratio (percentage of females), number of offspring per female, and offspring longevity (d).

The offspring longevity of the females *T. howardi* was evaluated by selecting 24h females (n = 20) from each treatment and placing them in individual in glass tubes that contained a droplet of honey solution (treatments), where they remained until dying. The sex of the adult parasitoids was determined according to antennae morphology (La Salle & Polaszek 2007). The percentage of parasitism was determined by counting the total number of host pupae that showed emergence of the parasitoid or emergence of moths. Those pupae that did not present emergence of either moths or parasitoids were dissected to determine if any parasitoid failed in the emergency.

Statistical Analyses

The experimental design was completely randomized with 3 diet treatments and 10 replicates; each replicate was composed of 5 *D. saccharalis* pupae individualized in glass tubes, being each pupae exposed to parasitism by 1 females of *T. howardi* for 24 hours, for a total of 50 pupae per treatment. These three treatments were compared to each other during five successive generations of the parasitoids.

The data from reproductive biology referring to the parameters diets and generations were subjected to analysis of variance for each one of these measured parameters, and the means were compared using Tukey's test ($P \leq 0.05$) in the Assistat program version 7.7 2016 (Silva, 2016).

3. Results

The analyses of variance resulted in interaction not significant between treatments (diets) and generations, evidencing that the factors are independent, that is, the behavior of the treatments is not affected by variation (absence or presence) of the generations.

The percent parasitism by *T. howardi* of *D. saccharalis* pupae was significantly lower in the Bt pollen-fed treatment ($F= 7.4348$, $DF=3$, $P= 0.0005$) relative to honey only-fed wasps ($F= 1.9412$, $DF= 3$, $P= 0.1404$), and the wasps fed with non-Bt pollen was not significantly different than the percent parasitism exposed by wasps fed with either Bt-pollen or honey during the first generation. However, there were no differences in the other generations evaluated, when considering the percentage of parasitism (Table 1).

Table 1 - Reproductive biology of *Tetrastichus howardi* fed transgenic maize pollen.

Biological parameters		Honey + Bt maize pollen	Honey + convencional maize pollen	Honey
Parasitism (%)	Generation 1	56.00 ± 7.55 bB	64.50 ± 6.16 abB	84.00 ± 4.98 aA
	Generation 2	88.00 ± 4.42 aA	92.00 ± 3.26 aA	92.00 ± 4.42 aA
	Generation 3	92.00 ± 3.26 aA	96.00 ± 4.0 aA	98.00 ± 2.0 aA
	Generation 4	92.00 ± 3.26 aA	94.00 ± 3.05 aA	92.00 ± 4.42 aA
Emergence (%)	Generation 1	100% ± 0.00 aA	100% ± 0.00 aA	100% ± 0.00 aA
	Generation 2	100% ± 0.00 aA	100% ± 0.00 aA	100% ± 0.00 aA
	Generation 3	100% ± 0.00 aA	100% ± 0.00 aA	100% ± 0.00 aA
	Generation 4	100% ± 0.00 aA	100% ± 0.00 aA	100% ± 0.00 aA
Duration (Cycle)	Generation 1	16.64 ± 0.17 aA	16.82 ± 0.26 aA	16.82 ± 0.24 aA
	Generation 2	17.52 ± 0.61 aA	16.75 ± 0.44 aA	16.75 ± 0.56 aA
	Generation 3	16.10 ±0.20 aA	15.41 ± 0.05 bB	15.30 ± 0.1 bB
	Generation 4	16.39 ± 0.17 aA	16.01± 0.05 aAB	16.17± 0.096 aAB
No.progeny	Generation 1	65.84 ± 5.6 aB	51.54 ± 4.22 aB	64.96 ± 3.37 aB
	Generation 2	86.60 ± 5.84 aA	87.64 ± 4.19 aA	96.53 ± 4.02 aA
	Generation 3	99.76± 2.82 aA	92.52 ± 3.90 aA	98.80 ± 3.22 aA
	Generation 4	83.92 ± 2.25 aA	80.49 ± 2.94 aA	69.28 ± 2.23 bB
Sex ration	Generation 1	0.70 ± 0.04 aAB	0.95 ± 0.004 aA	0.86 ± 0.039 aA
	Generation 2	0.74 ± 0.07 aAB	0.79 ± 0.07 aAB	0.69 ± 0.07 aB
	Generation 3	0.41 ± 0.07 bB	0.74 ± 0.05 aB	0.91 ± 0.01 aA
	Generation 4	0.94 ± 0.005 aA	0.94 ± 0.005aA	0.95 ± 0.003 aA
Progeny/ Females	Generation 1	54.46 ± 3.85 aB	49.38 ± 4.11aB	55.87 ± 3.88 aB
	Generation 2	66.27 ± 7.80 aAB	72.63 ± 5.57 aA	64.98 ± 8.40 aB
	Generation 3	41.34 ± 6.98 bB	71.10 ± 6.39 aA	91.25 ± 4.06 aA
	Generation 4	79.64 ± 2.05 aA	76.89 ± 2.73 aA	66.61± 2.22 bB
Longevity females (D)	Generation 1	14.10 ± 0.95 bA	15.05 ± 1.36 bA	22.20 ± 1.64 aA
	Generation 2	17.80 ± 1.37 aA	18.35 ± 1.58 aA	16.70 ± 1.00 aB
	Generation 3	18.30 ± 1.28 aA	19.85 ± 1.52 aA	20.25 ± 1.67 aAB
	Generation 4	18.65 ± 1.79 aA	15.45 ± 1.26 aA	19.45 ± 1.04 aAB

* Means followed by the same lowercase letter in the line within each generation, do not differ from each other, by the Tukey test at 5% probability.
* Means followed by the same capital letter in the column between each generation, do not differ from each other, by the Tukey test at 5% probability.
Source: Research data (2025).

The percentage of parasitoid emergence was not affected by treatment in any of the four generations, nor was the percentage of parasitoid emergence influenced by generation for any of the treatments. The development time of *T. howardi* cultured for 4 generations in *D. saccharalis* pupae exhibited little variation, and only during the third generation were the Bt pollen-fed parasitoids significantly slower to develop (Table 1).

The number of progeny per *T. howardi* females fed by Bt polen or conventional polen was lower in the first generation than in subsequent generations, and significant differences were observed for honey-fed wasps in the fourth and third generations, having a greater number of progeny. In the third generation, the sex ratio of offspring from Bt-fed wasps was significantly lower than the ratios from either non-Bt- or honey-fed wasps. Meanwhile, significant differences were also observed in the ratios of the fourth generation of Bt-fed wasps, and second generation of honey-fed wasps (Table 1).

In the third generation, the number of offspring per female was also significantly lower in Bt-fed wasps; however, in the fourth generation, the number of offspring per female was lower in honey-fed wasps. In addition, the number of offspring per female was also significantly greater in the second and fourth generations of Bt-fed wasps and the first generation of non-Bt-fed wasps was lower than the subsequent generations (Table 1).

The longevity of both Bt- and non-Bt-fed offspring was lower than in the honey-fed offspring in the first generation, but they were similar to each other. In addition, we only observed differences among the generations of honey-fed wasps, in which the longevity of the first generation was significantly different from the second (Table 1).

Discussion

The present study demonstrated that the consumption of Bt pollen had little effect on the biological characteristics of the parasitoid *T. howardi* when studied over four generations, and, although the dynamics of parasitism are affected by various factors (Koller et al., 2023), we found that *T. howardi* consistently demonstrated favorable characteristics, as a potential agent for the control of Lepidopteran pests. The parasitism rate, emergence, development time, and offspring number of *T. howardi* observed in this research indicated that the nutritional quality of the food resource provided to the parasitoid females did not have any effect on the parameters of the offspring biology throughout the four generations. At beginning, we considered there was a possibility that some biological parameters of adult parasitoids could be affected, since the food supplied contained transgenic pollen, and according to some studies found in the scientific literature, it is demonstrated that Bt toxin is expressed in the pollen (Yang et al., 2022; Kenis et al., 2024; Lohn et al., 2023; Otto et al., 2023).

The number of emerged pupae of *T. howardi* was not influenced over the generations, being one hundred percent of emergence. High emergency levels are a favorable feature in mass releases, especially when associated with high parasitism level (Borase et al., 2024; Lü et al., 2024). In this sense, the study indicated that *T. howardi* had a high emergency capacity independent of the maize cultivar.

The development time of parasitoids fed with transgenic or conventional maize pollen did not show major changes, what demonstrates the quality of the host, and also that the feeding source used by the female of the parasitoid did not interfere in the biological characteristics of its descendants. The development period of this parasitoid in different hosts is variable, but in general, the cycle lasts from 14 to 17 days. The variation in the development period may be due to nutritional availability, host response and pupal size, where the parasitoid has developed (Borase et al., 2024).

The efficiency of pest control in the field depends on the proportion between the sexes, since it is the females that control insects pests in the crop. In our study, we have demonstrated that the sexual ratio is not affected by transgenic pollen throughout the generations. In *T. howardi*, a sex ratio higher than 0.87 is ideal for laboratory cultivation that is intended for release into the field (Rodrigues et al., 2013), since the lower number of females may compromise the parasitism efficiency by this species (Yan & Zhang 2023; Pereira et al., 2011).

Longevity is an important characteristic in biological control programs, since it increases the permanency of the parasitoids in field, favoring the parasitism (Kishinevsky & Ives 2024). In our research, the adult females lived 22 days in the honey treatment, which is quite interesting for biological control programs, because these parasitoids will remain for a long time in the crop. Favoreto et al. (2021) observed that the longevity of *T. howardi* females is higher than that of other parasitoids, such as *Trichogramma* sp. and *Cotesia* sp. This demonstrates that *T. howardi* females have more time to locate and parasitize the host in the field, which constitutes an important advantage, since, under field conditions, the parasitism alone may suffice to prevent the continuation of the pest's life cycle.

The fact the Bt pollen only affected some of the biological characteristics of *T. howardi* indicates that transgenic maize crops do not compromise the potential of *T. howardi* as an excellent biological control agent and confirms that transgenic plants can be used along with this IPM strategy.

4. Conclusion

The present study demonstrated that Bt maize pollen has minimal influence on the biological parameters of *Tetrastichus howardi* across successive generations. The parasitoid maintained high emergence rates, stable development time, favorable sex ratios, and adequate longevity, indicating that exposure to transgenic pollen does not compromise its performance or potential as a biological control agent of *Diatraea saccharalis*. These results support the compatibility of Bt maize cultivation with integrated pest management (IPM) strategies that include the use of *T. howardi* for sustainable pest control.

Acknowledgments

We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Financial code 001) for granting a Master scholarship to the first author.

References

Bermúdez, N. C., (2023). Biological and behavioural responses of the sugarcane borer parasitoid *Tetrastichus howardi* to insecticide residues. *Journal of Economic Entomology*. Advance online publication. https://doi.org/10.1111/jen.13165.

Borase, D. N., Chaudhary, R. S., Yadav, S. K., & Patil, S. R. (2024). Parasitizing efficiency of *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae) on Galleria mellonella larvae and pupae. *Egyptian Journal of Biological Pest Control*, 34, Article 77. https://ejbpc.springeropen.com/articles/10.1186/s41938-024-00777-5.

Bruyn, M., Nel, A. & van Niekerk, J. (2024). The nutritional benefits of maize-soybean rotational systems in the North-Western Free State, South Africa. *Agric & Food Secur*, 13, 20 (2024). https://doi.org/10.1186/s40066-024-00473-5.

Bueno, A. de F., Sutil, W. P., Cingolani, M. F., & Colmenarez, Y. C. (2024). Using Egg Parasitoids to Manage Caterpillars in Soybean and Maize: Benefits, Challenges, and Major Recommendations. *Insects*, 15(11), 869. https://doi.org/10.3390/insects15110869.

Cabello, T., Gallego, J. R., Lopez, I., Gamez, M., & Garay, J. (2024). Importance of Host Feeding in the Biological Control of Insect Pests: Case Study of Egg Parasitoid Species (Hymenoptera: Chalcidoidea: Trichogrammatidae). *Insects*, 15(7), 496. https://doi.org/10.3390/insects15070496.

Conab / Soybean & Corn Advisor. (2025). Brazil corn production estimate 2024/25. Soybean & Corn Advisor. https://www.conab.gov.br/info-agro/safras/graos

Favoreto, A.L; Pavani, R.F; Ribeiro, M.F; Zanuncio, A.J.V; Soares, M.A; Zanuncio, J.C; Wilcken, C.F (2021). *Tetrastichus howardi* (Hymenoptera: Eulophidae): first report of parasitism in *Oxydia vesulia* (Lepidoptera: Geometridae). *Braz. J. Biol*. 81(2). https://doi.org/10.1590/1519-6984.228541.

Hensley, S.D., Hammond, A.H. (1968). Laboratory techniques for rearing the sugar cane borer on an artificial diet. *Journal of Economic Entomology* 61: 1742-1743.

Holst, N., Lang, A., Lövei, G., Otto, M. (2013). Increased mortality is predicted of *Inachis io* larvae caused by Bt-maize pollen in European farmland. *Ecological Modelling* 250:126–133.

Instituto Brasileiro de Geografia e Estatística (IBGE). (2025). Previsão da safra de cereais, leguminosas e oleaginosas / Junho 2025. Agência de Notícias IBGE.

Kenis, M., Zhong, Y., Fontes, J., et al. (2024). Pre-emptive augmentative biological control of *Spodoptera frugiperda* in Europe using *Trichogramma* spp. CABI *Agriculture and Bioscience*, 5, 96. https://doi.org/10.1186/s43170-024-00296-1.

Kishinevsky, M., & Ives, A. R. (2024). Longevity of hymenopteran parasitoids in natural versus agricultural habitats and implications for biological control. *Ecological Applications*, 34(6), e3009. https://doi.org/10.1002/eap.3009.

Koller, J., Sutter, L., Gonthier, J., Collatz, J., & Norgrove, L. (2023). Entomopathogens and Parasitoids Allied in Biocontrol: A Systematic Review. *Pathogens,* 12(7), 957. https://doi.org/10.3390/pathogens12070957.

La Salle J, Polaszek A. (2007). Afrotropical species of the *Tetrastichus howardi* species group (Hymenoptera: Eulophidae). *African Entomology* 15:45–56.

Lohn, A.F., Trtikova, M., Chapela, I. et al. (2023). Effect of herbivore stress on transgene behaviour in maize crosses with different genetic backgrounds: cry1Ab transgene transcription, insecticidal protein expression and bioactivity against insect pests. *Environ Sci Eur* 35, 106. https://doi.org/10.1186/s12302-023-00815-3.

Lü, X., Qiu, R., He, X. et al. (2024). Evaluation of key factors for mass rearing the egg parasitoid *Telenomus remus* Nixon (Hymenoptera: Scelionidae). CABI *Agric Biosci* 5, 55 (2024). https://doi.org/10.1186/s43170-024-00263-w.

Oliveira, J. S., Diniz, A. J. F., & Reigada, C. (2024). Effects of interactions between the parasitoids *Tetrastichus howardi* and *Cotesia flavipes* on biological control of sugar cane borer *Diatraea saccharalis*: insights from an experimental study. *Entomologia Generalis*, 44(5), 1283–1290. https://doi.org/10.1127/entomologia/2024/2629.

Otto, M., Papastefanou, P., & Fahse, L. (2023). Pressure from insect-resistant maize on protected butterflies and moths. *Conservation Biology*, 38, e14222. https://doi.org/10.1111/cobi.14222.

Parra, J.R.P. (2007). Técnicas de criação de insetos para programa de controle biológico, 6th edn. ESALQ/FEALQ- Fundação de Estudos Agrários Luiz de Queiroz Piracicaba, Brazil.

Pereira, F.F., Zanuncio, J.C., Oliveira, H.N., Grance, E.L.V., Pastori, P.L., Gava-Oliveira, M.D. (2011). Thermal requirements and estimate number of generations of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) in different *Eucalyptus* plantations regions. *Brazilian Journal of Biology* 71, 431–436.

Pereira, A. S. et al. (2018). Metodologia da pesquisa científica. [free ebook]. Santa Maria: Editora da UFSM.

Rodrigues, M.A.T., Pereira, F.F., Kassab, S.O., Pastori, P.L., Glaeser, D.F., Oliveira, H.N., Zanuncio, J.C. (2013). Thermal requirements and generation estimate of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) in sugarcane Producing regions of Brazil. *Florida Entomologist* 96, 154–159.

Silva, F.A.S., Azevedo, C.A.V. (2016). The Assistat Software Version 7.7 and its use in the analysis of experimental data. *African Journal of Agricultural Research*. Vol 11(39), pp.3733-3740, 29 September.

Shitsuka, R. et al. (2014). Matemática fundamental para a tecnologia. (2ed). Editora Érica.

Souza, M. W. R., de Andrade, M. P., dos Santos, M. M., de Oliveira Junior, G. G. S., da Silva, R. S., Demolin Leite, G. L., Zanuncio, J. C., & Soares, M. A. (2023). Side Effects of Single-Transgene or Pyramided Genetically Modified Maize on the Generalist Endoparasitoid *Palmistichus elaeisis* (Hymenoptera: Eulophidae). *Sustainability,* 15(23), 16525. https://doi.org/10.3390/su152316525.

Souza, T. de, Sanches, A. C., Cruz, A., Prado, T. J., Savi, P. J., & Polanczyk, R. A. (2025). Mortality of *Diatraea saccharalis* is affected by the pH values of the spore suspension of *Beauveria bassiana* and *Metarhizium anisopliae*. *Revista Ceres*, 69(4), 483-487. https://ojs.ceres.ufv.br/ceres/article/view/8049.

Vargas, E.L., Pereira, F.F., Tavares, M.T., Pastori, P.L. (2011). Record of *Tetrastichus howardi* (Hymenoptera: Eulophidae) parasitizing *Diatraea* sp. (Lepidoptera: Crambidae) in surgarcane crop in Brazil. *Entomotropica* 26, 135–138.

Vieira, S. (2021). Introdução à bioestatística. Editora GEN/Guanabara Koogan.

Wang, Z.Y., Wu, Y., He, K.L., Bai, S.X. (2007). Effects of transgenic Bt maize pollen on longevity and fecundity of *Trichogramma ostriniae* in laboratory conditions. *Bulletin of Insectology* 60, 49–55.

Yan, Z., Yue, J.J., & Zhang, Y.Y. (2023). Biotic and abiotic factors that affect parasitism in *Trichogramma pintoi* (Hymenoptera: Trichogrammatidae) as a biocontrol agent against *Heortia vitessoides* (Lepidoptera: Pyralidae). *Environmental Entomology*, 52(3), 301–308. https://doi.org/10.1093/ee/nvad040.

Yang, Y., Zhao, J., Li, X., & Zhang, Z. (2022). Impact of transgenic Cry1Ab/Cry2Aj maize on abundance and community composition of non-target arthropods. *Plants*, 11(19), 2520. https://www.mdpi.com/2223-7747/11/19/2520.

Zhang, L., Wang, Y., & Liu, X. (2025). Effects of Transgenic Bt Maize on Development, Body Weight, and Parasitism Efficiency of *Trichogramma* spp. *Journal of Insect Science*, 16(5), 150. https://academic.oup.com/jinsectscience/article/16/5/150.