ECO^2-TUTA - Ecological and economic feasibility of mass production of biological agents to control tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera, Gelechiidae) in protected culture

(ACORES-01-0145-FEDER-000081)

- Final Report -

Coordinator team

António Onofre Soares

Maria Luís Oliveira

Ponta Delgada, 12 July 2023

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1. Involved Institutions

1.1. Principal Contractor

Fundação Gaspar Frutuoso (FGF) (http://www.fgf.uac.pt/)

1.2. Participating institutions associated with the researcher team (Figure 1.).

cE3c- Centre for Ecology, Evolution and Environmental Changes, Azorean Biodiversity Group, CHANGE – Global Change and Sustainability Institute, Faculty of Sciences and Technology, University of the Azores, PT-9500-321 Ponta Delgada, Portugal.

CBA – Biotechnology Centre of Azores, Faculty of Agricultural Sciences and Environment, University of the Azores, PT-9500-321 Ponta Delgada, Portugal

LEAF-Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.

Laboratório Associado TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.

CEF-Forest Research Centre, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.

Laboratoire de Lutte Biologique, Département des Sciences Biologiques, Université du Québec à Montréal (UQAM), CP 8888, succursale Centre-Ville, Montréal (Québec, Canada) H3C 3P8.

University of the Azores, School of Business and Economics, Rua da Mãe de Deus, s/n, 9501-801 Ponta Delgada, Portugal.

Business Research Unit (BRU-IUL), Avenida das Forças Armadas, 1649-026 Lisboa, Portugal.

Centre of Applied Economics Studies of the Atlantic (CEEAplA), Rua da Mãe de Deus, s/n, 9501-801 Ponta Delgada, Portugal.

Anatis Bioprotection Inc., 278 rang Saint-André, St-Jacques-le-Mineur, Quebec J0J 1Z0 Canada

Direção de Serviços de Agricultura / Serviço de Desenvolvimento Agrário de São Miguel, Quinta de São Gonçalo, 9500-343 Ponta Delgada, S. Miguel, Açores, Portugal

1.3. Research units

cE3c- Centre for Ecology, Evolution and Environmental Changes, Azorean Biodiversity Group, CHANGE – Global Change and Sustainability Institute, Faculty of Sciences and Technology, University of the Azores, PT-9500-321 Ponta Delgada, Portugal.

1.4. Host institution

Faculty of Sciences and Technology, University of the Azores.

Azorean Biodiversity Group (cE3c).

Figure 1. Brief presentation of the institutions involved in the project, as it was projected in the initial proposal. One additional institution [Business Research Unit (BRU-IUL)] was added after the integration of DR. Tiago Dutra in the research team.

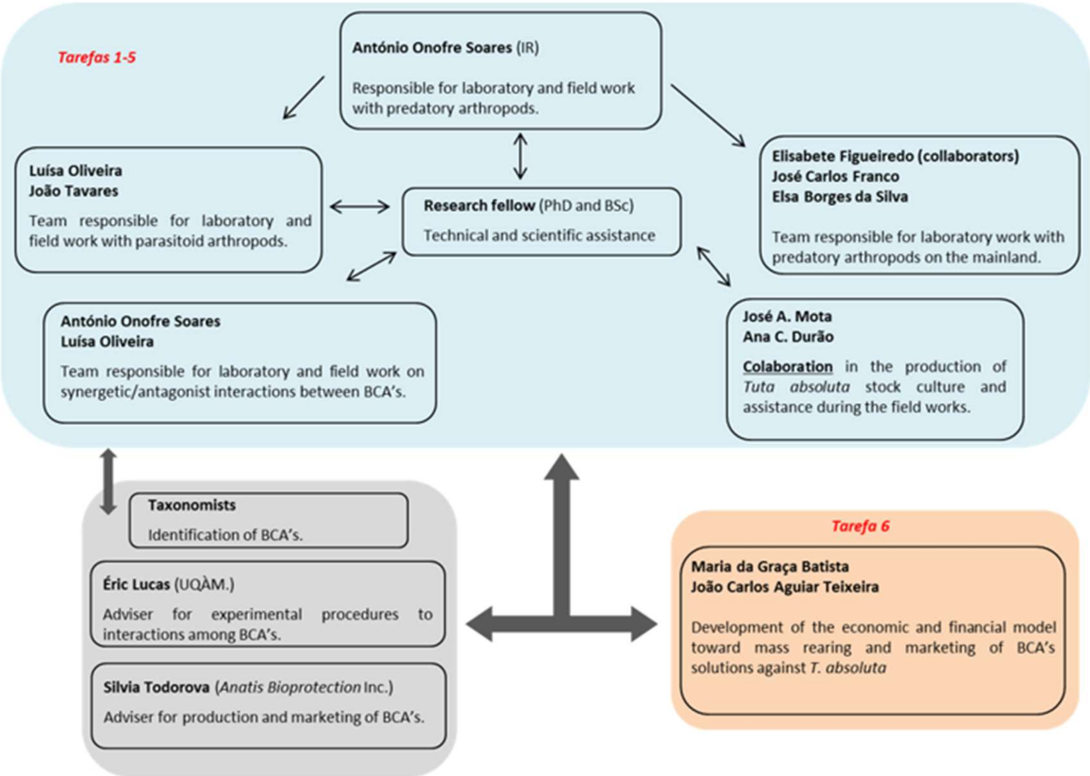

Figure 2. Chronogram of the partnership between the main tasks and participants / collaborators.

2. Participating Researchers

2.1. Main Research Team

Coordinators: António Onofre Soares[1], Luísa Oliveira[2]

Team members: Ana C. Durão[3], Francisco Barbosa[4], Elisabete Figueiredo[5,6], José Carlos Franco[6,7], Eric Lucas[8], Tiago Mota Dutra[9,10], Maria da Graça Batista[9,11], João C. A. Teixeira[9,11], Silvia Todorova[12], João Tavares[2]

Team members (Contracted): Isabel Borges[1], Patrícia Arruda[4] (Technician)

[1]cE3c- Centre for Ecology, Evolution and Environmental Changes, Azorean Biodiversity Group, CHANGE – Global Change and Sustainability Institute, Faculty of Sciences and Technology, University of the Azores, PT-9500-321 Ponta Delgada, Portugal.

[2]CBA – Biotechnology Centre of Azores, Faculty of Agricultural Sciences and Environment, University of the Azores, PT-9500-321 Ponta Delgada, Portugal

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[4]Faculty of Science and Technology, University of the Azores, 9500-321 Ponta Delgada, Portugal.

[5]LEAF-Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.

[6]Laboratório Associado TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.

[7]CEF-Forest Research Centre, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.

[8]Laboratoire de Lutte Biologique, Département des Sciences Biologiques, Université du Québec à Montréal (UQAM), CP 8888, Succursale Centre-Ville, Montréal, NA H3C 3P8, Canada.

[9]University of the Azores, School of Business and Economics, Rua da Mãe de Deus, s/n, 9501-801 Ponta Delgada, Portugal.

[10]Business Research Unit (BRU-IUL), Avenida das Forças Armadas, 1649-026 Lisboa, Portugal.

[11]Centre of Applied Economics Studies of the Atlantic (CEEAplA), Rua da Mãe de Deus, s/n, 9501-801 Ponta Delgada, Portugal.

[12]Anatis Bioprotection Inc., 278 rang Saint-André, St-Jacques-le-Mineur, Quebec J0J 1Z0 Canada.

3. Project outcomes

3.1. Main achievements

The Eco2-Tuta project has allowed to expand our knowledge of the arthropod diversity and ecosystem services provide by natural enemies on tomato crops produced in greenhouses. For most of the expected indicators, we went beyond what was expected (Table I and II). In particular, we have reached the following main achievements.

i) We present a comprehensive annotated checklist of the ladybeetle of Portugal, including the Azores and Madeira archipelagos, once several species of Coccinellidae are described as potential natural enemies of *T. absoluta*. Moreover, we suggest mitigating actions for ladybird conservation and recovery.

ii) We present data about the biodiversity of ladybeetles and their distribution and abundance in five Islands of the Azores (Faial, Graciosa, Pico, São Jorge and São Miguel). Surveys included herbaceous and arboreal habitats from native to anthropogenic-managed habitats: ruderal road vegetation, vegetable garden, mixed forest of endemic and non-native host plants, coastal prairies, coastal mixed vegetation, cornfields and urban areas. This study overcome the deficiency of information on faunal composition across the Azorean islands and their habitats, using standardized inventories.

iii) In this study, we also present an extensive checklist of selected arthropods and their distribution in five Islands of the Azores (Santa Maria. São Miguel, Terceira, Flores and Pico). Habitat surveys included five herbaceous and four arboreal habitat types, scaling up from native to anthropogenic managed habitats. Selection of Arthropoda groups for the current checklist was based on their known richness and abundance (Arachnida, Collembola, Hemiptera, Neuroptera, Coleoptera, Hymenoptera), in almost all terrestrial ecosystems, as well as their importance in current Integrated Pest Management and alternative Biocontrol protocols at large (i.e. hymenopteran parasitoids and beneficial Coleoptera).

iv) We compare the abundance of *T. absoluta* (eggs and larvae) and its native natural enemies, in greenhouses of tomato crops produced under different production modes (biological, traditional and intensive). Considering the major concerns of the growers by the infestation levels of their crops, together with low abundance of native natural enemies, we suggest the use of biological production mode or an inoculative or augmentative strategy to control the pest in intensive and traditional productions modes.

v) We contrasted the life-history traits and population growth parameters of two feral populations of *M. pygmaeus*, one from Portugal mainland and one from the Azores archipelago. The predators were tested on single prey diet, either of *Ephestia kuehniella* eggs, a factitious prey used for mass rearing of mirids, or *T. absoluta* eggs. We predicted that populations would express differences in its phenotypic characteristics, with the Azorean population displaying low performance due to likely low genetic diversity, as expected for insular populations. Our results revealed the inexistence of phenotypic differences in several life history traits, such as immature developmental time, female longevity, males' body weight and sex ratio. However, Azorean *M. pygmaeus* females were larger, matured earlier and reproduced at a higher rate for longer periods, than mainland females.

vi) We evaluated, under laboratory and semi-field conditions, the voracity and feeding preference of *M. pygmaeus* females provided with mixed diets of *T. absoluta* eggs unparasitized and parasitized by T. achaeae and the effect of competitive and intraguild interactions between *M. pygmaeus* and *T. achaeae* on the number of *T. absoluta* eggs consumed and/or parasitized. *Macrolophus pygmaeus* prefers unparasitized eggs of *T. absoluta* but inflicts intraguild predation on *T. achaeae*. In conspecific experiments, mutual interference between *M. pygmaeus* predators intensifies as the number of individuals increases, but for *T. achaeae*, it occurs in an unpredictable manner. Adding *T. achaeae* could significantly increase the level of control of *T. absoluta* compared to what could be achieved when only *M. pygmaeus* is present in greenhouse tomatoes.

vii) We assessed and compared the voracity, weight gain and conversion efficiency in females of two populations of *M. pygmaeus*, one from Portugal mainland and the other from the Azores, fed on single prey diets of *E. kuehniella* eggs, or *T. absoluta*.

viii) We developed an economic and financial model to the mass-rearing of *M. pygmaeus*, a biological control agent against the tomato moth T*. absoluta* in protected culture. Our results are in line with (i) the more recent European Commission proposals for a new Regulation on sustainable use of plant protection products, which includes the reduction of 50% the use and risk of chemical pesticides by 2030 and (ii) most of the existing literature which conclude that new projects on BCA production are worth investments.

Table I. A comparison between the Expected and Realized Indicator are as follows (* New Indicators):

Indicators	Expected	Realized
A – Publications		
A1 – Articles in international journals	5	13*
A2 - Articles in national journals	2	0**
B – Oral presentations in international scientific meetings	2	12
C – Reports	3	3
D – Organization of scientific meetings	2	2
E – Education (PhD; MSc)	2	2***
F – Models	1	1
G – Pilot facilities	1	1

* 4 manuscripts are in preparation.

** The articles planned to be published in national journals (2) were published in international journals with an impact factor. They are articles 01 and 03.

*** The two master's theses are still ongoing.

Table II. Achievement indicators not planned but realized.

Indicators	Expected	Realized
Organization of outreach activities	0	3
Poster presentations in outreach activities	0	3
Poster presentations at international scientific meetings	0	2
Publication of datasets on GBIF	0	2
News published in the media related to the execution of the project.	0	3

3.1.1. Brief summary of the project's impact

With the ECO^2-TUTA project, we gained a deeper knowledge on the population dynamics of *T. absoluta* and their natural enemies in protected cultures of tomato crops in intensive, traditional and organic production modes. Additional surveys on other islands, provided a comprehensive list of arthropod species with potential to be used in futures biological control programs. The extended comparation of life-history and population growth parameters of feral populations of *M. pygmaeus*, one from Portugal mainland and one from the Azores archipelago fed on single prey diet, either of *E. kuehniella* eggs, a factitious prey used for mass rearing of mirids, or *T. absoluta* eggs, together with the results of the semi-field tests carried out in the greenhouses, allowed (i) to optimize the mass-rearing conditions of natural enemies and (ii) to better predict the efficacity of *M. pygmaeus* (with and without additional natural enemies) under natural conditions.

We highlight the positive socio-economic contribution of the project. The project can give rise to the establishment of the first bio-factory in Portugal, with predictable benefits, such as the creation of jobs. The initial project approved by the PO-Açores, only envisaged the study and development of an economic and financial model for its implementation. However, the ECO^2-TUTA project managed to go further than expected and, we already developed its financing framework toward making it a reality.

3.2. Future strategy, direction, and questions

Pupae of *T. absoluta* spend a part of life cycle in the soil, but till today, no studies are available on the potential of soil epigean arthropods with the potential to control the species when is in the soil. A recent study, for Terceira Island, already recorded some species providing this ecosystem services (BORGES et al., 2022).

The assessment of the taxonomical and functional biodiversity of soil arthropods and its generalist predators' potential role in the biological management control of pest complexes species requires as well as measuring the predation range of these animals, are important steps in conservation biological control. An accurate estimation of diet is crucial to determine realistic estimates of prey consumption and potential impact of predators on prey dynamics. Presently, the most common method of reconstructing insect predator diets involves morphological identification of prey hard parts or prey fragments into the stomach of the predators is often difficult due to damage during capture, ingestion and digestion by predators. Moreover, can be laborious and time-consuming from, a logistical standpoint, particularly in dynamic vegetable growing systems where both prey and predators have brief life cycles and are of small size. The development of new techniques such as the use of molecular genetic analyses provides alternate means of assessing the diet of predators. NGS approaches, have been used to assess a more comprehensive understanding of the specific types of arthropods and plants targeted having a clearer view of their food webs (ROY et al., 2021; BATUECAS et al. 2022; SAQIB et al., 2022). Nonetheless, specialized laboratory equipment and personnel are required, and the budget needed to process the data makes it impractical in many research settings. Targeted molecular methods, such as conventional PCR (PCR) and quantitative PCR (qPCR), can provide an effective and shipper way to detect prey from stomach contents of predators. The combination of target species-specific primers with PCR-based methods provides greater detection than metabarcoding because they only target the species of interest. Besides, the potential of qPCR to quantify DNA in samples makes it a valuable molecular tool to infer the predation levels on specific prey by analyzing predators' stools or stomach contents. Molecular tools could help in the establishment of novel pest control approaches for pests with a high rate of propagation and expansion, as in the example of *T. absoluta*. Choosing PCR strategy will involve a trade-off between the financial costs, logistical feasibility, and the risk of inaccurate results.

References:

BATUECAS I, ALOMAR, CASTAÑE C, et al (2022) Development of a multiprimer metabarcoding approach to understanding trophic interactions in agroecosystems. Insect Science 29:1195–1210.

BORGES, P.A.V., LAMELAS-LÓPEZ, L., FERRANTE, M., MONJARDINO, P., LOPES, D.H., SORES, A.O., GIL, A., NUNES, R., GABRIEL, R., ARROZ, A.M., RIGAL, F., BACHER, S. & LOVEI, G.L. (2022). Guia Prático da Fauna de Artrópodes Predadores Ecossistemas Agrícolas dos Açores. Universidade dos Açores, Angra do Heroísmo.

ROY, K., EWING, C.P., PRICE, D.K. (2021) Diet Analysis of Hawai‘i Island’s *Blackburnia hawaiiensis* (Coleoptera: Carabidae) Using Stable Isotopes and High-Throughput Sequencing1. Pacific Science 74, 3:245–256.

SAQIB, H.S.A., SUN, L., POZSGAI, G., et al (2022) DNA metabarcoding of gut contents reveals key habitat and seasonal drivers of trophic networks involving generalist predators in agricultural landscapes. Pest Management Science 78:5390–5401.

3.3. Webpages

ECO2 – TUTA webpage:

https://ce3c.ciencias.ulisboa.pt/research/projects/ver.php?id=202

3.4. Publications

3.4.1. Papers already published or *in press* in international journals with impact factor.

1. Soares, A.O., H.R. Calado, J.C. Franco, A.F. Aguiar, M.M. Andrade, V. Zina, O.M.C.C. Ameixa, I. Borges & A. Magro (2021). An annotated checklist of ladybeetle species (Coleoptera: Coccinellidae) of Portugal, including the Azores and Madeira Archipelagos. *Zookeys*, 1053: 107–144. doi: 10.3897/zookeys.1053.64268. **Q2 Zoology. Fator de impacto (*JRS©2021*): 1.492.**

   Main achievement: Detailed updated list of ladybird species collected in Portugal with potential use as natural enemies of agricultural pests.

2. Marcelino, J.A.P., P.A.V. Borges, I. Borges, E. Pereira, V. Santos & A.O. Soares (2021). Standardized arthropod (Arthropoda) inventory across natural and anthropogenic impacted habitats in the Azores archipelago. *Biodiversity Data Journal*, 9: e62157. doi: 10.3897/BDJ.9.e62157. **Q3 Biodiversity and Conservation. Fator de impacto (*JRS©2021*): 1.540.**

   Main achievement: Detailed list of arthropod species collected in several Azorean habitats with potential use as natural enemies of agricultural pests.

3. Soares, A.O., I. Borges H.R. Calado & P.A.V. Borges (2021). An updated checklist to the biodiversity data of ladybeetles (Coleoptera: Coccinellidae) of the Azores archipelago (Portugal). Biodiversity Data Journal, 9: e77464. https://doi.org/10.3897/BDJ.9.e77464. **Q3 Biodiversity and Conservation. Fator de impacto (*JRS©2021*): 1.540.**

Main achievement: Detailed updated list of ladybird species across the Azores with potential use as natural enemies of agricultural pests.

4. Soares, A.O., D. Haelewaters, O.M.C.C. Ameixa, I. Borges, P.M.J. Brown, P. Cardoso, M.D. de Groot, E.W. Evans, A.A. Grez, A. Hochkirch, M. Holecová, A. Honěk, J. Kulfan, A.I. Lillebø, Z. Martinková, J.P. Michaud, O. Nedvěd, Omkar, H.E. Roy, S. Saxena, A. Shandilya, A. Sentis, J. Skuhrovec, S. Viglášová, P. Zach, T. Zaviezo & J.E. Losey (2023). A roadmap for ladybird conservation and recovery. *Conservation Biology*, DOI: 10.1111/cobi.13965. **Q1 Ecology. Fator de impacto (JRS©2022): 6.3.**

   Main achievement: We suggest mitigating actions for ladybird conservation and recovery.

5. Oliveira, L., I. Borges, D. Silva, A.C. Durão & A.O. Soares (2023). Abundance of *Tuta absoluta* (Meyrick, 1917) and its natural enemies on tomato crops in greenhouses of different production modes (Azores, Portugal) (Lepidoptera: Gelechiidae). SHILAP Revista de lepidopterologia, 51 (201): 59-70. https://doi.org/10.57065/shilap.437. **Q4 Entomology. Fator de impacto (JRS©2022): 0.3.**

   Main achievement: Two-year study of population dynamics *T. absoluta* and its natural enemies recorded in Azorean tomato crops greenhouses from different production modes.

6. Borges, I., L. Oliveira, A.C. Durão, P. Arruda, E. Figueiredo, J.C. Franco, E. Lucas, A.O. Soares (2023). Contrasting phenotypic variability of life-history traits of two feral populations of *Macrolophus pygmaeus* (Hemiptera: Miridae) under two alternative diets. *Agronomy*, 13, 118. https://doi.org/10.3390/agronomy13010118. **Q1 Agronomy. Fator de impacto (*JRS©2022*): 3,7.**

Main achievement: We found differences in life-history traits and population growth parameters in populations of M. pygmaeus from Portugal mainland and Azores archipelago.

7. Dutra, T.M., Batista, M.G., Teixeira, J.C.A., Todorova, S. Oliveira, L., Tavares, J., Borges, I., **Soares, A.O.** (available online). Economic and financial model to the mass-rearing of *Macrolophus pygmaeus* (Rambur) (Heteroptera: Miridae), a biological control agent against the tomato moth *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in protected culture. *Pest Management Science*, https://onlinelibrary.wiley.com/doi/full/10.1002/ps.7552. **Q1 Entomology. Fator de impacto (*JRS©2022*): 4.1.**

Main achievement: Our results are in line with most of the existing literature which conclude that new projects on biological control agents production are worth investments.

8. Borges, I., L. Oliveira, A. C. Durão, P. Arruda & A. O. Soares (available online). Feeding preference and intraguild interactions between the parasitoid *Trichogramma achaeae* and the predator *Macrolophus pygmaeus*, two biological agents of *Tuta absoluta*. *Pest Management Science*. DOI: 10.1002/ps.7635. **Q1 Entomology. Fator de impacto (JRS©2022): 4.1.**

Main achievement: *Macrolophus pygmaeus* prefers unparasitized eggs of *T. absoluta* but inflicts intraguild predation on *T. achaeae*. Despite inter and intra-guild antagonistic interactions, the simultaneous use of these natural enemies are not incompatible.

Under review

9. Borges, I., L. Oliveira, F. Barbosa, E. Figueiredo, J.C. Franco, A.C. Durão & A.O. Soares (Submitted). Voracity and conversion efficiency in females of two feral populations of *Macrolophus pygmaeus*, a biocontrol agent of *Tuta absoluta*. *Phytoparasitica*. **Q2 Plant Science. Fator de impacto (*JRS©2022*): 1.4.**

In preparation

10. Borges, I., L. Oliveira, A. C. Durão & A. O. Soares (in prep.). *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs as a surrogate host to test feeding preference and intraguid interactions between and *Macrolophus pygmaeus* (Hemiptera: Miridae) and *Trichogramma achaeae* (Hymenoptera: Trichogrammatidae).

11. Borges, I., L. Oliveira, E. Figueiredo, J.C. Franco & A.O. Soares (in prep.). Contrasting genetic variability feral populations of *Macrolophus pygmaeus* (Hemiptera: Miridae) in Mediterranen area.

12. Franco, C., I. Borges, L. Oliveira, M. Brum, R. Calado, P.A.V. Borges & A. O. Soares (in prep.). Monitoring epigeal arthropods in greenhouses of tomato crops in Azorean Agroecosystems: the project ECO^2-TUTA.

13. Franco, C., I. Borges, L. Oliveira, M. Brum, D. Toubarro, P.A.V. Borges & A. O. Soares (in prep.). Interactions of arthropod soil predators with *T. absoluta* by using molecular tools like PCR and quantitative PCR.

Figure 3. Summary listing of articles published/submitted/in preparation, and its position by quartiles (Q_n) according to the ranking of impact factors.

3.5. Oral presentations in international scientific meetings

3.5.1 International

“XIX Congresso Ibérico de Entomologia” (online), 21-24 September 2021, Coimbra, Portugal **(Attached: abstract published in the book of abstracts and first slide with the acknowledgement to funding).**

Borges, I., L. Oliveira & A.O. Soares (2021). Suitability of *Tuta absoluta* (Lepidoptera: Gelechiidae) and *Ephestia kuehniella* (Lepidoptera: Pyralidae) eggs for the predator *Macrolophus pygmaeus* (Hemiptera: Miridae). “XIX Congresso Ibérico de Entomologia: os Insetos e o Homem” (online), 21-24 de setembro. Livro de resumos. Coimbra, Portugal. 209pp. (Oral Presentation)

Suitability of *Tuta absoluta* (Lepidoptera: Gelechiidae) and *Ephestia kuehniella* (Lepidoptera: Pyralidae) eggs for the predator *Macrolophus pygmaeus* (Hemiptera: Miridae).

Isabel Borges [(1)]; Luísa Oliveira [(2)]; António Onofre Soares [(1)]

1 - Centre for Ecology, Evolution and Environmental Changes and Azorean Biodiversity Group, Faculty of Sciences and Technology, University of the Azores, Portugal; 2 - Biotechnology Centre of Azores (CBA), University of the Azores, Portugal

Tuta absoluta (Meyrick, 1917) (Lepidoptera: Gelechiidae) is a major pest attacking tomato that may lead to 60-100% of crop losses. This invasive pest species native to South America landed in Europe in 2006, in Spain, and 3 years later it spread to Portugal. In 2012, it was recorded for the first time in the Azores archipelago. *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) is a predator used as biological control agent against the tomato leaf miner. The biological performance of a feral population of the Azores was studied on single diets of a potential natural prey (*T. absoluta* eggs) and of a substitute prey (*Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs) used for predator mass rearing in biofactories. Except for fecundity, both food regimes seem equally suitable for immature and adult traits. *Macrolophus pygmaeus* eggs took 11.5±0.1 days to hatch and nymphal development was completed in approximately 18 days for both diets with high survival rate. To complete development 239.6±7.7 T. absoluta eggs were consumed whereas when fed *E. kuehniella*, 147.25±4.6 eggs were preyed. The diet did not influence female and male weights nor the pre-oviposition time but on *E. kuehniella* the predator females laid less eggs than on *T. absoluta* (91.6±12.9 eggs during 27.8±2.5 days vs. 140.8±9.6 eggs in 28.3±2.7 days). On the other hand, adult females lived longer on *E. kuehniella* than on *T. absoluta* (52.3±5.1 vs. 42.8±6.3 days). Biological data obtained were used to calculate the population growth parameters for each diet, as well.

Contrarily to what is documented in the bibliography, the biological performance of *M. pygmaeus* fed the tomato leaf miner eggs may represent a rapid shift in the mirid predator food range in the Azores archipelago.

Palavras chave: Biological control; *Ephestia kuehniella*; *Macrolophus pygmaeus*; Tomato pest; *Tuta absoluta*

“XXVI International Congress of Entomology”, 17-22 de July, 2022, Helsinki, Finland **(Attached: abstract published in the book of abstracts, photo of presenting author and slide with the acknowledgement to funding).**

Soares, A.O., D. Haelewaters, O.M.C.C. Ameixa, I. Borges, P.M.J. Brown, P. Cardoso, M.D. de Groot, E.W. Evans, A.A. Grez, A. Hochkirch, M. Holecová, A. Honěk, J. Kulfan, A.I. Lillebø, Z. Martinková, J.P. Michaud, O. Nedvěd, Omkar, H.E. Roy, S. Saxena, A. Shandilya, A. Sentis, J. Skuhrovec, S. Viglášová, P. Zach, T. Zaviezo & J.E. Losey (2022). Assessing the conservation status of ladybirds: A roadmap towards conservation. “*XXVI International Congress of Entomology*”, 17-22 de julho, Helsinquía, Finlândia. Livro do programa, 122pp (abstract virtually presented in the congress app). (Oral presentation).

ICE 2022 HELSINKI

Entomology for our planet

XXVI International Congress of Entomology

HELSINKI, FINLAND, JULY 17–22, 2022

Daily schedules

10:45 **1300** Early establishment of the omnivorous predator Nesidiocoris tenuis increased uptake of biological control in tomato. Is it an alternative for others? **Francisco Javier Calvo**[1], Jesús Moreno[1] and José David Soriano[1], [1]R&D Koppert Biological Systems

11:00 **1301** Zoophytophagous predator-induced defences restrict infection of plant viruses. **Alberto Urbaneja**[1], Luis Rubio[1], Meritxell Pérez-Hedo[1] and Sarra Bouagga[1], [1]Instituto Valenciano de Investigaciones Agrarias (IVIA). Valencia, Spain

11:15 **1302** Can artificial diets support cost-effective mass production of omnivorous predators? **Patrick De Clercq**, Laboratory

10:15-12:15

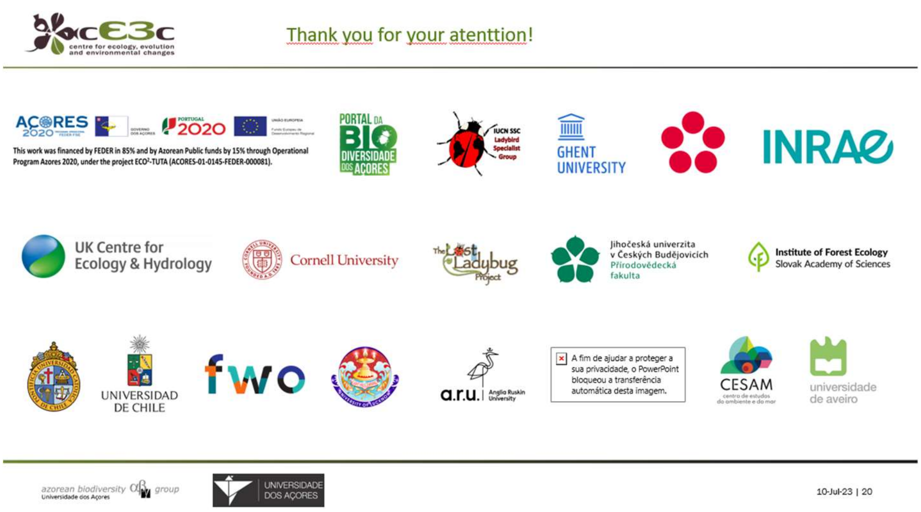
Conservation of ladybirds

Room 208

Moderators and Organizers: Danny Haelewaters, Ghent University

10:15 **1076** Assessing the conservation status of ladybirds: A roadmap towards conservation. **António Onofre Soares**[1], Ahmad Pervez, Danny Haelewaters et al, [1]CE3C, Centre for Ecology, Evolution and Environmental Changes, Azorean Biodiversity Group, Faculty of Sciences and Technology, University of the Azores, Portugal


cE3c
centre for ecology, evolution and environmental changes
IUCN SSC Ladybird Specialist Group
A roadmap for ladybird conservation and recovery
António O. Soares, Danny Haelewaters, Olga M.C.C. Ameixa, Isabel Borges, Peter M.J. Brown, Pedro Cardoso, Michiel D. de Groot, Edward W. Evans, Audrey A. Grez, Axel Hochkirch, Milada Holecová, Alois Honek, Ján Kulfan, Ana I. Lillebø, Zdenka Martinkova, J.P. Michaud, Oldřich Nedvěd, Omkar, Helen E. Roy, Swati Saxena, Apoorva Shandilya, Arnaud Sentis, Jiri Skuhrovec, Sandra Viglášová, Peter Zach, Tania Zaviezo and John E. Losey
azorean biodiversity group
Universidade dos Açores
UNIVERSIDADE DOS AÇORES
10-Jul-23 | 1


cE3c
centre for ecology, evolution and environmental changes
Thank you for your atenttion!
AÇORES 2020
PORTUGAL 2020
This work was financed by FEDER in 85% and by Azorean Public funds by 15% through Operational Program Azores 2020, under the project ECO²-TUTA (ACORES-01-0145-FEDER-000081).
PORTAL DA BIODIVERSIDADE DOS AÇORES
IUCN SSC Ladybird Specialist Group
GHENT UNIVERSITY
INRAE
UK Centre for Ecology & Hydrology
Cornell University
The Lost Ladybug Project
Jihočeská univerzita v Českých Budějovicích
Přírodovědecká fakulta
Institute of Forest Ecology
Slovak Academy of Sciences
UNIVERSIDAD DE CHILE
fwo
a.r.u. Anglia Ruskin University
A fim de ajudar a proteger a sua privacidade, o PowerPoint bloqueou a transferência automática desta imagem.
CESAM
universidade de aveiro
azorean biodiversity group
Universidade dos Açores
UNIVERSIDADE DOS AÇORES
10-Jul-23 | 20

3.5.2 National

Workshop "*ECO²-TUTA - Ecological and economic feasibility of mass production of biological agents to control tomato moth, Tuta absoluta (Meyrick) (Lepidoptera, Gelechiidae) in protected culture: Workshop 1 – Experimental Design and Planning of Laboratory and Field Work*", 27-28 June 2019, Ponta Delgada, Portugal.

Note: **We include the program and photos of the event.**

Soares, A.O. (2019). General Presentation of the Project. (Oral presentation).

Oliveira, M.L. (2019). What do we already know about *Tuta absoluta* in the Azores? (Oral presentation).

Figueiredo, E. (2019). What do we already know about *Tuta absoluta* in Portugal mainland? (Oral presentation).

Mota, J.A. & A.C. Durão (2019). The tomato production in the Azores. (Oral presentation).

Tordorova, S. (2019). Transferring science into business: the example of biofactories. (Oral presentation).

Teixeira, J. & M.G. Batista (2019). Economic feasibility of launching the biofactory in the Azores. (Oral presentation).

Participação no Encontro "*9th Frontiers in E3, cE3c's Annual Meeting*", Organizado pelo Center of Ecology, Evolution and Environmental Changes, 7-9 de junho, Lisboa, Portugal e no qual apresentou a seguinte comunicação oral:

Soares, A.O., L. Oliveira, I. Borges, D. Silva, A. C. Durão, P. Arruda, F. Barbosa, E. Figueiredo, J. C. Franco, E. Lucas, T. M. Dutra, M. G. Batista, João C. A. Teixeira, S. Todorova & J. Tavares (2023). From research to business: sharing some deliveries of the ECO^2-Tuta Project.

ECO²-TUTA - Ecological and economic feasibility of mass production of biological agents to control tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera, Gelechiidae) in protected culture

(ACORES-01-0145-FEDER-000081)

Workshop 1 – Experimental Design and Planning of Laboratory and Field Work

27-28 June 2019- Ponta Delgada

Venue: Faculty of Science and Technology, University of the Azores, Campus of Ponta Delgada

Meeting Room: A. 022

Coordinating Team:

António Onofre Soares

Maria Luís Oliveira

Ponta Delgada, 19 June 2019

Timeline

27 June 2019 - Thursday

9h00 – Informal meeting with presentation of project researchers.

9h30 – General Presentation of the Project (**António Onofre Soares**).

10h00 – What do we already know about *Tuta absoluta* in the Azores? (**Maria Luísa Oliveira**)

10h30 - Coffee Break

10h45 - What do we already know about *Tuta absoluta* in Portugal mainland? (**Elisabete Figueiredo**)

11h15 – The tomato production in the Azores (**José Adriano Mota and Catarina Durão**)

12h00 – Lunch

13h30 – 15h00

Visit to field stations in agroecosystems (**Guided by José Adriano Mota and Catarina Durão**)

15h00-15h15 – Coffee Break

15h15 – Group Photo

15h30– Transferring scientific into business: the example of biofactories (**Silvia Tordorova**)

16h00 – Economic feasibility of launching the biofactory in the Azores (**João Teixeira and Maria da Graça Batista**)

16h00 – 17h30 - Discussion about the experimental design (**Guided by João Teixeira and Maria da Graça Batista**)

AÇORES 2020 PROGRAMA OPERACIONAL FEDER FSE
GOVERNO DOS AÇORES
PORTUGAL 2020
UNIÃO EUROPEIA
Fundo Europeu de Desenvolvimento Regional

28 June 2019 - Friday

9h00 – Discussion about the experimental design (**Guided by Elisabete Figueiredo, Elsa Silva and José Carlos Franco**)

10h00 – Discussion about the experimental design (**Guided by António Onofre Soares, Isabel Borges and Éric Lucas**)

11h00 – 11h15-– Coffee Break

11h15- Discussion about the experimental design (**Guided by António Onofre Soares and Maria Luísa Oliveira**)

12h15 – Lunch

14h00 – 15h00

-Publication strategy

-Logistics of the project

Meeting financing: This work was financed by FEDER in 85% and by Azorean Public funds by 15% through Operational Program Azores 2020, under the project ECO^2-TUTA (ACORES-01-0145-FEDER-000081).

“ECO2-TUTA - Ecological and economic feasibility of mass production of biological agents to control tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera, Gelechiidae) in protected culture: Status of the Project Development”, 13 October 2021, Ponta Delgada, Portugal

Note: We include the program and photos of the event.

Borges, I., L. Oliveira & A.O. Soares (2021). Suitability of *Tuta absoluta* (Lepidoptera: Gelechiidae) and *Ephestia kuehniella* (Lepidoptera: Pyralidae) eggs for the predator *Macrolophus pygmaeus* (Hemiptera: Miridae). (Oral presentation)

Oliveira, L., I. Borges, D. Silva & A.O. Soares (2021). Abundance of *Tuta absoluta* (Lepidoptera: Gelechiidae) and its natural enemies on tomato crops of different production modes. (Oral presentation).

Soares, A.O. & L. Oliveira (2021) Ecological and economic feasibility of mass production of biological agents to control tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera, Gelechiidae) in protected culture: presentation of the project. (Oral presentation).

ECO^2-TUTA - Ecological and economic feasibility of mass production of biological agents to control tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera, Gelechiidae) in protected culture

(ACORES-01-0145-FEDER-000081)

Workshop 2 – Status of the Project Development

13 october 2021- Ponta Delgada

Venue: **Serviço de Desenvolvimento Agrário de São Miguel**

Meeting Room: **Sala Polivalente**

Organizing Committee

António Onofre Soares

Maria Luís Oliveira

Isabel Borges

Catarina Durão

Timeline

9h30 - Opening Ceremony (Hosted by Engº **António Ventura,** Secretário Regional da Agricultura e do Desenvolvimento Rural).

9h45 - The tomato production in the Azores (**António Cordeiro**)

10h00 - Sustainable tomato production in greenhouse - Case of study in the SDASM (**Catarina Durão**)

10h15 - General presentation of the ECO^2-TUTA project (**António Onofre Soares, Maria Luísa Oliveira**).

10h30 - What do we already know about *Tuta absoluta* in the Azores? (**Dário Silva**)

10h45 - Biology and ecology of native biological control agents *Tuta absoluta* (**Isabel Borges**)

11h00 - Development of an economic feasibility model toward mass rearing and marketing of biological control agents solutions against *Tuta absoluta* (**Maria da Graça Batista, João Teixeira, Tiago Dutra**)

11h15 – Discussion

Acknowledgments

Meeting financing: This work was financed by FEDER in 85% and by Azorean Public funds by 15% through Operational Program Azores 2020, under the project ECO^2-TUTA (ACORES-01-0145-FEDER-000081).

3.6. Reports

These reports, which are attached and were sent to the FGF, correspond to the 3 progress reports.

3.7. Organization of scientific meetings

Workshop "*ECO^2-TUTA - Ecological and economic feasibility of mass production of biological agents to control tomato moth, Tuta absoluta (Meyrick) (Lepidoptera, Gelechiidae) in protected culture: Workshop 1 – Experimental Design and Planning of Laboratory and Field Work*", 27-28 June 2019, Ponta Delgada, Portugal.

Workshop "*ECO^2-TUTA - Ecological and economic feasibility of mass production of biological agents to control tomato moth, Tuta absoluta (Meyrick) (Lepidoptera, Gelechiidae) in protected culture: Status of the Project Development*", 13 October 2021, Ponta Delgada, Portugal.

Note: We include in this report the program and photos of the event.

3.8. Students theses (ongoing)

2022 Cecília de Jesus Soares Franco. "Effect of tomato agricultural production systems on the biodiversity of epigeal arthropod communities".

2019 Orientador do plano de trabalho do Licenciado Dário Miguel Gonçalves Silva, intitulado "Avaliação do potencial de *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) e *Trichogramma achaeae* Nagaraja and Nagarkatti (Hymenptera: Trichogrammatidae) no controlo de *Tuta absoluta* (Meyrick (Lepidoptera: Gelechiidae) na cultura do tomate em estufa".

3.6. Models

Dutra, T.M., Batista, M.G., Teixeira, J.C.A., Todorova, S. Oliveira, L., Tavares, J., Borges, I., **Soares, A.O.** (accepted). Economic and financial model to the mass-rearing of *Macrolophus pygmaeus* (Rambur) (Heteroptera: Miridae), a biological control agent against the tomato moth *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in protected culture. *Pest Management Science*, https://onlinelibrary.wiley.com/doi/full/10.1002/ps.7552. **Q1 Entomology. Fator de impacto (*JRS©2022*): 4.1.**

Link: https://onlinelibrary.wiley.com/doi/full/10.1002/ps.7552

Research Article

Received: 17 November 2022 Revised: 21 March 2023 Accepted article published: 11 May 2023 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/ps.7552

Economic and financial model to the mass-rearing of *Macrolophus pygmaeus* (Rambur) (Heteroptera: Miridae), a biological control agent against the tomato moth *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in protected culture

Tiago Mota Dutra,[a,b] Maria da Graça Batista,[a,c] João CA Teixeira,[a,c] Silvia Todorova,[d] Luísa Oliveira,[e] João Tavares,[e] Isabel Borges[f] and António Onofre Soares[f*]

3.10. Material acquired under the project

USB Datalogger for Temperature e Humidity

3.11. Pilot facilities

The document transcribed below is a "**Descriptive Memorandum**" prepared by Doctor Isabel Borges and submitted to the financing and municipal entities of the project to install the biofactory to mass reared agents for the biological control of *T. absoluta*. It should be noted that Doctor Isabel Borges was the element hired within the scope of ECO^2-Tuta. The document transcribed below presents the aforementioned project with information that includes:

1. TITLE
2. PROJECT FRAMEWORK
3. FINANCING JUSTIFICATION
4. DESCRIPTION OF ACTIVITIES TO BE DEVELOPED
5. TECHNICAL AND PHYSICAL MEANS NECESSARY TO PROPERLY PERFORM THE PROVIDED ACTIVITIES
6. CHARACTERIZATION OF THE TARGET POPULATION AND ITS INVOLVEMENT IN THE PROJECT
7. PROJECT DISSEMINATION AND PROMOTION PLAN
8. PHASES AND SCHEDULE OF ACTIVITIES.
9. PROJECT SUSTAINABILITY
10. PROJECT COORDINATOR
11. EXPECTED IMPACT
12. ESTIMATED BUDGET
13. ESTIMATED INVESTMENTS

MEMÓRIA DESCRITIVA

Instalação de biofábrica de produção de insetos para utilização como agentes de controlo biológico

Isabel Marisa Mateus Borges

(PhD)

1. Título

Instalação de biofábrica de produção de insetos para utilização como agentes de controlo biológico.

2. Enquadramento do projeto

A sustentabilidade dos ecossistemas em geral, e dos agro-ecossistemas em particular, bem como a segurança alimentar estão na ordem do dia. Os efeitos negativos dos pesticidas utilizados no combate a insetos pragas na agricultura e silvicultura na saúde humana e no ambiente levaram à retirada do mercado de diversas substâncias ativas, pelo que é urgente encontrar meios alternativos, mais ecológicos, para o seu controlo. A diretiva comunitária 2009/128/CE DO PARLAMENTO EUROPEU E DO CONSELHO de 21 de Outubro de 2009 obriga a adoção dos princípios da Proteção Integrada com vista à redução do recurso à luta química, sendo o controlo biológico uma alternativa ecológica, amiga do ambiente, e considerado pela FAO um meio para atingir o seu objetivo de Desenvolvimento Sustentável 15. Para além disso, a presente ideia de negócio poderá contribuir com novas soluções para a Agricultura biológica que se pretende promover nos Açores, enquadrando-se nos eixos 2 e 3 da Estratégia para o Desenvolvimento da Agricultura Biológica e Plano de Ação para a Produção e Promoção de Produtos Biológicos na Região Autónoma dos Açores.

A implementação da empresa surge na sequência do projeto de investigação, ECO2TUTA - *Avaliação da viabilidade ecológica e económica da produção em massa de agentes biológicos para combate à traça-do-tomateiro, Tuta absoluta (Meyrick) (Lepidoptera, Gelechiidae), em cultura protegida, nos Açores*, código da operação ACORES-01-0145-FEDER- 000081, financiado pelo Governo Regional dos Açores e pelo FEDER no âmbito do PO2020, após o previsto estudo de viabilidade económica apontar para boas perspetivas de negócio.

A escolha específica de *M. pygmaeus* como inseto predador da lagarta mineira do tomateiro como primeiro produto da empresa não é arbitrária. Portugal é o maior produtor de tomate para indústria e um dos principais produtores de tomate para consumo em fresco. Com as restrições impostas pela Comunidade Europeia nos pesticidas autorizados, e por outro lado, a obrigatoriedade da implementação dos princípios da Proteção Integrada, os produtores de tomate deparam-se com a falta de soluções para controlar a lagarta mineira do tomateiro, uma praga altamente nociva que invadiu Portugal em 2009 e, mais recentemente, chegou aos Açores em 2012. *Macrolophus pygmaeus* tem sido o predador mais utilizado para combater a traça do tomateiro pelo que há um nicho de mercado com grande potencial.

Como projeto regional, é de salientar a valorização dos recursos naturais e da Biodiversidade dos Açores, sendo utilizados indivíduos nativos para produção em massa. Todo o ciclo de produção do auxiliar biológico será feito na empresa, pelo que não haverá dependência de terceiros em nenhuma fase do processo.

À parte o objetivo principal de comercialização de agentes de controlo biológico, a empresa de biotecnologia poderá alargar a sua área de intervenção à formação avançada (Mestrados e Doutoramentos em contexto empresarial), projetos de investigação e colaboração com entidades do governo regional. Em alinhamento com a sua missão ecológica, a empresa está comprometida com a sustentabilidade recorrendo a painéis solares, recolha de água das chuvas e compostagem de material vegetal com vista à minimização da sua pegada carbónica.

3. Justificação de financiamento

A especificidade técnico-científica do projeto requer a utilização de material entomológico para multiplicação e manuseamento dos insetos, laboratórios devidamente apetrechados e a montagem de câmaras climatizadas para produção em massa de insetos em condições abióticas controladas em instalações estanques.

Os recursos financeiros necessários abrangem a compra de material e equipamento de laboratório, aquisição de terreno e construção das instalações fabris.

4. Descrição das atividades a desenvolver

O produto a comercializar como ponto de partida da empresa será *Macrolophus pygmaeus*, um inseto predador de *Tuta absoluta*, a lagarta mineira do tomateiro. Como inseto zoofitófago, a sua dieta compreende uma componente vegetal, o tomateiro, e uma componente animal, o ovo de *Ephestia kuehniella*, a traça da farinha, como alimento de substituição. Assim, será necessário plantar tomateiros, bem como produzir *E. kuehniella* em larga escala. Será necessário ainda cultivar milho regional produzido em modo biológico. Tendo em conta que a própria traça da farinha tem elevado valor comercial como alimento de muitos agentes de controlo biológico e também como atrativo para auxiliares biológicos nas culturas, o excesso produzido poderá ser comercializado. Poder-se-á ainda aumentar a sua produção para rentabilizar a empresa em caso de procura no mercado.

5. meios técnicos e físicos necessários à adequada realização das atividades previstas;

Todas as atividades previstas serão desempenhas por pessoal de elevada qualificação técnico-científica, altamente especializados e com experiência comprovada na área. As instalações foram concebidas para permitir concluir eficientemente todas as tarefas necessárias, de acordo com a experiência adquirida ao longo do projeto de investigação ECO2TUTA.

6. Caracterização da população alvo e do seu envolvimento no projeto

Os produtos comercializados na fase de arranque da biofábrica destinam-se, predominantemente, a produtores de tomate em estufa ou ar livre, predominantemente em Portugal. No entanto, dado a polifagia do predador, o inseto tem potencial para uso em culturas atacadas por afídeos e mosca branca, entre outras. Pretende-se, posteriormente, estabelecer uma rede de parcerias entre os produtores, os Serviços Agrários, a Universidade dos Açores e a empresa no sentido de dar respostas às necessidades específicas do mercado.

7. Plano de divulgação e promoção do projeto

A divulgação do projeto far-se-á principalmente através da webpage e redes sociais, no entanto contatos na comunidade científica poderão traduzir-se em valorização do projeto.

8. Fases e calendarização das atividades.

Estima-se um período de 6 meses após a construção das instalações fabris para que a produção dos insetos se desenvolva em pleno. As atividades iniciar-se-ão com a produção de *E. kuehniella*, um mês depois o plantio dos tomateiros, um mês depois o *M. pygmaeus* prevendo-se, no espaço de 3 meses, obter o nível de produção do agente de controlo biológico pretendido.

9. Sustentabilidade do projeto

A tendência é de crescimento na utilização do controlo biológico na agricultura. A produção de *E. kuehniella* por si só pode ser rentável para manter a empresa. Prevê-se alargar a gama de agentes de controlo biológico ou outros insetos com interesse em produzir, como por exemplo joaninhas, abelhões e abelhas. A participação em projetos de investigação, bem como na formação avançada (Mestrados, Doutoramentos) em contexto empresarial em parceria com a Universidade dos Açores e outros eventuais interessados também está em aberto.

10. Coordenador do projeto

Isabel Borges, Doutorada em Ecologia pela Universidade dos Açores com 20 anos experiência em investigação científica no âmbito do controlo biológico. Dado a participação no projeto ECO2TUTA como Doutorada contratada responsável pelo trabalho de campo e de laboratório, reúne toda a *expertise* e *know-how* requeridos.

11. Impacto esperado

Contributo para promover o controlo biológico como método para gestão de pragas de insetos.

Orçamento estimado

Instalações com um total de 320 m2 com um custo de 255.000 €, considerando uma estimativa de 750€/m2, incluindo:

i) terreno com 400 m2, para permitir construção de edifício de 320m2
ii) 1 sala de sujo com 10m2
iii) 1 sala de triagem com 50 m2
iv) 10 câmaras climatizadas num total de 50 m2

v) 1 dispensa com 30 m2

vi) 1 sala de produção de *E. kuehniella* com 100 m2

vii) 1 casa de banho 10 m2

viii) 1 vestiário com 10 m2

ix) 1 escritório com 30 m2

x) Hall + corredores 30 m2

13. Investimentos estimados

Prevê-se um investimento aproximado de 150.000 € para equipar as instalações, incluindo:

i) Equipamento e material de laboratório

ii) Material de escritório

iii) 2 arcas congeladoras

iv) 1 frigorífico

v) 2 computadores

vi) 1 viatura

vii) Webpage

viii) 1 trituradora de milho

ix) 1 esterilizador UV

x) 1 debulhadora

xi) 1 máquina lavar roupa

xii) 1 máquina de secar roupa

3.12. Achievement indicators not planned but realized.

Organization and participation of outreach activities

Organization of experimental field activities to Mr. Regional Secretary for Agriculture and Rural Development, Engº António Lima Cardoso Ventura, and to the Agrarian Development Services Technicians. June de 2021. **(Em anexo: fotos da atividade).**

During the Workshop "ECO2-TUTA - Ecological and economic feasibility of mass production of biological agents to control tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera, Gelechiidae) in protected culture: Status of the Project Development", 13 October 2021, Ponta Delgada, Portugal, we **organize** an interactive exhibition was installed to show farmers *T. absoluta* and natural enemies **(Em anexo: foto da atividade).**

Organization of an exhibition as part of the research project ECO2-TUTA - Avaliação da viabilidade ecológica e económica da produção em massa de agentes biológicos para combate à traça-do-tomateiro, *Tuta absoluta* (Meyrick) (Lepidoptera, Gelechiidae), em cultura protegida, nos Açores, na Feira Agrícola Açores, de 17 to 19 June de 2022, Ribeira Grande, São Miguel. Açores **(Em anexo: fotos da atividade).**

Poster presentations in outreach activities

Soares, A.O., L. Oliveira, A.C. Durão, F. Barbosa & I. Borges. *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae): a lagarta mineira do tomateiro. Feira Agrícola Açores, 17 to 19 June de 2022, Ribeira Grande, São Miguel. Açores.

Oliveira, L., I. Borges, A. C. Durão, F. Barbosa & A.O. Soares. *Trichogramma achaeae* (Hymenoptera: Trichogrammatidae:) um inseticida vivo. Feira Agrícola Açores, 17 to 19 June de 2022, Ribeira Grande, São Miguel. Açores.

Borges, I., Oliveira, L., A. C. Durão, F. Barbosa & A.O. Soares. *Macrolophus pygmaeus* (Hemiptera: Miridae:) um predador para o controlo biológico de pragas de tomateiro. Feira Agrícola Açores, 17 to 19 June de 2022, Ribeira Grande, São Miguel. Açores.

Poster presentations at international scientific meetings

Oliveira, L., I. Borges, D. Silva & A.O. Soares (2021). Abundance of *Tuta absoluta* (Lepidoptera: Gelechiidae) and its natural enemies on tomato crops of different production modes. "XIX Congresso Ibérico de Entomologia: os Insetos e o Homem" (online), 21-24 de setembro. Livro de resumos. Coimbra, Portugal. 190pp. (Poster).

XIX Congresso Ibérico de Entomologia **21-24 de setembro** 2021 ONLINE

Abundance of *Tuta absoluta* (Lepidoptera: Gelechiidae) and its natural enemies on tomato crops of different production modes

Luisa Oliveira (1), Isabel Borges (2), Dário Silva (3), António O. Soares (2)

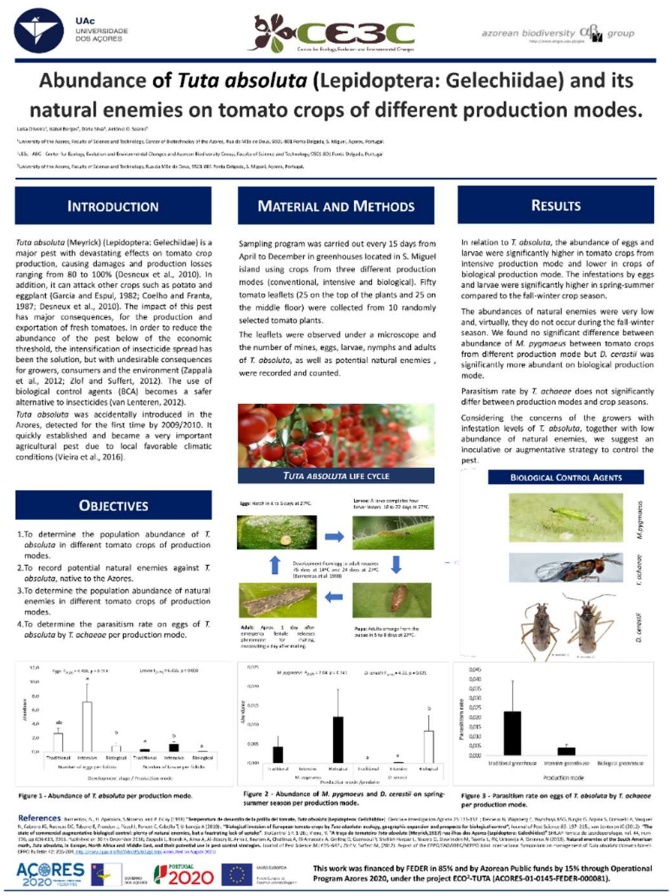
1 - University of the Azores, Faculty of Science and Technology, Center of Biotechnoloy of the Azores, Portugal; 2 - cE3c - ABG - Center for Ecology, Evolution and Environmental Changes and Azorean Biodiversity Group, Department of Biology, Faculty of Science and Technology, Portugal; 3 - University of the Azores, Faculty of Science and Technology, Portugal

Currently, *Tuta absoluta* (Lepidoptera: Gelechiidae) is one of the major phytosanitary concerns of tomato crops produced in the Azorean greenhouses. To contrast the abundance of the pest and its natural enemies of crops from different production modes, a sampling program was carried out during 2020-2021. Tomato plants from traditional, intensive and biological production modes were sampled including crop seasons of spring-summer and fall-winter.

In relation to *T. absoluta*, the abundance of eggs and larvae are higher in tomato crops from intensive production modes and lower in crops of biological production mode. The infestations by eggs and larvae are higher in spring-summer compared to the fall-winter crop season. Regarding natural enemies of *T. absoluta*, we recorded the presence of *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae), *Dicyphus cerastii* Wagner (Hemiptera: Miridae) and *Trichogramma achaeae* (Hymenoptera: Trichogrammatidae). The abundances of natural enemies were very low and, virtually, they do not occur during the fall-winter season. We found no significant difference between abundance of M. pygmaeus between tomato crops from different production mode but *D. cerastii* was more abundant on biological production mode. Parasitism rate by *T. achaeae* does not differ between production modes and crop seasons.

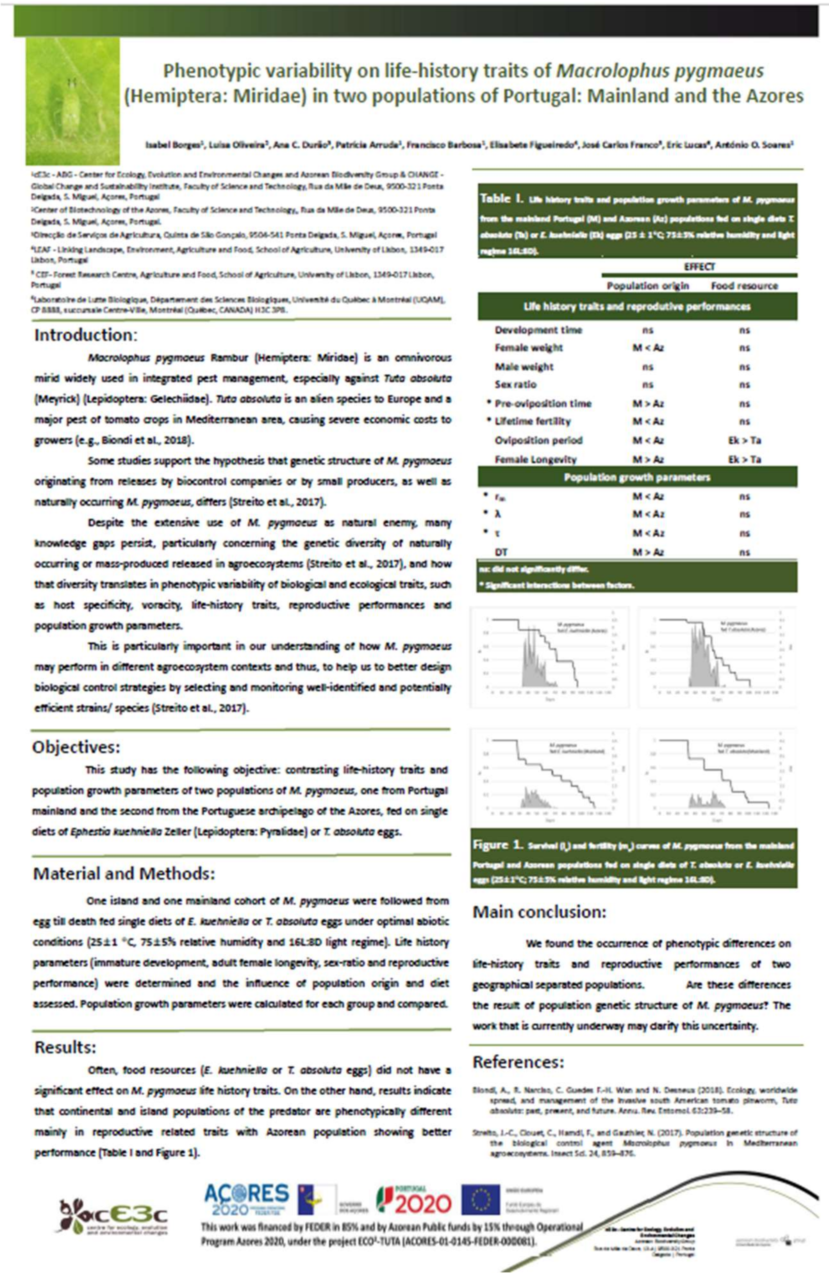
Considering the concerns of the growers by the infestation levels of their crops, together with low abundance of natural enemies, we suggest an inoculative or augmentative strategy to control the pest.

Palavras chave| palabras clave: Crop season; Infestation level; Natural enemies; Production mode; Tomato crop



Borges, I., L. Oliveira, A.C. Durão, P. Arruda, F. Barbosa, E. Figueiredo, J.C. Franco, E. Lucas, A.O. Soares (2022). Phenotypic variability of life-history traits and voracity of *Macrolophus pygmaeus* (Hemiptera: Miridae) in two populations of Portugal: Mainland and Azores. "*XXVI International Congress of Entomology*", 17-22 de julho, Helsinquía, Finlândia. Livro do programa, 182pp (abstract virtually presented in the congress app). (Poster).

P494 Phenotypic variability of life-history traits and voracity of Macrolophus pygmaeus (Hemiptera: Miridae) in two populations of Portugal: Mainland and Azores
António Onofre Soares; Isabel Borges



Phenotypic variability on life-history traits of *Macrolophus pygmaeus* (Hemiptera: Miridae) in two populations of Portugal: Mainland and the Azores

Isabel Borges[1], Luisa Oliveira[2], Ana C. Durão[3], Patrícia Arruda[1], Francisco Barbosa[1], Elisabete Figueiredo[4], José Carlos Franco[5], Eric Lucas[6], António O. Soares[1]

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Introduction:

Macrolophus pygmaeus Rambur (Hemiptera: Miridae) is an omnivorous mirid widely used in integrated pest management, especially against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Tuta absoluta* is an alien species to Europe and a major pest of tomato crops in Mediterranean area, causing severe economic costs to growers (e.g., Biondi et al., 2018).

Some studies support the hypothesis that genetic structure of *M. pygmaeus* originating from releases by biocontrol companies or by small producers, as well as naturally occurring *M. pygmaeus*, differs (Streito et al., 2017).

Despite the extensive use of *M. pygmaeus* as natural enemy, many knowledge gaps persist, particularly concerning the genetic diversity of naturally occurring or mass-produced released in agroecosystems (Streito et al., 2017), and how that diversity translates in phenotypic variability of biological and ecological traits, such as host specificity, voracity, life-history traits, reproductive performances and population growth parameters.

This is particularly important in our understanding of how *M. pygmaeus* may perform in different agroecosystem contexts and thus, to help us to better design biological control strategies by selecting and monitoring well-identified and potentially efficient strains/ species (Streito et al., 2017).

Objectives:

This study has the following objective: contrasting life-history traits and population growth parameters of two populations of *M. pygmaeus*, one from Portugal mainland and the second from the Portuguese archipelago of the Azores, fed on single diets of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) or *T. absoluta* eggs.

Material and Methods:

One island and one mainland cohort of *M. pygmaeus* were followed from egg till death fed single diets of *E. kuehniella* or *T. absoluta* eggs under optimal abiotic conditions (25±1 °C, 75±5% relative humidity and 16L:8D light regime). Life history parameters (immature development, adult female longevity, sex-ratio and reproductive performance) were determined and the influence of population origin and diet assessed. Population growth parameters were calculated for each group and compared.

Results:

Often, food resources (*E. kuehniella* or *T. absoluta* eggs) did not have a significant effect on *M. pygmaeus* life history traits. On the other hand, results indicate that continental and island populations of the predator are phenotypically different mainly in reproductive related traits with Azorean population showing better performance (Table I and Figure 1).

Table I. Life history traits and population growth parameters of *M. pygmaeus* from the mainland Portugal (M) and Azorean (Az) populations fed on single diets *T. absoluta* (Ta) or *E. kuehniella* (Ek) eggs (25 ± 1°C; 75±5% relative humidity and light regime 16L:8D).

	EFFECT	
	Population origin	Food resource
Life history traits and reprodutive performances		
Development time	ns	ns
Female weight	M < Az	ns
Male weight	ns	ns
Sex ratio	ns	ns
* Pre-oviposition time	M > Az	ns
* Lifetime fertility	M < Az	ns
Oviposition period	M < Az	Ek > Ta
Female Longevity	M > Az	Ek > Ta
Population growth parameters		
* r_m	M < Az	ns
* λ	M < Az	ns
* τ	M < Az	ns
DT	M > Az	ns

ns: did not significantly differ.
* Significant interactions between factors.

Figure 1. Survival (l_x) and fertility (m_x) curves of *M. pygmaeus* from the mainland Portugal and Azorean populations fed on single diets of *T. absoluta* or *E. kuehniella* eggs (25±1°C; 75±5% relative humidity and light regime 16L:8D).

Main conclusion:

We found the occurrence of phenotypic differences on life-history traits and reproductive performances of two geographical separated populations. Are these differences the result of population genetic structure of *M. pygmaeus*? The work that is currently underway may clarify this uncertainty.

References:

Biondi, A., R. Narciso, C. Guedes F.-H. Wan and N. Desneux (2018). Ecology, worldwide spread, and management of the invasive south American tomato pinworm, *Tuta absoluta*: past, present, and future. Annu. Rev. Entomol. 63:239–58.

Streito, J.-C., Clouet, C., Hamdi, F., and Gauthier, N. (2017). Population genetic structure of the biological control agent *Macrolophus pygmaeus* in Mediterranean agroecosystems. Insect Sci. 24, 859–876.

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Datasets

Marcelino, J., Borges, P.A.V., Borges, I. & Soares, A.O. (2022). Eden Arthropod Azores Database. v1.10. Universidade dos Açores. Sampling event dataset. DOI:10.15468/38ccb3.

Soares A O, Borges I, Calado H, Borges P A V (2021). Biodiversity data of ladybeetles (Coleoptera: Coccinellidae) of the Azores archipelago (Portugal). Version 1.5. Universidade dos Açores. Sampling event dataset https://doi.org/10.15468/m74aqv accessed via GBIF.org on 2023-07-10.

News published in the media related to the execution of the project.

REGIÃO AUTÓNOMA DOS AÇORES
SECRETARIA REGIONAL DA AGRICULTURA E DO DESENVOLVIMENTO RURAL

Folha Informativa SRADR 2021-10-14

LEGISLAÇÃO DIÁRIA

Diploma	Data	Emissor	Sumário
Regulamento (UE) 2021/1807	2021.10.14	Comissão Europeia	Altera os anexos II, III e IV do Regulamento (CE) n.º 396/2005 do Parlamento Europeu e do Conselho no que se refere aos limites máximos de resíduos de acibenzolar-S-metilo, extrato aquoso de sementes germinadas de Lupinus albus doce, azoxistrobina, clopiralide, ciflufenamida, fludioxonil, fluopirame, fosetil, metazacloro, oxatiapiprolina, tebufenozida e tiabendazol no interior e à superfície de determinados produtos.
Regulamento de Execução (UE) 2021/1809	2021.10.14	Comissão Europeia	Altera o Regulamento de Execução (UE) 2020/1191 que estabelece medidas para impedir a introdução e a propagação na União do vírus do fruto rugoso castanho do tomateiro (ToBRFV).
Comunicação da Comissão	2021.10.14	Comissão Europeia	Orientações para o encerramento dos programas operacionais aprovados para intervenção do Fundo Europeu de Desenvolvimento Regional, do Fundo Social Europeu, do Fundo de Coesão e do Fundo Europeu dos Assuntos Marítimos e das Pescas (2014-2020).

OUTROS ASSUNTOS

Região Autónoma dos Açores

Notícias

"Investigação científica é parceira essencial" do Governo dos Açores na Agricultura, defende António Ventura

O Secretário Regional da Agricultura e Desenvolvimento Rural, António Ventura, realçou ontem, em Ponta Delgada, o papel da investigação científica enquanto "parceira fundamental do Governo Regional na procura de soluções para os problemas que assolam a agricultura Açoriana".

"Estamos hoje aqui para ver resultados, ouvir resultados e projetar o futuro" sustentou o Secretário Regional.

António Ventura falava na sessão de abertura do encontro "ECO2-TUTA: Meeting Programe", dedicado a fazer a avaliação da viabilidade ecológica e económica da produção em massa de agentes biológicos para combate a *Tuta absoluta* (Meyrick) (Lepidoptera, *Gelechiidae*), em cultura protegida.

Este programa desenvolveu, ao longo de três anos, trabalhos de campo na cultura do tomate e respetivos registos, na firme convicção de que podem residir nos organismos nativos as respostas ao controlo de algumas pragas-chave das culturas no ecossistema insular, sensível à perda de biodiversidade.

Cumulativamente, foram recolhidos outros dados que se podem revestir de utilidade para enriquecer o caminho do agricultor na agricultura sustentável, nomeadamente, na adoção de meios de luta alternativos à luta química no que respeita ao combate de pragas e doenças e ainda a observação da adaptação das variedades instaladas às condições locais existentes.

1

Legislação diária e outros assuntos de interesse para o setor da agricultura e do desenvolvimento rural regional

REGIÃO AUTÓNOMA DOS AÇORES
SECRETARIA REGIONAL DA AGRICULTURA E DO DESENVOLVIMENTO RURAL
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"Investigação científica é parceira essencial" do Governo dos Açores na Agricultura, defende António Ventura

Correio dos Açores, 14 de Outubro de 2021 — regional/opinião 15

"Investigação científica é parceira essencial na Agricultura de futuro", defende António Ventura

Arquipélago promove *open studio* com a artista Berta Teixeira

A talho de foice...

Homens de barba rija

Por: Manuel Carreiro

4. Scientific Report

Task 1. Title: Population dynamics of *T. absoluta* and their BCA's.

ZooKeys 1053: 107–144 (2021)
doi: 10.3897/zookeys.1053.64268
https://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

An annotated checklist of ladybeetle species (Coleoptera, Coccinellidae) of Portugal, including the Azores and Madeira Archipelagos

António Onofre Soares[1], Hugo Renato Calado[2], José Carlos Franco[3], António Franquinho Aguiar[4], Miguel M. Andrade[5], Vera Zina[3], Olga M.C.C. Ameixa[6], Isabel Borges[1], Alexandra Magro[7,8]

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http://zoobank.org/79A20426-803E-47D6-A5F9-65C696A2E386

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Biodiversity Data Journal 9: e77464
doi: 10.3897/BDJ.9.e77464

Data Paper

An updated checklist to the biodiversity data of ladybeetles (Coleoptera: Coccinellidae) of the Azores Archipelago (Portugal)

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Academic editor: Pedro Cardoso

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Citation: Soares AO, Borges I, Calado HR, Borges PAV (2021) An updated checklist to the biodiversity data of ladybeetles (Coleoptera: Coccinellidae) of the Azores Archipelago (Portugal). Biodiversity Data Journal 9: e77464. https://doi.org/10.3897/BDJ.9.e77464

Biodiversity Data Journal 9: e62157
doi: 10.3897/BDJ.9.e62157

Data Paper

Standardised arthropod (Arthropoda) inventory across natural and anthropogenic impacted habitats in the Azores archipelago

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Academic editor: Pedro Cardoso

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Citation: Marcelino J, Borges PAV, Borges I, Pereira E, Santos V, Soares AO (2021) Standardised arthropod (Arthropoda) inventory across natural and anthropogenic impacted habitats in the Azores archipelago. Biodiversity Data Journal 9: e62157. https://doi.org/10.3897/BDJ.9.e62157

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Conservation Biology

REVIEW

A roadmap for ladybird conservation and recovery

António O. Soares[1,2] | Danny Haelewaters[2,3,4,5] | Olga M. C. C. Ameixa[6] | Isabel Borges[1] | Peter M. J. Brown[7] | Pedro Cardoso[8] | Michiel D. de Groot[3,9] | Edward W. Evans[10] | Audrey A. Grez[11] | Axel Hochkirch[12,13] | Milada Holecová[14] | Alois Honěk[15] | Ján Kulfan[16] | Ana I. Lillebø[6] | Zdenka Martinková[15] | J. P. Michaud[17] | Oldřich Nedvěd[4,5] | Omkar[18] | Helen E. Roy[19] | Swati Saxena[18] | Apoorva Shandilya[18] | Arnaud Sentis[20] | Jiri Skuhrovec[15] | Sandra Viglášová[16] | Peter Zach[16] | Tania Zaviezo[21] | John E. Losey[2,22]

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https://doi.org/10.57065/shilap.437

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

Luísa Oliveira, Isabel Borges, Dário Silva, Ana C. Durão & António O. Soares

Task 2. Title: Phenotypic and genetic variability between insular and mainland populations of *M. pygmaeus*.

agronomy

MDPI

Article

Contrasting Phenotypic Variability of Life-History Traits of Two Feral Populations of *Macrolophus pygmaeus* (Hemiptera: Miridae) under Two Alternative Diets

Isabel Borges [1], Luísa Oliveira [2], Ana C. Durão [3], Patrícia Arruda [4], Elisabete Figueiredo [5,6], José Carlos Franco [6,7], Eric Lucas [8] and António O. Soares [1,*]

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Voracity and conversion efficiency in females of two feral populations of *Macrolophus pygmaeus*, a biocontrol agent of *Tuta absoluta*

Isabel Borges[1], Luísa Oliveira[2], Francisco Barbosa[3], Elisabete Figueiredo[4,5], José Carlos Franco[5,6], Ana C. Durão[7], António Onofre Soares[1*]

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Abstract

Macrolophus pygmaeus (Rambur, 1839) (Hemiptera: Miridae) is a polyphagous predator used in programs of biological control and integrated pest management as natural enemy against small arthropod pest populations, including whiteflies, thrips, spider mites and aphid. This mirid is also successfully mass reared under factitious prey for commercial purposes. In the present study we assessed and compared the voracity, weight gain and conversion efficiency in females of two populations of *M. pygmaeus*, one from Portugal mainland and the other from the Azores, fed on single prey diets of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs, or *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) eggs. We found that female's voracity on eggs of *T. absoluta* was higher than that of *E. kuehniella*, presumably due to the comparative size/amount of biomass of the eggs. Translating voracity in biomass ingested, we only find a significant difference for consumption on *E. kuehniella* eggs by *M. pygmaeus* from the Azores. Despite the smaller size of females from the Azores, they were able to consume more prey and ultimately reaching a similar body weight and conversion efficiency compared with females from mainland, independently of the prey provided. Our results are in line with a previous study, showing differences in the life-history traits of *M. pygmaeus* from Portugal mainland and Azorean populations fed on alternative preys. From an applied perspective, our results are a contribution to the decision-making on the use of the most effective biocontrol agents for different preys and/or agroecosystem contexts.

Keywords: Biological control; predators; Miridae; biological traits; egg-prey; *Ephestia kuehniella*

1. Introduction

The South American leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) was firstly reported outside its native range in Eastern Spain in 2006 and, in 2007, dispersed to Africa via Algeria, Morocco and Tunisia (Desneux et al., 2011; Desneux et al., 2022). Now, it is widely distributed in more than 100 countries (EPPO 2023). The primary host of this pest seems to be *Solanum lycopersicum* L., although it can feed and develop on other plants within Solanaceae and Convolvulaceae, such as the bittersweet nightshade (*Solanum dulcamara* L.), black nightshade (*Solanum nigrum* L.), cape gooseberry (*Physalis peru*viana L.), common thorn (*Datura stramonium* L.), eggplant (*Solanum melongena* L.), pepper (*Capsicum annuum* L.), potato (*Solanum tuberosum* L.), sweet potato (*Ipomea batatas* (L.) Lam.), tobacco (*Nicotiana tabacum* L.) and wild tomato (*Lycopersicon hirsutum* Dunal) (Veira, 2016). *Tuta absoluta* is a key pest of tomato crops, causing devastating economic impacts to growers (Biondi et al., 2018), sometimes reaching 100% of economics losses fruits (Rostami et al., 2020). Foliage damages are the primary cause of the economic losses, especially when populations are abundant. The damages result predominantly from feeding activity of larvae on leaf tissues where they produce mines. When attacks are extreme, mines may merge causing destruction of the leaves. The reduction of leaf tissues induces dispersion to new organs, including the fruits. The larvae penetrate fruits, often below the calyx, facilitating fruit infection by pathogens (Chermiti et al., 2009; Balzan & Moonen, 2012; Guedes & Picanço, 2012).

The zoophytophagous *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) is recognized as one of the most promising biological control agents against *T. absoluta* (Arnó et al., 2021). This mirid has been successfully mass reared for commercial purposes (van Lenteren, 2012) and used in the biological control of different arthropod pests, such as aphids, thrips, whiteflies, and spider mites (Fauvel et al., 1987; Alvarado et al., 1997;

Barnadas et al., 1998; Riudavets & Castañé, 1998; Margaritopoulos et al., 2003; Perdikis et al., 2008; Arnó et al., 2009; Calvo et al., 2009; Urbaneja et al., 2009; Castañé et al., 2011; Urbaneja et al., 2012). *Macrolophus pygmaeus* is widely distributed across the Mediterranean area, including Portugal. It was firstly referred to the Azores, in 2012 (Kerzhner & Josifov, 1999), but no information is available on the origin and date of colonization. Most likely, the Azorean populations have originated from Portugal mainland. Although *Dicyphini* mirids are zoophytophagous, the level of damage they can originate in host plants is variable, depending on the species. *Macrolophus pygmaeus* has been seen as a candidate to replace *Nesidiocoris tenuis* (Reuter), in biological control in tomato crops, because the damage inflicted on plants is much less. Nevertheless, we should take in consideration that plant feeding allows mirids being present in the field, when prey is scarce, which is a critical aspect in biological control, due to a faster build-up of their populations. Thus, mirid zoophytophagy can be seen as an advantage in biological control tactics if the originated crop damage is tolerable (Abraços-Duarte et al., 2021; Perez-Hedo et al., 2021; Coppel & Mertins, 1977).

Biological and ecological traits of the pests and their natural enemies should be accessed to predict how successful a biocontrol agent can be for augmentative biological control (Coppel & Mertins, 1977). The effectiveness of pest control is largely determined by the relationship between biocontrol agent voracity and pest population growth (Coppel & Mertins, 1977), especially the females that, in general, are the most voracious developmental stage and, depending on the availability of prey, biomass consumption may translate in a positive reproductive numerical response. That is, changes in the number of predators on prey colonies may result from two different mechanisms: attraction of predators to prey aggregations (aggregational response) and increased rate of predator reproduction when prey is abundant (reproductive numerical response). Predation or parasitism rate must be higher than the growth rate of prey/host (Coppel & Mertins, 1977). In insect pests with fast population growth, such as aphids, it is essential that biocontrol agents must have a high voracity (Borges et al., 2011; 2013). However, prey may differ in their suitability driving alterations on predator's survival and development rate. The consumption of different prey species can have consequences for predator's reproductive numerical responses changing, by this way, the success of biological control. Predator egg production also requires nutritional intake beyond a maintenance level, and thus high-quality food sources are mandatory for supporting predator reproduction (Seagraves, 2009; Sebastião et al., 2015; Hodek, 1962; Hodek & Honěk, 1996; Hodek & Evans, 2012).

Recently, Borges et al. (2023) provided experimental evidence that *T. absoluta* eggs can be considered essential food for *M. pygmaeus*. That is, when fed only with *T. absoluta* eggs, nymphs of *M. pygmaeus* can complete development and the originated adults are able to reproduce. From an applied point of view, the assessment of the number of eaten (killed) eggs of *T. absoluta* is an important predictor of a predator's potential as a biological control agent. Prey consumed by insects can vary greatly in quality. Prey quality affects growth, development and reproduction. The percentage of consumed prey biomass converted into predator biomass (the so-called efficiency of conversion of ingested material or relative growth rate) is a useful proxy to test food quality (Waldbauer, 1968). Expressing predator voracity as the amount of biomass intake, allows i) to compare similar biological traits among different prey, given that species do not have the same body weight and ii) to estimate some physiological parameters, as the conversion efficiency. Following biomass consumption, weight increases and conversion efficiency are good predictors of energy intake and associated costs (Waldbauer, 1968; Odum, 1956). According to the universal model of energy flow (Odum, 1956), conversion efficiency corresponds to the proportion of biomass consumed allocated to growth.

In the present study, we assessed the voracity, the weight gain and conversion efficiency of *M. pygmaeus* females when fed on *T. absoluta* eggs. We compared two feral populations of *M. pygmaeus*, i.e., one from Portugal mainland and one from Azores archipelago. We intended to estimate the predatory activity of *M. pygmaeus*, assessing to what extent will it be possible to mass-reared under laboratory conditions, using a factitious prey, for later use in IPM of *T. absoluta*, as well as investigating if its predatory performance may vary among populations of different geographical origin.

2. Materials and Methods

2.1. Biological materials

Specimens of *M. pygmaeus* were obtained from two feral populations of different geographical origin: 1) Portugal mainland population, whose specimens originated mainly from the Western region of Portugal (Azueira, Mafra: 39°00′43.8″ N 9°16′49.1″ W; Silveira, Torres Vedras: 39°07′02.5″ N 9°21′54.1″ W; Tapada da Ajuda, Lisbon: 38°42′45.8″ N 9°11′02.8″ W), collected both in greenhouse and open field crops, and maintained in laboratory conditions, during several generations; 2) Azorean population, based on specimens collected in S. Miguel Island, on *S. lycopersicum* plants, in five different locations (Pico da Pedra: 37°47′48.1″ N 25°35′51.7″ W; Lagoa: 37°45′4.3″ N 25°34′27.2″ W; Ribeira Grande: 37°48′49.59″ N 25°31′41.10″ W; two locations on Arrifes: 37°45′1.54″ N 25°41′28.70″ W; 37°45′1.54″ N 25°41′28.70″ W). Laboratory populations were mantained, separately, in rearing cages (40 x 40 x 40 cm) covered by voile. One potted tomato plant, as well as *E. kuehniella* eggs, provided as a source of protein, were offered twice a week to the mirids within the cages. The eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) are used as a reference diet. *Ephestia kuehniella* is an important pest of stored products (Cox & Bell, 1991; Hill, 2006) and its eggs has been commonly used to mass-rear different biocontrol agents (Hamasaki & Matsui, 2003; Vandekerkhove & De Clercq, 2010; Moghaddassi, et al., 2019; Gallego et al., 2022). Additionally, the mirids were fed on honey droplets (50% in water) placed over the leaflets. Tomato plant was substituted by a fresh one, every two days. The mirids were reared at 25 ± 1° C, 75 ± 5% relative humidity and 16L:8D light regime.

Ephestia kuehniella eggs were provided by CBA (Centre of Biotechnology of the Azores), where the lepidopteran is mass reared. *Tuta absoluta* was collected in a greenhouse in S. Miguel Island and used to start a small-scale mass rearing program in the laboratory, as described in Borges et al. (2023).

2.2. Voracity

Several rearing cages were made to obtain enough nymphs of the mirid populations for the experiments. Approximately, 50 *M. pygmaeus* adults from the stock population were placed in a rearing cage, and *E. kuehniella* eggs and honey diluted in water were provided over a tomato leaf placed in a water container. These procedures were the same for both mirid populations. After 10-days exposure, all adult mirids were removed and each cage was kept for nymphal development. Twice a week, fresh *E. kuehniella* eggs were provided, as well as honey diluted in water. Recently emerged adults were picked up every two days and transferred to a separate rearing cage, until reaching 9 to 12 days old. At that time, females were collected to be used in the voracity tests. The sex of adult specimens was identified based upon the abdomen shape: rounded, thick, and symmetric, ventrally with a central groove for females; and thin, asymmetric, without groove and tapered near the end for males.

Twenty-four hours prior to testing, females were fed only with a tomato plant and a droplet of honey diluted in water. For the voracity test, 50 eggs of *E. kuehniella* or 80 eggs of *T.*

absoluta were offered over a tomato leaflet to a single predator female in an acrylic box (3 cm diameter x 2 cm height), for 24 hours. The weight of the eggs of *E. kuehniella*, *T. absoluta* and females of *M. pygmaeus* were accessed before and after the 24 hour-test, using Sartorius ultra-microbalance. Female voracity was expressed in terms of the number of prey eggs and the amount of biomass ingested. Weight gain by females of *M. pygmaeus* expresses the difference of the body weight assessed before and after the 24 hour-test and the percentage of biomass ingested was calculated by balancing the difference in the weight loss of the eggs, relative to the initial weight. Biomass ingested (BI), relative growth rate (RGR), and conversion efficiency (CE) by females were calculated according to the formulas (Soares et al., 2004):

$$\mathbf{BI(mg) = EWi - EWf}$$

$$\mathbf{RGR}\ \% = \frac{\mathbf{PWf - PWi}}{\mathbf{PWi}} * \mathbf{100}$$

$$\mathbf{CE}\ \% = \frac{\mathbf{PWf - PWi}}{\mathbf{BI}} * \mathbf{100}$$

where, EWi and EWf are the initial and final weight of the eggs, and PWi and PWf are the initial and final weights of the predator. The experiment was conducted with 10 replicates.

2.3. Statistical analysis

For statistical analysis, we considered the following independent variables: i) prey species (*T. absoluta* or *E. kuehniella* eggs); and ii) origin of population (Azores or Portugal mainland). Generalized Linear Models (GZLM) were used to test statistical significances of the biological parameters for prey, regions and interactions between independent factors. Data were firstly examined for normal distribution, using the Kolmogorov-Smirnov test. When the previous condition of normality was not verified, the data were log-transformed.

If normal distribution was confirmed, GZLM analyses considered the normal error distribution and the identity link function. If not, we used Poisson error distribution and the Log link function Pairwise multi-comparisons were carried out and P values corrected using Bonferroni test.

Mean values were considered significantly different when $P < 0.05$. SPSS v. 27 was used to perform the statistical analyses (IBM Corp 2017).

3. Results

3.1. Initial weight of the eggs

No significant interaction was found between prey species (*T. absoluta* or *E. kuehniella* eggs) and the population origin of *M. pygmeus* (Azores or Portugal mainland) in relation to the initial weight of the eggs provided to *M. pygmaeus* females (Chi-Square = 2.926, df = 1, P = 0.087; Table 1). The eggs of *E. kuehniella* (1.32 ± 0.01 mg) were significantly heavier than those of *T. absoluta* (0.83 ± 0.01 mg) (Chi-Square = 1516.76, df = 1, $P \leq 0.0001$) and the eggs provided to *M. pygmaeus* females of mainland population was significantly heavier (1.10 ± 0.01 mg) than those provided to Azorean females (1.05 ± 0.01 mg) (Chi-Square = 14.89, df = 1, P = 0.001).

3.2. Final weight of the eggs

A significant interaction was found between prey species (*T. absoluta* or *E. kuehniella* eggs) and the population origin of *M. pygmeus* (Azores or Portugal mainland) in relation to the final weight of the eggs (mg) provided to *M. pygmaeus* females (Chi-Square = 11.84, df = 1, P = 0.001; Table 1). We only found a significant difference for the final weight of *E. kuehniella* after being consumed by *M. pygmaeus* females from Portugal mainland, that is, the final weight of the eggs was significant higher (Table 1).

3.3. Voracity

No significant interaction was found between prey species (*T. absoluta* or *E. kuehniella* eggs) and population origin of *M. pygmeus* (Azores or Portugal mainland) with respect to the voracity of *M. pygmaeus* females (Chi-Square = 3.456, df = 1, P = 0.063). The females of *M. pygmaeus* consumed a significantly higher number of eggs of *T. absoluta* (40.70 ± 4.56) in comparison to *E. kuehniella* (26.00 ± 3.56) (Chi-Square = 9.018, df = 1, P = 0.003). Females of *M. pygmaeus* from the Azores were significantly more voracious (42.70 ± 3.53 eggs consumed) more than those from mainland (24.00 ± 4.48 eggs consumed) (Chi-Square = 14.593, df = 1, P < 0.001) (Table 1).

3.4. Amount of biomass ingested

We found a significant interaction between prey species (*T. absoluta* or *E. kuehniella* eggs) and population origin of *M. pygmeus* (Azores or Portugal mainland) (Chi-Square = 13.74, df = 1, P = 0.001). A significant difference between populations of *M. pygmaeus* on the amount of ingested mass was observed in the case of *E. kuehniella* eggs only, with a higher performance shown by *M. pygmaeus* females from the Azores (Table 1).

3.5. Percentage of biomass ingested

A significant interaction was found between the prey species (*T. absoluta* or *E. kuehniella* eggs) and the population origin of *M. pygmaeus* (Azores or Portugal mainland) (Chi-Square = 5.374, df = 1, P = 0.02; Table 1). The *M. pygmaeus* females from the Azores ingested a higher rate of biomass from eggs of *E. kuehniella* in comparison to those from mainland.

3.6. Initial body weight of M. pygmaeus females

No significant interaction was found between prey species (*T. absoluta* or *E. kuehniella* eggs) and the population origin of *M. pygmaeus* (Azores or Portugal mainland) on the initial body weight of *M. pygmaeus* females (Chi-Square = 3.219, df = 1, P = 0.073; Table 2). Initial body weight of *M. pygmaeus* females fed with *T. absoluta* eggs (1.16 ± 0.04 mg) did not significantly differ from that of females fed with *E. kuehniella* eggs (1.16 ± 0.03 mg) (Chi-Square = 0.008, df = 1, P = 0.928). Initial body weight of *M. pygmaeus* females from the mainland (1.24 ± 0.03 mg) was significantly higher that of the females from Azores (1.09 ± 0.03) (Chi-Square = 18.153, df = 1, P < 0.001).

3.7. Final body weight of M. pygmaeus females

No significant interaction was found between prey species (*T. absoluta* or *E. kuehniella* eggs) and population origin of *M. pygmaeus* (Azores or Portugal mainland) on the final body weight of *M. pygmaeus* females (Chi-Square = 3.721, df = 1, P = 0.0543; Table 2). Final body weight of *M. pygmaeus* females fed with *T. absoluta* eggs (1.26 ± 0.04 mg) did not significantly differ from that of females provided with *E. kuehniella* eggs (1.33 ± 0.04 mg) (Chi-Square = 2.173, df = 1, P = 0.140). Final body weight of females of *M. pygmaeus* from the mainland (1.32 ± 0.03 mg) did not differ from that of the females of the Azores (1.27 ± 0.04 mg) (Chi-Square = 0.998, df = 1, P = 0.318) (Table 2).

3.8. Weight gain

No significant interaction was found between prey species (*T. absoluta* or *E. kuehniella* eggs) and the population origin of *M. pygmaeus* (Azores or Portugal mainland) on the weight gain of *M. pygmaeus* females (Chi-Square = 0.567, df = 1, P = 0.451; Table 2). Weight gain *M. pygmaeus* females fed with *E. kuehniella* eggs (0.17 ± 0.03 mg) was significantly higher than that of females fed with *T. absoluta* eggs (0.09 ± 0.04 mg) (Chi-Square = 3.894, df = 1, P = 0.048). Weight gain of Azorean *M. pygmaeus* females (0.18 ±

0.03 mg) was significantly higher than that of females from mainland (0.08 ± 0.03 mg) (Chi-Square = 78.293, df = 1, P = 0.007).

3.9. Relative growth rate

No significant interaction was found between prey species (*T. absoluta* or *E. kuehniella* eggs) and the population origin of *M. pygmaeus* (Azores or Portugal mainland) on the relative growth rate of *M. pygmaeus* females (Chi-Square = 0.497, df = 1, P = 0.481; Table 2). Relative growth of *M. pygmaeus* females fed with *E. kuehniella* eggs (14.90 ± 2.98 %) was significantly higher than that of females fed with *T. absoluta* eggs (0.09 ± 0.04 %) (Chi-Square = 8.593, df = 1, P = 0.003). Relative growth rate of Azorean *M. pygmaeus* females (16.80 ± 3.00 mg) did not significantly differ of females from mainland (6.64 ± 2.04 %) (Chi-Square = 3.374, df = 1, P = 0.066).

3.10. Conversion efficiency

No significant interaction was found between prey species (*T. absoluta* or *E. kuehniella* eggs) and population origin of *M. pygmaeus* (Azores or Portugal mainland) on the conversion efficiency of *M. pygmaeus* females (Chi-Square = 2.513, df = 1, P = 0.113; Table 2). Conversion efficiency of *M. pygmaeus* females fed with *E. kuehniella* eggs (0.25 ± 0.06) did not significantly differ from that of females fed with *T. absoluta* eggs (0.17 ± 0.07) (Chi-Square = 0.990, df = 1, P = 0.320). No significant differences were observed between *M. pygmaeus* females from the Azores (0.28 ± 0.04) and those from mainland (0.14 ± 0.08) (Chi-Square = 2. 923, df = 1, P = 0.087) for the same variable.

4. Discussion

In this study, we assessed and compared the number of eggs of *E. kuehniella* or *T. absoluta* consumed by females of *M. pygmaeus* from populations of two different geographical origins. We found that the number of eggs of *T. absoluta* consumed by *M. pygmaeus* females was higher than that of *E. kuehniella*. This may be related with the relative amount of biomass of the two prey-species eggs. The eggs of *T. absoluta* are very small, even compared with other Lepidopteran species (0.36 mm long and 0.22 mm in diameter [44]). In our study, the weight of *E. kuehniella* eggs was 1.59 times higher than that of *T. absoluta* eggs (1.32 vs 0.83, respectively). Using the same comparative relationship, the voracity of *M. pygmaeus* females on *T. absoluta* eggs was 1.56 higher than on *E. kuehniella* eggs.

We also found that the eggs provided to *M. pygmaeus* females of mainland population was significantly heavier (1.10 ± 0.01 mg) than those provided to Azorean females (1.05 ± 0.01 mg). Despite that statistical difference, this represents a residual difference of 4% in egg.

In our study,the number of *T. absoluta* eggs consumed in 24h by *M. pygmaeus* females (40.70 ± 4.56) is within the range of values reported by previous studies. Urbaneja et al., reported that when females were provided with 60 eggs of *T. absoluta*, they were able to consume in 24h all the eggs. Chailleux et al., for an experimental period of 12 h, recorded a mean value of about 10 eggs of *T. absoluta*. In a more recent study, the daily voracity of *M. pygmaeus* females was 60.7 ± 5.3 eggs of *T. absoluta* (Borges, personal communication).

Our results suggest a higher performance of the females from the Azorean population of *M. pygmaeus* in comparison to those of mainland population. In fact, we found that *M. pygmaeus* females from the Azores consumed about 78% more eggs of both preys than those from mainland (42.7 vs 24.0 eggs). In respect to the biomass ingested, the Azorean females also showed a higher performance, with about 54% to 68% of the prey egg biomass ingested, in comparison with 25% to 41%, in the case of mainland females. Nevertheless, no differences were observed between the two populations in relation to the relative growth rate and conversion efficiency.

These results are in line with Borges et al. (2023), which was aimed at contrasting life-history variables of two feral populations of *M. pygmaeus* (Azores vs Portugal mainland) under two alternative preys (*T. absoluta* vs *E. kuehniella* eggs). Data revealed that variables with direct impact on fitness, such as lifetime fertility, give a better performance to the Azorean population of the predator. Azorean *M. pygmaeus* females were bigger in size, matured earlier and had a higher reproduction rate for longer periods than those of the mainland population. Genetic structure differs between feral populations of *M. pygmaeus*, as well as between naturally occurring or mass-reared ones (Sanchez, 2012; Streito et al., 2017). Evolutionary context may have caused variation in the genetic structure in several populations of Mediterranean region. For example, during glaciations, populations geographically isolated by ice fields or permafrost that covered northern and center of Europe (Hewitt, 2000) might have evolved independently and become genetically differentiated (Sanchez, 2012). In the previous study (Borges, 2023) we found that the origin can affect the initial body weight of mirid. However, in the present study, we found no differences in the initial body weight between the two studied populations *of M. pygmaeus*, reared with the same diet, i.e., *E. kuehniella*.

In the present study, we also assessed the effect of population origin (Azores vs Portugal mainland) and prey diets (*T. absoluta* vs *E. kuehniella* eggs) on the weight gain and conversion efficiency of *M. pygmaeus* females. We found that the weight gain of *M. pygmaeus* females was similar in both tested diets, i.e., *T. absoluta* eggs and *E. kuehniella* eggs, but differed between the two studied populations of *M. pygmaeus*, with the Azorean females showing higher values than mainland females. Analyzing some results obtained in this study, it comes evident that, in general, females from the Azores were able to consume more prey (Azores: 42.70 ± 3.53 eggs consumed *vs* mainland: 24.00 ± 4.48 eggs consumed) and ultimately reaching a similar body weight comparing with females from mainland (mainland: 1.32 ± 0.03 mg *vs* Azores: 1.27 ± 0.04 mg), independently of the prey provided. Considering the general model of biomass/energy flow (Odum, 1956), we estimated that about 3.9 % to 20.5% of egg-prey biomass ingested is converted to body biomass by *M. pygmaeus* females, corresponding to a relative growth rate between 3 to 31%, depending on prey species and population origin of the predator. Our results provide new information on how *M. pygmaeus* may perform under different food resources and what are the physiological consequences in terms of each of the resource used for growth and basal metabolism maintenance. As far as we know, our study is the first aiming to access conversion efficiency of *M. pygmaeus* females. A previous study on *Lygus hesperus* Knight (Miridae) reported that females ingested in 24 hours an amount of prey corresponding to 169% of their body weight, while males ingested only 98% (Strong & Landes, 1965). A study on food requirement of *Blepharidopterus angulus* (Fallen) (Miridae) as a predator of *Eucallipterus tiliae* L., under low temperatures (14 °C), reported that the efficiency in the transformation of the weight of aphids absorbed into the weight of the body of the predator decreases from the 3rd larval stage (from 46% for the 1st stage to 19% in adult females) (Glen, 1973). Body weight of Azorean females were similar to females of *M. pygmaeus* reared for 30 generations either on tobacco leaves and fed on eggs of *E. kuehniella* and without plants (1.36±0.03) (Vandekerkhove, 2011).

From an applied point of view, our study provides estimates of predation rates of *T. absoluta* by adult females *of M. pygmaeus* and new insights on how its predation performance may vary among populations. With regard to these traits, some differences were significant, including differences between females of the two populations. *Tuta absoluta* and *E. kuehniella* were readily consumed by *M. pygmaeus*. In terms of the number of eggs consumed, females of the Azores were more voracious against *T. absoluta* but, when voracity was expressed in the amount of biomass ingested, females from mainland were more voracious against *E. kuehniella*. The consumption of *E. kuehniella* translates in a higher weight gain and relative growth rate and Azorean female, gain more weigh independently of the prey but conversion was marginally not significant. Further studies on the nymphal performance of the predator (Lykouressis, 2009; Perdikis et al., 1999) are needed for a more complete idea about the potential of *M. pygmaeus* as a biological control agent of *T. absoluta*. Finally, gathering data on the biology and ecology of biocontrol agents feeding on different prey species are vital to implemented sustainable pest management programs of *T. absoluta* in Portugal. In this context, a recent study shows the economic and financial commercial viability of a continuous mass production of *M. pygmaeus* to implement an augmentative biological control approach (Dutra et al., 2023).

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Declarations

Competing interests The authors have no financial or non-financial conflict interests to disclose.

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Table 1. Ponderal characteristics (mean ± SE) of *T. absoluta* or *E. kuehniella* eggs before and after consumption, voracity (number of eggs consumed per 24h ± SE), and the amount of biomass ingested (mean ± SE) by *M. pygmaeus* females from the Azores or Portugal mainland populations fed on single diets of *E. kuehniella* or *T. absoluta*. Different letters in the same row mean significant differences (*GZLM, p < 0.05).

	Azorean population		**Portugal mainland population**	
	T. absoluta	***E. kuehniella***	***T. absoluta***	***E. kuehniella***
Initial weight of the eggs (mg)	0.80±0.01	1.33±0.01	0.87±0.01	1.34±0.02
Final weight of the eggs (mg)	0.37±0.06a*	0.42±0.05a	0.52±0.07a	1.00±0.07b
Voracity (number of eggs)	45.50±6.57	39.90±1.87	35.90±6.42	12.10±2.97
Biomass ingested (mg)	0.43±0.07a*	0.89±0.04b	0.35±0.07a	0.34±0.08a
Biomass ingested (%)	0.54±0.08bc*	0.68±0.03c	0.41±0.08ab	0.25±0.06a

Table 2. Ponderal characteristics (mean ± SE), relative growth rate (mean ± SE) and conversion efficiency (% ± SE) of *M. pygmaeus* females from the Azores or Portugal mainland populations fed on single diets of *E. kuehniella* or *T. absoluta*.

	Azorean population		**Portugal mainland population**	
	T. absoluta	***E. kuehniella***	***T. absoluta***	***E. kuehniella***
Initial weight of *M. pygmaeus* (mg)	1.05±0.03	1.12±0.04	1.28±0.004	1.21±0.03
Final weight of *M. pygmaeus* (mg)	1.18±0.05	1.35±0.06	1.33±0.05	1.31±0.04
Weight gain (mg)	0.13±0.04	0.24±0.05	0.05±0.04	0.10±0.03
Relative growth rate (%)	12.39±3,65	21.21±4,51	4.67±2,91	8.59±2,71
Conversion efficiency (%)	0.31±0.08	0.26±0.05	0.03±0.10	0.25±0.10

Note: The component on genetic variability between insular and mainland populations of *M. pygmaeus* of the task 2 is ongoing. However, here we present a brief description of the tasks already carried out as well as the results already obtained:

Thirty-eight specimens of *Macrolophus* sp. and 44 specimens of *Dicyphus* sp. from multiple locations in the Azores, Portugal and Spain mainland and 4 commercialized *M. pygmaeus* samples from different companies were successful used for DNA extraction with the Dneasy® Blood & Tissue Kit (QIAGEN). The entire specimen(s) was(were) used and macerated inside a tube with a plastic pestle.

A fragment of about 650 bp near the 5'-end of the COI gene, corresponding to the DNA barcode sequence, was amplified using the primers LEPF and LEPR developed for butterflies and moths (Hajibabaei et al., 2006). PCR reaction volume contained 5x Green Buffer, 2 mM MgCl2, 0.25 mM of each dNTP, 1 uM of each primer and 0.02 U of Taq DNA polymerase. The PCR conditions were: 94 °C for 1 min, 5 cycles of 94 °C for 30 s, 45 °C for 1 min and 72 °C for 1 min, followed by 30 cycles of 94 °C for 1 min, 50 °C for 1.5 min and 72 °C for 1 min, and a final extension of 5 min at 72 °C. PCR products were purified with VWR® ExoCleanUp FAST and sent to STABvida (www.stabvida.com) for sequencing. Forward and reverse sequences were aligned and edited using Sequencher 4.05 (Gene Codes Corporation) and the consensus sequences were aligned in AliView v1.28.

The sequences were blasted using NCBI's blastn suite (blast.ncbi.nlm.nih.gov).

In the case of *Dicyphus* sp., four species were identified: *D. cerastii*, the most abundant, *D. pallicornis*, *D. bolivari* and *D. escalarae*. *D. bolivari* was collected in *Solanum villosum*; *D. escalarae* in *Anthirinum* sp.; *D. pallicornis* in *Digitalis* sp.; and *D. cerastii* in *Solanum lycopersicum*, *Physalis peruviana*, *Anthirinum* sp., *Borago officinalis*, *Helianthus anuus*, *Lactuca sativa*, *Halimium halimifolium*, and *Verbascum* sp.

Regarding *Macrolophus* sp. only two species, *M. pygmaeus* and *M. melanotoma*. *M. pygmaeus* was collected in *Solanum lycopersicum*, *Physalis peruviana*, *Anthirinum* sp., *Calendula arvensis*, *Cistus monsielensis*, and Phacelia tanacetifolia; *M. melanotoma* was collected in *Dittrichia viscosa*, *Ononis* sp., and *Verbascum* sp.

There was almost no variability among *D. cerastii* samples, being the Azorean specimens between mainland groups (Fig. 3). In contrast, we found higher variability among *M. pygmaeus* samples. All the Azorean samples were placed in the same group, but the Iberian Peninsula samples were grouped in different groups. Several samples collected at the same or nearby locations were placed in different groups. The commercial samples are all together in a more distant group together with a Catalonian (probably from a contaminated population with insects that were released in greenhouses and not a wild one).

In face of those results, we are performing an all genome sequencing method with 20 samples of *M. pygmaeus* from different locations. We are also analyzing 5 *Dicyphus cerastii* and 5 *M. pygmaeus* samples using the method of Oh et al. (2023) to place these two genera in the phylogenetic tree presented by these authors.

Minsuk Oh, M., Kim, S. Lee, S. (2023). Revisiting the phylogeny of the family Miridae (Heteroptera: Cimicomorpha), with updated insights into its origin and life history evolution. Molecular Phylogenetics and Evolution, 184, 107796. https://doi.org/10.1016/j.ympev.2023.107796

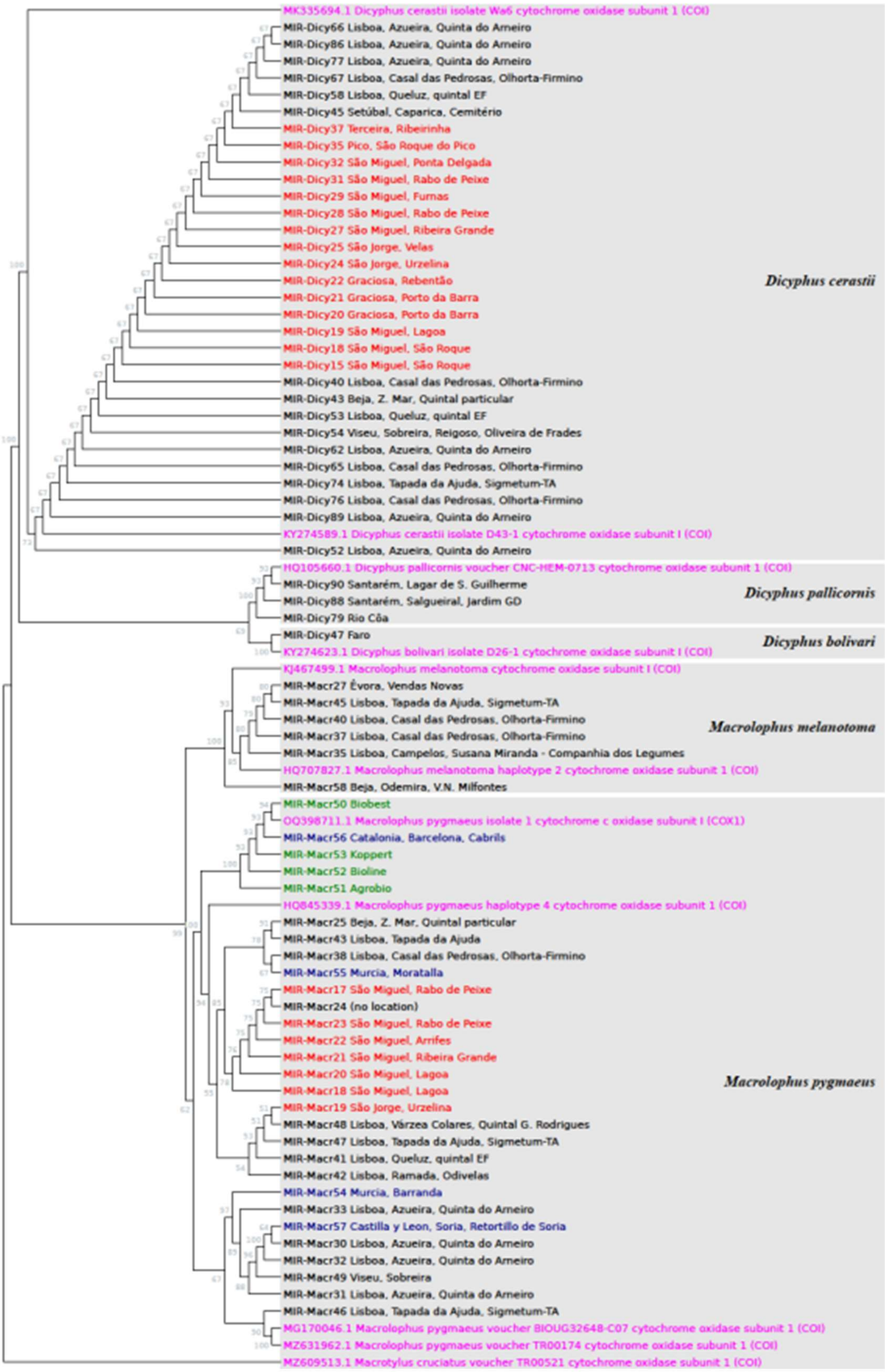

Figure 2 - Phylogenetic tree of populations of *Dicyphus cerastii* and *Macrolophus pygmaeus* from Iberia peninsula and Azores.

Task 3. Evaluation of feeding preference by M. pygmaeus for host eggs parasitized by *Tr. achaeae* and eggs of *T. absoluta*.

Task 4. Evaluation of occurrence of synergetic/additive/antagonist interactions between BCA on the success of the suppression of *T. absoluta*

Task 5. *In-situ* evaluation of the efficacy of *M. pygmaeus* and *Tr. achaeae* against *T. absoluta*.

The results projected to the task 5 (In-situ evaluation of the efficacy of *M. pygmaeus* and *Tr. achaeae* against *T. absoluta*) were published on the accepted manuscript reference:

Borges, I., L. Oliveira, A. C. Durão, P. Arruda & A. O. Soares (accepted). Feeding preference and intraguild interactions between the parasitoid Trichogramma achaeae and the predator Macrolophus pygmaeus, two biological agents of Tuta absoluta. Pest Management Science. DOI: 10.1002/ps.7635. Q1 Entomology. Fator de impacto (JRS©2022): 4.1.

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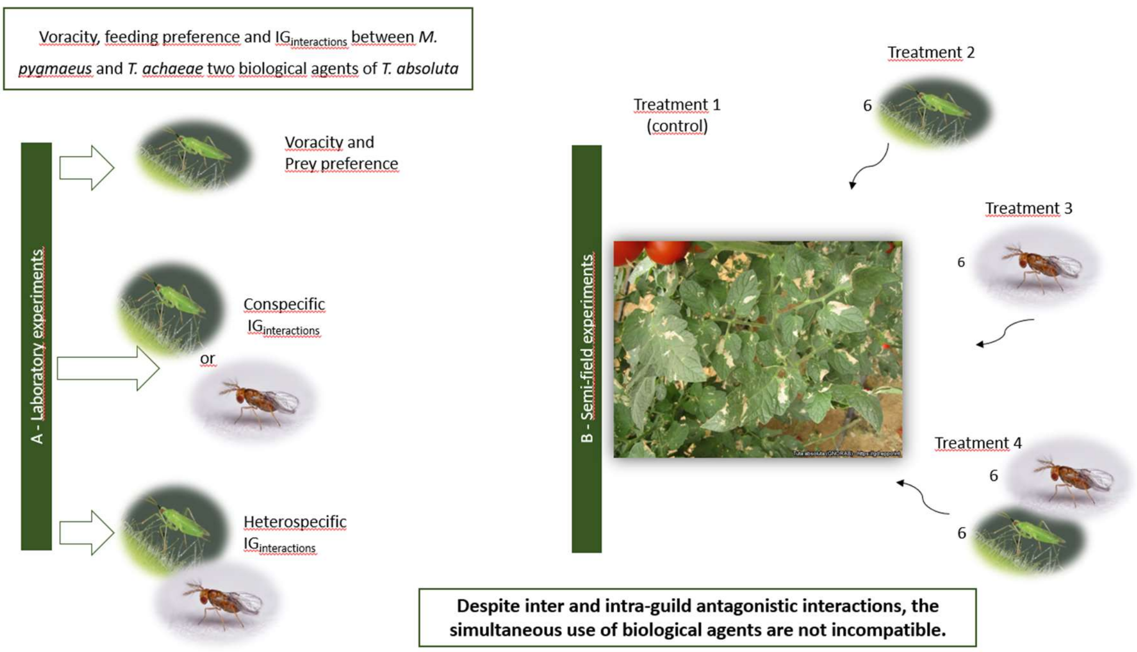

Feeding preference and intraguild interactions between the parasitoid *Trichogramma achaeae* and the predator *Macrolophus pygmaeus*, two biological agents of *Tuta absoluta*

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Abstract

BACKGROUND: *Tuta absoluta* is an exotic species and a major pest of tomato crops in Europe. *Macrolophus pygmaeus* and *Trichogramma achaeae* are two biocontrol agents widely used in integrated pest management programs of the South American tomato pinworm *T. absoluta*. In this study, we evaluated under laboratory conditions the i) voracity of *M. pygmaeus* females fed on single diets of *T. absoluta* eggs parasitized or unparasitized by *T. achaeae*, ii) voracity and feeding preference of *M. pygmaeus* females provided with mixed diets of *T. absoluta* eggs unparasitized and parasitized by *T. achaeae* and iii) effect of competitive and intraguild interactions between *M. pygmaeus* and *T. achaeae* on the number of *T. absoluta* eggs consumed and/or parasitized. Lastly, we assessed under field conditions the effect of inter- and intraspecific interactions between natural enemies on the number of *T. absoluta* eggs consumed and/or parasitized.

RESULTS: *Macrolophus pygmaeus* consumed more unparasitized than parasitized eggs of *T. absoluta*. Under mixed diet regimes, Manly indices revealed a feeding preference for unparasitized eggs, and a decrease in the total number of eggs consumed, as the proportion of available parasitized eggs increased, whereas the unparasitized eggs were consumed in direct proportion to their availability. Conspecific interactions between *M. pygmaeus*, in contrast to *T. achaeae*, revealed the possible occurrence of intraspecific competition. For intraguild heterospecific interactions, the number of *T. absoluta* eggs consumed by *M. pygmaeus* and parasitized by *T. achaeae* was lower than that predicted for additive and non-interactive scenarios. Under field conditions, a significant difference between the conspecific treatment and heterospecific treatments revealed a slightly higher success rate in controlling *T. absoluta* when both *M. pygmaeus* and *T. achaeae* were used simultaneously.

CONCLUSION: *Macrolophus pygmaeus* prefers unparasitized eggs of *T. absoluta* but inflicts intraguild predation on *T. achaeae*. In conspecific experiments, mutual interference between *M. pygmaeus* predators intensifies as the number of individuals increases, but for *T. achaeae*, it occurs in an unpredictable manner. Adding *T. achaeae* could significantly increase the level of control of *T. absoluta* compared to what could be achieved when only *M. pygmaeus* is present in greenhouse tomatoes.

Keywords: Augmentative biological control; South American tomato pinworm; predatory mirid bug; egg parasitoid; feeding preference; intraguild interactions

1. Introduction

Biological control using native natural enemies has proven to be an environmentally sound, sustainable alternative in a wide range of cropping systems[1], given that exotic natural enemies may pose important concerns for local biodiversity.[2] An optimal augmentative strategy should consider the simultaneous use of more than one natural enemy. For many agroecosystems, there is growing evidence that more diverse guilds enhance biological control efficacy.[3,4] The few empirical studies reveal variable results, however, as intraguild predation and/or behavioral interference may disrupt pest control. These varying outcomes are due to idiosyncrasies that demand case-by-case consideration, once it may be dependent on the agroecosystems, landscape complexity, predators' identity and species abundance and the mechanisms underlying their functioning.[5-10] Interactions between multiple biocontrol agents can be (i) antagonistic, (ii) additive/non-interactive, or (iii) synergistic. Additive/non-interactive interactions result in a summation effect, while an antagonistic interaction results in a decreased overall effect of biocontrol agents, that is less than additive effect. Synergetic effect occurs when the combined effect is greater than the expected additive effect. Despite the possibility that any of these three general scenarios may occur between biocontrol agents in integrated pest management programs, extrapolative experiments are rarely conducted to determine which of these various scenarios may apply.

When confronted with two alternative preys, biocontrol agents often display a feeding preference for one of them and that response is strongly affected by the relative abundance of the two preys.[11-13] When tested with different prey proportions, a predator can display four types of response: (i) a constant preference for one prey species, (ii) no preference, wherein the ratio of consumed prey is equal to the ratio of prey individuals in the environment (i.e., null switching)[14], (iii) a switching behavior, wherein the predator eats disproportionally more of the more abundant prey[11] and (iv) an anti-switching behavior, wherein the predator eats disproportionably more of the less abundant prey.[14] However, testing for such responses is rarely carried out before initiating a program of inoculative or augmentative biological control.

The South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a major pest of tomatoes, causing considerable fruit quality reduction[15] and worldwide economic losses to growers.[16] This species, native to South America, has already invaded more than 100 countries.[17] In the Portuguese mainland, this invasive pest was reported for the first time in 2009 in greenhouse tomato crops.[18-20] *Tuta absoluta* was accidentally introduced in the Azores archipelago (Portugal); it was first reported in São Miguel Island, during the 2009-2010 growing season[21] and it has spread to Terceira, Faial and Pico Islands[22] and Graciosa Island (António O. Soares, personal observations).

In the Mediterranean area, native parasitoids and predators can play an important role in attacking *T. absoluta* eggs.[23-26] This seems to be the case of *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae)[27-30] and *Trichogramma achaeae* (Hymenoptera: Trichogrammatidae).[31-33] Laboratory and field studies using *T. achaeae* against *T. absoluta* have shown promising results in greenhouses tomatoes of the Azores[33], but an effective control of pest populations was not achieved due to a low rate of parasitism. In this context, it is crucial to explore more effective solutions, by augmentation of one single biocontrol candidate or, alternatively, by multispecific releases.

The general aim of our study was to evaluate the potential of feral populations of *M. pygmaeus* and *T. achaeae*, collected in the Azores, Portugal, as biocontrol agents against *T. absoluta*. For such, we (i) compared the voracity of *M. pygmaeus* fed on single diets of *T. absoluta* eggs unparasitized or parasitized by *T. achaeae*, (ii) compared the total voracity and feeding preference of *M. pygmaeus* when provided with mixed diets of *T. absoluta* eggs both unparasitized and parasitized, (iii) assessed, under controlled laboratory conditions, the effect of the density of either *M. pygmaeus* or *T. achaeae* on the number of *T. absoluta* eggs consumed and/or parasitized (Conspecific experiments vs. Heterospecific experiments) and (iv) evaluated, under field conditions, the effect of *M. pygmaeus* and/or *T. achaeae* on the number of *T. absoluta* eggs consumed and/or parasitized (Conspecific experiments vs. Heterospecific experiments).

2. Methodology

2.1. Biological material

Macrolophus pygmaeus individuals were collected on tomato plants, in S. Miguel Island, in five locations (Pico da Pedra: 37°47′48.1″ N 25°35′51.7″ W; Lagoa: 37°45′4.3″ N 25°34′27.2″ W; Ribeira Grande: 37°48′49.59″ N 25°31′41.10″ W; two locations in Arrifes: 37°45′1.54″ N 25°41′28.70″ W; 37°45′1.54″ N 25°41′28.70″ W). A laboratory stock population was maintained under controlled conditions. The insects were kept in rearing cages (40 cm x 40 cm x 40 cm) covered with mousseline fabric. Eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) and a potted tomato plant were provided as food sources. *Ephestia kuehniella* eggs were placed every two days onto the tomato plant leaflets. To supplement the diet, droplets of honey diluted in water were placed every week on the tomato plant leaflets.

For all experiments, predatory females were 9-12-day-old to ensure that they were ovipositing. To obtain these females, approximately 20 couples of *M. pygmaeus* adults were transferred from the stock population to a new rearing cage containing a tomato plant leaf inserted in a water pot on which *E. kuehniella* eggs and droplets of honey diluted in water were provided. The new females used in our experiments were obtained from the eggs laid by these couples. After 10 days, the adults were removed to another rearing cage.

In the days following removal of adults, predatory nymphs started to hatch, completing development approximately 18 days later. At this time, newly emerged adults were collected every 2 days and transferred to new cages. Females that were 9 to 12-day-old were then selected to be used in the experiments. This procedure was repeated for as long as necessary to obtain enough predatory females for all the experiments.

Ephestia kuehniella eggs and *T. achaeae* parasitoids were supplied by a facility of the Biotechnology Centre of Azores (University of the Azores, Portugal) and *T. absoluta* was collected in a greenhouse in S. Miguel Island to establish a stock population in the laboratory, as described in Borges et al.[30] All experiments and laboratory populations were maintained at 25±1º C, 75±5% relative humidity and 16L:8D photoperiod.

2.2. Voracity of *M. pygmaeus* fed on single diets of *T. absoluta* eggs unparasitized or parasitized by *T. achaeae*

To obtain *T. absoluta* eggs parasitized by *T. achaeae*, fresh eggs (< 24 h) were carefully removed from the tomato plant leaf used for egg collection (see Borges et al.[30]) and spread over individual tomato plant leaflets (500 to 1000 eggs per leaflet), using a dissection needle under the microstereoscope. The leaflets bearing the eggs were then put in plastic boxes (10 cm diameter x 2 cm height) along with *T. achaeae* individuals that had emerged that day (approximately 400 individuals per leaflet). After 6-7 days, parasitized eggs turned black and thus were ready for use in the experiments. During the 24 h before experiments began, *M. pygmaeus* females were only fed on tomato and honey diluted in water. The next day, at the start of the experiment, these 9 to 12-day-old predatory females were offered 100 parasitized (treatment 1) or unparasitized (treatment 2) eggs of *T. absoluta* eggs (N=15 per treatment) evenly distributed over a tomato plant leaflet, for 24 h. The number of parasitized and unparasitized *T. absoluta* eggs consumed was recorded.

2.3. Voracity and feeding preference of *M. pygmaeus* fed on mixed diets of *T. absoluta* eggs unparasitized or parasitized by *T. achaeae*

Females of *M. pygmaeus* that were 9-12-day-old were offered parasitized/unparasitized eggs in 3 ratios: 30:70, 50:50 and 70:30 in a total of 100 eggs. Eggs were spread over the tomato plant leaflet surface. No lepidopteran eggs were provided to females in the previous 24 h of the experiment. Females were allowed to feed for 24 h and then the remaining number of eggs was counted. Feeding preference was calculated using the formula of "Manly's preference index" (α)[34]:

$$\alpha = \frac{\mathrm{Ln}\,\dfrac{(n1 - r1)}{n1}}{\mathrm{Ln}\,\dfrac{(n1 - r1)}{n1} + Ln\,\dfrac{(n2 - r2)}{n2}}$$

where:

n1 and *n2* are the number of *T. absoluta* parasitized or unparasitized eggs provided.

r1 and *r2* are the number of *T. absoluta* parasitized or unparasitized eggs eaten in 24h by *M. pygmaeus*.

2.4. Effect of intraguild interactions between *M. pygmaeus* and *T. achaeae* on the number of *T. absoluta* eggs consumed and/or parasitized under laboratory conditions.

The methodology was adopted from Northfield et al.[35] We carried out a set of 3 experiments in which 500 unparasitized *T. absoluta* eggs were offered to different numbers of *M. pygmaeus* and/or *T. achaeae* for 24 h. Experiment 1 (conspecific tests with parasitoid females) and Experiment 2 (conspecific tests with predatory adult females) consisted in 4 treatments in which 1, 2, 4 or 6 natural enemies were offered *T. absoluta* eggs. Experiment 3 (heterospecific treatments with predator + parasitoid females) consisted in 4 treatments in which 1+1, 2+2, 4+4 or 6+6 natural enemies were offered to *T. absoluta* eggs.

To provide for the very large number of *T. absoluta* eggs needed for this experiment (500 eggs x 15 replicates x 12 treatments), we placed 250 fresh eggs (< 24 h) on one leaflet and 250 eggs that had been kept at 5ºC for a maximum of 10 days on another leaflet. Treatments were replicated 15 times. As in the previous experiments, *M. pygmaeus* females were only fed on tomato and honey diluted in water before the tests. Tests were performed for 24 h, and the number of eggs eaten were counted. The remaining eggs were kept at 25ºC for 6-7 days until parasitized eggs had turned black, to determine the number of parasitized eggs.

2.5. Effect of intraguild interactions between *M. pygmaeus* and *T. achaeae* on the number of *T. absoluta* eggs consumed and/or parasitized in semi-field conditions

The semi-field experiment was carried out in a biologically managed tomato crop greenhouse. Using sleeve cages, tomato leaves from the mid and high strata of the plant were isolated. Twenty to thirty *T. absoluta* adults were introduced inside the sleeve cage to oviposit for 2-3 days. After that time, the lepidopteran was removed and the predators and/or the parasitoids were released and kept for 24 h inside the sleeve cage, according to the respective experimental treatment. A control treatment was performed wherein the sleeve cage was removed, leaving the tomato plant leaf containing *T. absoluta* eggs exposed to environmental conditions in the greenhouses, including naturally occurring natural enemies, the most abundant of which were *M. pygmaeus*, *Dicyphus cerastii* Wagner (Hemiptera: Miridae) and *T. achaeae*.[36] To assess the presence of naturally occurring *T. absoluta* in the greenhouse during experiments, a random tomato plant leaf was cut daily and brought to the laboratory to check for the presence of *T. absoluta* eggs. Very few lepidopteran eggs were found, attesting to the low population density of the pest in the greenhouse. Predators and parasitoids were obtained in the laboratory as previously described. Each treatment was repeated 15 times. At the end of the experiments, the tomato plant leaves were cut and brought back to the laboratory for observation under microstereoscope to determine the number of eggs consumed. The tomato plant leaves of the treatments containing *T. achaeae* were kept at 25ºC until any parasitized egg turned black, to determine parasitism rate. The experiments started on July 24th 2021, and were completed by August 19th 2021.

2.6. Statistical analysis

Voracity of *M. pygmaeus* fed on single diets of parasitized or unparasitized eggs of *T. absoluta* was analyzed using a t-test. Univariate general linear model (GLM) with one-way ANOVA was used to test the effect of i) mixed diets of parasitized and unparasitized eggs on the voracity of *M. pygmaeus* ii) the densities of *M. pygmaeus* and/or *T. achaeae* on the consumption of parasitized and/or unparasitized *T. absoluta* eggs, iii) *in-situ* efficacy of *T. achaeae* and/or *M. pygmaeus* against *T. absoluta*. Pairwise multi comparisons were performed and P values were corrected using Bonferroni test. When two factors were in consideration, a Generalized Linear Model (GZLM) was performed. Wilcoxon's matched-pairs signed rank test (WMPSR; $P < 0.05$) was used to compare the feeding preference (Manly index).

Normal distribution and homogeneity of variances of data were assessed using the Kolmogorov-Smirnov and the Levene tests, respectively. In cases where data were not distributed normally, the Kruskal-Wallis test ($P < 0.05$), was performed applying the Bonferroni correction. Data were log transformed (consumption +1) when zero values occurred in the data set. Mean values were considered significantly different when $P < 0.05$. All statistical analyses were done using SPSS v. 27.[37]

3. Results

3.1. Voracity of *M. pygmaeus* fed on single diets of *T. absoluta* eggs unparasitized or parasitized by *T. achaeae*

There was a significant difference between the voracity of *M. pygmaeus* adults fed on parasitized (13.6 ± 3.5) and unparasitized (60.7 ± 5.3) *T. absoluta* eggs (t-test = -7.311, df = 28, p = 0.001; Figure 1).

3.2. Voracity and feeding preference of *M. pygmaeus* fed on mixed diets of *T. absoluta* eggs unparasitized or parasitized by *T. achaeae*.

We found a significant interaction between the proportion of eggs provided that were parasitized, and the proportion of parasitized versus unparasitized eggs consumed (Chi-Square = 23.032, df = 2, P < 0.001). Because of this, we analyzed the independent factors separately. Concerning total voracity, we found a significant difference between the three mixed diets (Chi-Square = 18.248, df = 2, P < 0.001). When the proportion of parasitized to unparasitized eggs was 70:30, the mean number of eggs consumed was 21.4 ± 2.9; for the proportion 50:50, the mean number of eggs consumed was 33.3 ± 4.5; and finally for the proportion 30:70, the mean number of consumed eggs was 48.2 ± 4.6. Pairwise comparison showed a significant difference between all proportions (Figure 2A). Significantly larger numbers of unparasitized eggs were eaten when they were offered in the proportion 30:70 than in the two other proportions (70:30 = 16.6 ± 2.1 unparasitized eggs eaten, 50:50 = 25.8 ± 3.9 and 30:70 = 42.7 ± 4.0; Chi-Square = 24.848, df = 2, P < 0.001) (Figure 2A), but the number of parasitized eggs eaten did not differ significantly among treatments (70:30 = 4.8 ± 1.0, 50:50 = 7.5 ± 1.3 and 30:70 = 5.5 ± 1.2; Chi-Square = 2.647, df = 2, P < 0.266) (Figure 2A).

Regarding feeding preference, *M. pygmaeus* showed a significant preference for unparasitized eggs (WMPSR; 70:30, $Z = -2.803$, $P = 0.005$; 50:50, $Z = -2{\cdot}344$, $P < 0.019$; 30:70, $Z = -3.408$, $P < 0.001$) (Figure 2B).

3.3. Effect of intraguild interactions between *M. pygmaeus* and *T. achaeae* on the number of *T. absoluta* eggs consumed and/or parasitized under laboratorial conditions

3.3.1. Conspecific experiments

We found significant differences in the number of eggs of *T. absoluta* eaten by *M. pygmaeus* between the four treatments (ANOVA: $F = 39.083$, $df = 3$, $P < 0.001$), and voracity significantly increased with predator density. No differences were found between single and two predator treatments, but they differed from the other two treatments (Figure 3). The number of eggs consumed is lower than predicted for additive and non-interactive scenarios (Figure 3).

There was a significant difference in the number of eggs of *T. absoluta* parasitized by *T. achaeae* between the four treatments (Kruskal-Wallis test: $Z = 13.423$, $df = 3$, $P = 0.004$), and parasitism significantly increased with parasitoid density. No differences were found between treatments with single and two parasitoids, but these two treatments differed significantly from the treatments with four and six parasitoids (Figure 3). The number of *T. absoluta* eggs parasitized by *T. achaeae* and the expected parasitism predicted for additive and non-interactive scenarios, are similar (Figure 3).

3.3.2. Heterospecific experiments

We found a significant interaction between factors (feeding mode: parasitism by *T. achaeae* vs predation by *M. pygmaeus*, and natural enemy densities: 1+1, 2+2, 4+4 or 6+6) in the parasitism/consumption of *T. absoluta* eggs (Wald Chi-Square = 56.699, df = 3, $P < 0.001$). There was a significant difference between the number of eggs parasitized (7.52 ± 1.29) and the number of eggs consumed (139.10 ± 10.57) (Wald Chi-Square = 5011.389, df = 1, $P \leq 0.0001$). We also found a significant increase in the overall parasitism and consumption with the increase in heterospecific abundance of *T. achaeae* and *M. pygmaeus* (Wald Chi-Square = 413.474, df = 3, $P \leq 0.0001$, 1+1: 26.87 ± 4.78, 2+2: 53.53 ± 10.46, 4+4: 81.67 ± 14.10, 6+6: 131.11 ± 22.55). No significant differences were found between treatment 1 (1 female of *M. pygmaeus* + 1 female of *T. achaeae*) and treatment 2 (2 females of *M. pygmaeus* + 2 females of *T. achaeae*), but treatments 3 and 4 (4 females of *M. pygmaeus* + 4 females of *T. achaeae* and 6 females of *M. pygmaeus* + 6 females of *T. achaeae*, respectively) differ between them. Pairwise comparisons only show the absence of a significant difference between the number of eggs parasitized by *T. achaeae* in the treatments 1 and 2 (Figure 4). At higher densities, the number of eggs of *T. absoluta* consumed by *M. pygmaeus* and parasitized by *T. achaeae* was lower than that predicted for additive and non-interactive scenarios (Figure 4).

3.4. Effect of intraguild interactions between *M. pygmaeus* and *T. achaeae* on the number of *T. absoluta* eggs consumed and/or parasitized under field conditions

We found a significant difference between the rate of parasitism and consumption between experimental treatments (Kruskal-Wallis test: $Z = 6.018$, df = 3, $P < 0.001$). Pairwise comparisons only showed the absence of a significant difference between the following treatments: conspecific treatment with 6 females of *T. achaeae* and the heterospecific treatment (Figure 5).

Discussion

We evaluated the voracity of *M. pygmaeus* females on *T. absoluta* eggs unparasitized or parasitized by *T. achaeae*. Predatory females were able to consume a high number of unparasitized eggs but consumed significantly fewer parasitized eggs. Urbaneja et al.[28] observed identical values, but Chailleux et al.[38], recorded a much lower mean number of eggs for an experimental period of 12h; an individual predator consumed roughly 10 unparasitized eggs and only roughly 2 parasitized eggs. These results provide evidence that intraguild predation occurs on *T. achaeae* and suggests that releases of *T. achaeae* against *T. absoluta* in tomatoes may slightly decrease the level of pest control when the predatory mirids are present. However, only in choice test experiments it is possible to assess the extent to which decreases in level of pest control may occur.

On mixed diets of unparasitized and parasitized eggs, the predator showed an overall decrease in voracity, even when 70% of the diet was composed by unparasitized eggs. Voracity decreased as the proportion of parasitized eggs increased. Independently of the mixed diet provided, no significant differences were found in the voracity of *M. pygmaeus* on parasitized eggs. Overall, fewer eggs were consumed when parasitized and unparasitized eggs were offered as a mixed diet than as single diets. These results highlight that, under mixed diets, even including *ad libitum* unparasitized eggs of *T. absoluta*, females of *M. pygmaeus* also consumed eggs parasitized by *T. achaeae*. The consumption of oophagous parasitoids, including *Trichogramma* spp. by predators is common[39] even for *T. achaeae*.[38]

Macrolophus pygmaeus engages in intraguild predation on parasitized eggs of *T. absoluta*.[38] The occurrence of intraguild predation was also demonstrated in our study; however, *M. pygmaeus* preferred to consume unparasitized eggs of *T. absoluta*. Feeding preference toward unparasitized prey occurs in other phytophagous species. For instance, Malo et al.[40] conducted a choice test in which an equal number of unparasitized nymphs or adults of *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) *vs* nymphs or adults parasitized by the parasitoid *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) were provided to *M. pygmaeus*. These authors found that *M. pygmaeus* consumed more unparasitized than parasitized preys. Preference response, however, is strongly affected by the relative abundances of the prey offered[11-13] and, in contrast to the aforementioned study, our methodological procedures in which we provided to the predator varying ratios of parasitized/unparasitized eggs (30:70, 50:50 and 70:30 in a total of 100 eggs), guards against this possibility.

In the conspecific experiments, as the number of *M. pygmaeus* females increased, both the number of eggs consumed as well as mutual interference between mirids increased. In comparison with the expected number of eggs consumed in the absence of mutual interference among mirids, decreases of 29.0, 32.8 and 37.6% in egg consumption were recorded for the treatments with two, fourth and six predators, respectively. For *T. achaeae*, mutual interference seems to occur in an unpredictable manner. That is, if we consider the number of parasitized eggs predicted for an additive scenario, 58.1 and 13.9% fewer parasitized eggs were recorded for the treatments with two and six parasitoids, respectively, but 70.2 % more parasitized eggs were recorded for the treatment with four parasitoids. From an applied point of view, our results indicate that augmentative biological control programs against *T. absoluta* using monospecific releases should always take into consideration the density of eggs of *T. absoluta*.

Efficacy of biological control programs may be enhanced by using a combination of different natural enemies. Simultaneous use of *M. pygmaeus* and *T. achaeae* against *T. absoluta*, however, may lead to increased, reduced, or similar efficacy. The results of our heterospecific experiments revealed that fewer *T. absoluta* eggs were parasitized or consumed than expected from conspecific treatments, that is, the rate of parasitism was decreased from a minimum of 46.7 to a maximum of 71.6% of that predicted for the additive scenario. The number of eggs consumed by females of *M. pygmaeus* was decreased from that predicted for the additive scenario, by 21.7 and 14.4% for the treatments with eight (4 *M. pygmaeus* + 4 *T. achaeae*) and twelve (6 *M. pygmaeus* + 6 *T. achaeae*) biological control agents, respectively, but was slightly increased by 6.8 % in the treatment with four (2 *M. pygmaeus* + 2 *T. achaeae*) biological control agents. If we combine the results of the four heterospecific treatments, there was an overall decrease in mortality of *T. absoluta* eggs from the rate predicted for the additive scenario, and this decrease intensifies as the number of natural enemies increases; by 0.4, 24.0 and 64.9% for the treatments with four, eight and twelve biological control agents, respectively. All of these results indicate a possible occurrence of antagonistic interactions, given that the rate of parasitism by females of *T. achaeae* and the consumption by females of *M. pygmaeus* were lower than predicted for non-interactive scenario. Previous results have similarly shown that *T. achaeae*, from south of France, contributed to control of *T. absoluta* compared to what could be achieved when only the mirid predator *M. pygmaeus* was present, but without full additive effects of the two natural enemies together.[38]

Our results do not clarify the mechanisms underlying the alteration in parasitism and consumption of *T. absoluta* eggs. However, it is likely that foraging activity of the mirids reduced the availability of *T. absoluta* eggs for the parasitoids, and that the mirids also preyed on eggs parasitized by *T. achaeae*. In a no-choice experiment, Chailleux et al.[38] noted that *M. pygmaeus* fed about tenfold less *T. achaeae* parasitized *T. absoluta* eggs (parasitized for more than 4–5 days) than unparasitized eggs. In the same study, authors found that feeding rates on *T. absoluta* eggs parasitized for 0–3 days, were similar to unparasitized eggs. A laboratory study in which *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) was exposed to *T. absoluta* eggs, then *Dolichogenidea gelechiidivoris* (Marsh)

(Hymenoptera: Braconidae) was exposed to hatching larvae, revealed that their combined efficacy significantly reduced *T. absoluta* adult eclosion compared with when either biological control agent acted alone.

The results obtained in the experiment under semi-field conditions, coupled with the results of laboratory experiments, confirm that *T. achaeae* may significantly increase the level of control of the pest over what could be achieved when only *M. pygmaeus* is present in tomatoes. The results obtained in a similar field study shows a better control of *T. absoluta*, by just over 20%, when *N. tenuis* and *T. achaeae* were both present, despite the occurrence of intraguild predation against the parasitoid.[32]

Importantly, other eco-friendly alternatives to chemical pesticides, such as the use of microbial and/or botanical pesticides alone or in combination with releases of *Trichogramma* parasitoids or predatory mirid bugs[42-43], could also be evaluated in the near future before to be implemented in sustainable pest management programs of *T. absoluta* in Portugal. In this context, a recent study shows the economic and financial commercial viability of a continuous mass production of *M. pygmaeus*.[44]

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Fig. 1. Voracity [mean number of eggs consumed (± SE)] by adults of *M. pygmaeus* fed on single diets of eggs of *T. absoluta* parasitized by *Tr. achaeae* or unparasitized. Different letters indicate significant differences (t-test; P < 0.05).

Fig. 2. Voracity (Panel A) and Manly's preference index (Panel B) for adults of *M. pygmaeus* fed on three different ratios of *T. absoluta* parasitized and unparasitized eggs (70 parasitized:30 unparasitized, 50 parasitized:50 unparasitized or 30 parasitized:70 unparasitized). For panel A), different letters indicate significant differences in total voracity between the three diets, and different number of asterisks indicate a significant difference between total voracity of unparasitized eggs (GZLM; P < 0.05). For panel B) Different letters indicate significant differences (Wilcoxon WMPSR test; P < 0.05).

Fig. 3. Number of *T. absoluta* eggs consumed by *M. pygmaeus* (white histograms) and number of *T. absoluta* eggs parasitized by *T. achaeae* (black histograms) in treatments in which the number of conspecifics was provided. Different letters in each panel indicate significant differences between treatments (GLM; P < 0.05). Black lines over the bars depicted the number of eggs consumed or parasitized predicted for the case of additive and non-interactive between conspecifics.

Fig. 4. Number of *T. absoluta* eggs parasitized by *T. achaeae* or consumed (± SE) by *M. pygmaeus* in treatments in which the number of heterospecifics provided increased. Different letters in each panel indicate significant differences between treatments (P < 0.05). Black lines over the bars depicted the number of consumed eggs predicted for the case of additive and non-interactive between conspecifics.

Fig. 5. *In-situ* impact of biological control agents against *T. absoluta* (proportion of *T. absoluta* eggs consumed by *M. pygmaeus* and parasitized by *T. achaeae*), after field release of conspecific or heterospecific guilds of *T. achaeae* and *M. pygmaeus*. Different letters in

the panel indicate significant differences between treatments (Kruskal-Wallis test; $P < 0.05$).

Fig. 1

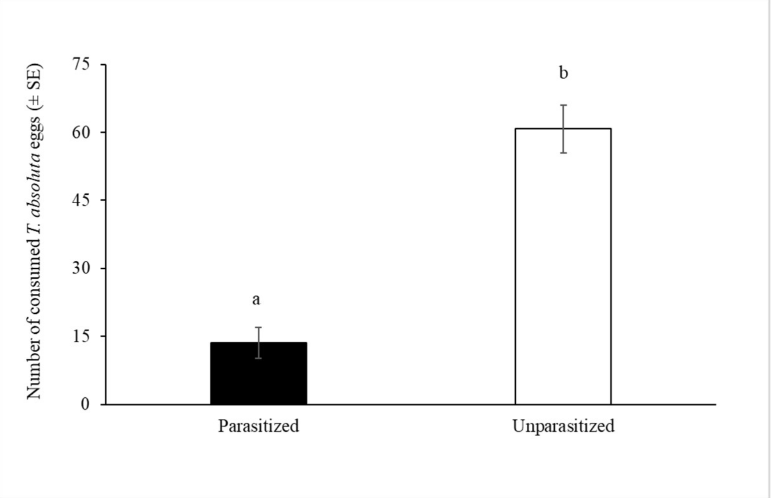
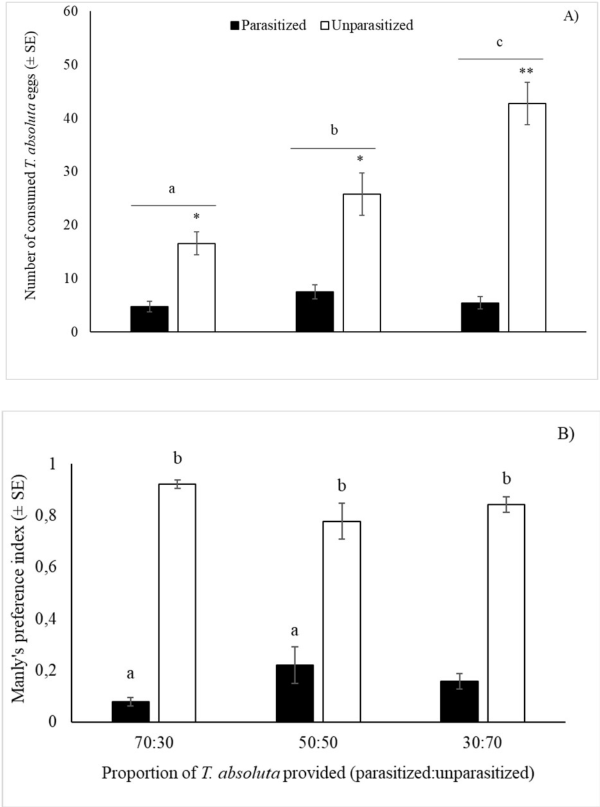
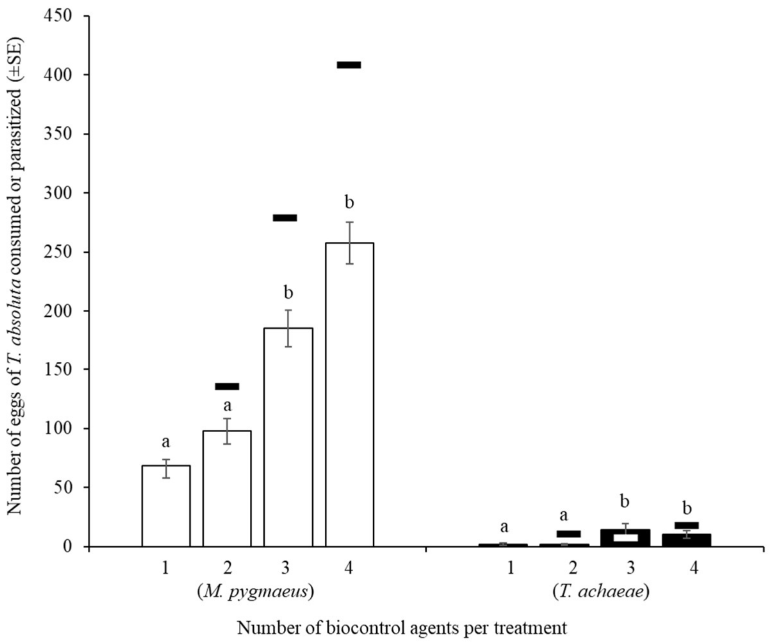

Number of consumed *T. absoluta* eggs (± SE)
75
60
45
30
15
0
a
b
Parasitized
Unparasitized

Fig. 2



A)
■Parasitized □Unparasitized
Number of consumed T. absoluta eggs (± SE)
0
10
20
30
40
50
60
a
b
c
*
*
**
B)
Manly's preference index (± SE)
0
0,2
0,4
0,6
0,8
1
a
b
a
b
b
70:30
50:50
30:70
Proportion of T. absoluta provided (parasitized:unparasitized)

Fig 3



450
400
350
300
250
200
150
100
50
0
Number of eggs of T. absoluta consumed or parasitized (±SE)
a
a
b
b
a
a
b
b
1
2
3
4
(M. pygmaeus)
1
2
3
4
(T. achaeae)
Number of biocontrol agents per treatment

Fig. 4

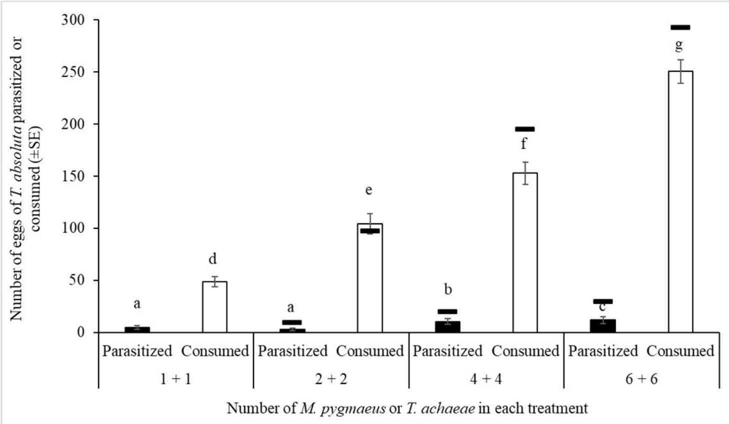
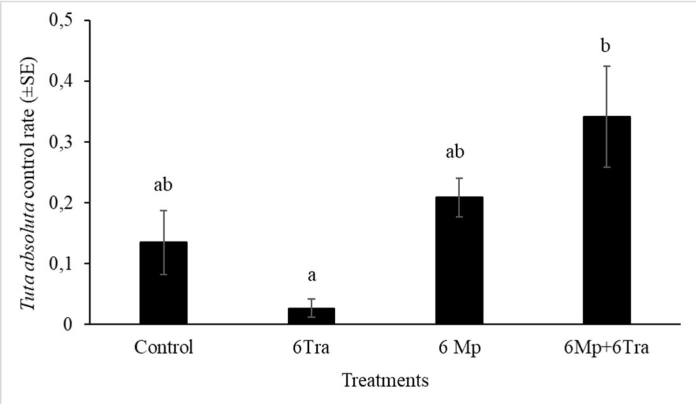

Number of eggs of T. absoluta parasitized or consumed (±SE)
300
250
200
150
100
50
0
a
d
a
e
b
f
c
g
Parasitized
Consumed
Parasitized
Consumed
Parasitized
Consumed
Parasitized
Consumed
1 + 1
2 + 2
4 + 4
6 + 6
Number of M. pygmaeus or T. achaeae in each treatment

Fig. 5


0,5
0,4
0,3
0,2
0,1
0
Tuta absoluta control rate (±SE)
ab
a
ab
b
Control
6Tra
6 Mp
6Mp+6Tra
Treatments

Task 6. Title: Development of an economic and financial model toward mass rearing and marketing of BCA solutions against *T. absoluta*.

Research Article

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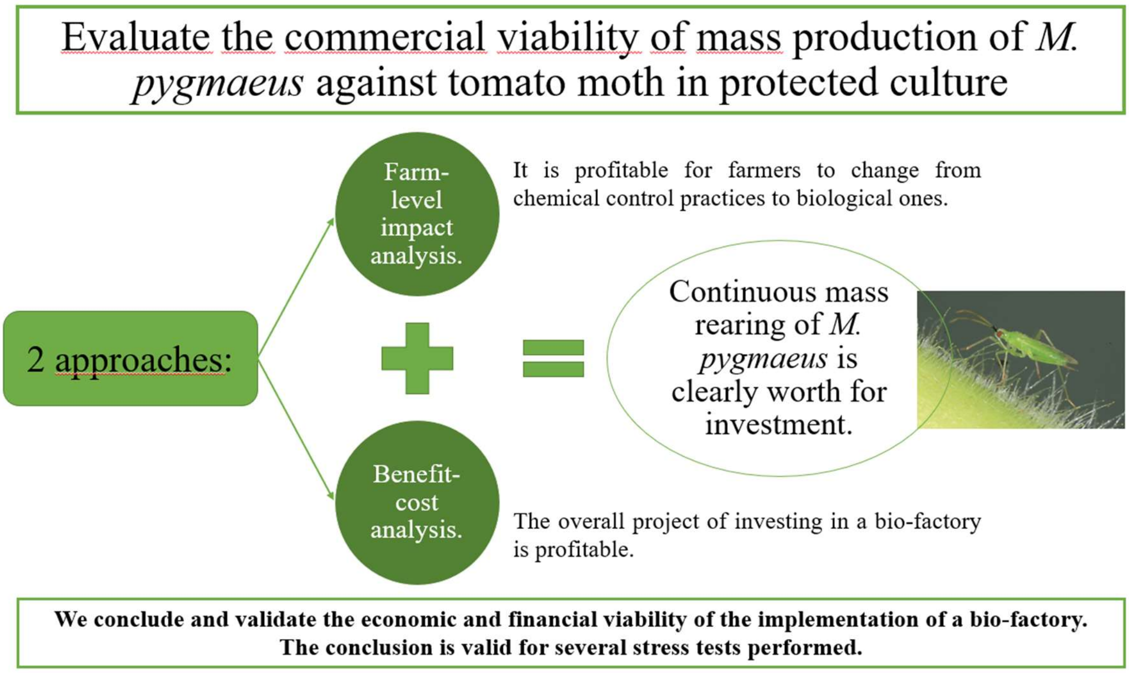

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