Management of *Tuta absoluta* with introduced and native biocontrol agents

S Mama Sambo

orcid.org 0000-0002-4470-5622

Thesis submitted in fulfilment of the requirements for the degree *Doctor of Philosophy in Science with Environmental Sciences* at the North-West University

Promoter:	Prof MJ du Plessis
Co-promoter:	Dr SA Mohamed
Co-promoter:	Dr S Ndlela

Examination November 2022

38961245

ACKNOWLEDGMENTS

This study was financially supported by several organizations and institutions. To name some of the organizations, institutions and individuals, my extreme gratitude goes to: the German Academic Exchange Service (DAAD), your program of African support made this dream a reality; my host university, North-West University (NWU) for accepting me as a student and offering me the chance to be taught from two different international organizations; the International Centre of Insect Physiology and Ecology (*icipe*) for intellectual, material, and financial support through the African Regional Post-graduate Programme in Insect Science (ARPPIS). Thank you to all the staff for the different forms of assistance. Thank you to the *icipe* capacity building team, the former head Dr. Rob Skilton, the current head Dr. Sunday Ekesi, Margaret Ochanda, Vivian Atieno, and Esther N'dungu; the Biovision Foundation grant number BV DPP-012/ 2019-2022 and Norwegian Agency for Development Cooperation-(NORAD), the section for research, innovation, and higher education grant number RAF-3058 KEN-18/0005 whose materially and financially supported this project.

Additionally, the results could not have been achieved without the support, assistance, and guidance of my supervisors. My sincere gratitude to my university supervisor, Prof Hannalene du Plessis, researcher and lecturer at North-West University, Potchefstroom campus, South Africa, for having accepted me as a student. She followed and encouraged my work with dynamism. During these three years, she granted me moral, scientific, and even financial support and assistance with any administrative issues concerning registration and other university requirements. My recognition to my host institution primary supervisor, Dr Samira Abuelgasim Mohamed, Senior Scientist under the plant health team at *icipe*, Nairobi, Kenya, who initiated and supervised this thesis. She gave me scientific support, while allowing free rein to my curiosity and initiatives. My sincere appreciation to my host institution co-supervisor, Dr Shepard Ndlela, post-doctoral fellow under the plant health team at *icipe*, Nairobi, Kenya, who supervised this thesis, and followed the activities and progress. Thank you for your encouragement, advice, and support. I would also like to acknowledge Dr Akutse Komivi, Senior Scientist under the plant health team at *icipe*, Nairobi, Kenya, for his mentorship during execution work to fulfil some of the objectives of this thesis. I appreciated your guidance.

Many other staff members contributed to this work. I sincerely appreciate the efforts of all of you. My extreme gratitude goes to: Miss Esther Awuor Owino, for all the technical support during data collection, thank you for all the hard work during this project; Mr. Francis Obala, for all the critical discussions and technical assistance; Dr. Abdelmutalab GA Azrag in particular for his availability and his valuable advice especially with statistical analysis; Dr. H Michael G Lattorff, Visiting Professor at the University of Nairobi, Kenya, and his technical assistant Miss Vivian Hinga for their support during the molecular identification of insects; all the technical staff of the African Fruit Fly Programme (AFFP) of the *icipe*, Nairobi, Kenya, Patrick Koech Kipkorir, Alex Muthembwa, Peterson Nderitu, and Omar Jimali Ibrahim. You provided more than technical assistance, you have been good collaborators and good friends. The technical support of the Arthropod Pathology Unit (APU) staff is highly appreciated. I am particularly thankful to Sospeter Wafula for his assistance; the Kirinyaga and Nakuru farmers, and agriculture extensionist officers, particularly Simon Ndambiri at Kenya Agricultural and Livestock Research Organization (KALRO), Mwea, Kirinyaga County.

My profound gratitude to Dr. Pascal M. Ayelo, Dr. Pascal O. Aigbedion-Atalor, and Mr. Ayaovi Agbessenou for their advice and comments on the thesis progress; my PhD colleagues of ARPPIS/DRIP, particularly 2020 and 2021 streams, their support in many ways added fun to this journey. Thanks to you Rehemah Gowkyalya and Bashiru Adams; my cohort mates Kavengi Kitonga, Birhanu Sisay, Ong'wen Fedinand, and Aklilu Belay.

Finally, thanks Dr. Miriam Frida Karlsson, Dr. Oduor Bonaventure Omondi Aman, Prof. Aimé H. Bokonon-Ganta, and Dr. Antonio Sinzogan, my career in research started with you and I am grateful of your support.

ABSTRACT

Tomato, *Solanum lycopersicum* L. (Solanaceae) is severely damaged by the South American tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Surveys among producers confirmed that the use of synthetic insecticides increased since the invasion of *T. absoluta*. The risks associated with the residual effect of insecticides in edible food and the negative effects it may have on ecosystem services, also increased. The aim of this project was to investigate the management of *T. absoluta* with introduced and native biocontrol agents in Kenya. These included a parasitoid introduced from South America, *Dolichogenidea gelechiidivoris* Marsh (Hymenoptera: Braconidae), native parasitoids, and the entomopathogenic fungus, *Metarhizium anisopliae* ICIPE 20. Release of *D. gelechiidivoris* was preceded by studies of its functional responses and the tritrophic interactions between the parasitoid and native parasitoids. For the functional responses, densities of *T. absoluta* larvae (20, 50, 100, 150, and 200) were tested with two densities of parasitoid (1 and 3 females). For the interaction with the native parasitoids, short term interspecific competition assays between *D. gelechiidivoris* and *Stenomesius* sp. near *japonicus* (Ashmead) (Hymenoptera: Eulophidae) have been conducted. Bioassays were also performed to investigate long term interaction between *D. gelechiidivoris* and a native parasitoid, *Bracon nigricans* Szépligeti (Hymenoptera: Braconidae). The susceptibility of *D. gelechiidivoris* to *M. anisopliae* ICIPE 20 was also assessed, followed by testing of the parasitism performance of *D. gelechiidivoris* and *M. anisopliae* individually as well as in combination under greenhouse conditions. The exotic parasitoid was also released in open-field and its dispersal was evaluated.

Parasitism by *D. gelechiidivoris* was positively correlated with density of the larval host, regardless of whether the wasp occurred individually or in groups. The highest emergence of these wasps from parasitized host larvae was at a high larval pest density of 100 larvae. Two native endogenous parasitoids species *viz*. *Bracon nigricans*, and *Stenomesius* sp. near *japonicus*, were identified and successfully maintained on *T. absoluta* larvae. Using an ecological niche prediction, high to very high suitability was shown for occurrence of *B. nigricans* in major parts of Africa. In a short-term interaction between *D. gelechiidivoris* and *Stenomesius* sp. near *japonicus*, the exotic parasitoid performed much better than the native species. However, in a long-term interaction study between *D. gelechiidivoris* and *B. nigricans*, the native parasitoid negatively affected population growth of the exotic *D. gelechiidivoris*. The same level of pest control was achieved where both species co-occurred as well as where each species of parasitoid was individually present. The investigation of the potential use of *M. anisopliae* ICIPE 20 with *D. gelechiidivoris* showed the fungus to negatively affect the longevity of the adult parasitoids as well as the survival of parasitised larvae. However, the percentage parasitism by fungus-infected female wasps, remained high (> 70%). Fungus infection of parasitized larvae at different ages, reduced parasitoid emergence by 35% and 23% for infection at one and five days post-parasitisation. Exposure of healthy-*D. gelechiidivoris* adults to a plant-sprayed with fungus did not affect their longevity, and no discriminatory host selection was found. The parasitoid did also not differentiate between sprayed and non-sprayed host plants. Significantly fewer *T. absoluta* moths eclosed from host plants sprayed with *M. anisopliae* ICIPE 20, prior to being exposed to *D. gelechiidivoris* compared to infested plants sprayed with the fungus only or exposed to the parasitoid only. These results were confirmed in a greenhouse trial with fewer fruits infested with *T. absoluta* when both agents were combined for control of the pest. Establishment of *D. gelechiidivoris* after its release was confirmed with recovery from the area of release, one year after its introduction. The findings of this thesis offer promising tactics including the use of the exotic parasitoid *D. gelechiidivoris*, native parasitoids, and an entomopathogenic fungus to develop sustainable IPM methods to control *T. absoluta*.

Keywords: Exotic parasitoid, native parasitoid, entomopathogenic fungus, co-occurrence, integrated pest management, South American tomato moth

PREFACE

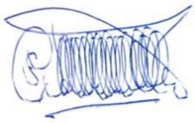
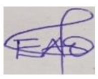
This thesis follows the article style format as specified by the North-West University (NWU) guidelines. The format of the published articles follows the instructions to authors of the respective internationally accredited, scientific journals. Chapters that are not intended to be published were prepared according to the general guidelines for theses as prescribed by the NWU (Table A).

Table A: Publication status of manuscripts and articles in this thesis.

Chapter	Status	Journal
Chapter 1	-	NWU general guidelines
Chapter 2	-	NWU general guidelines
Chapter 3	Article 1: Published	*Biocontrol Science and Technology* (Taylor & Francis)
Chapter 4	Article 2: Published	*Insects* (MDPI)
Chapter 5:	Article 3: Submitted	*Biological control* (Elsevier)
Chapter 6:	Article 4: Submitted	*International Journal of Tropical Insect Science* (Springer)
Chapter 7:	Article 5: Published	*Biology* (MDPI)
Chapter 8:	Article 6: Prepared for submission	*Entomologia Generalis* (Schweizerbart Science Publishers)
Chapter 9:	-	NWU general guidelines

The instructions to authors of the respective journals are included as Appendix A-E. The contributions of authors to each article/manuscript and their consent to use it as part of this thesis, are provided in Table B.

Table B: Author contributions and consent for use of the respective articles/manuscripts in this thesis.

Authors	Articles	Contribution	Consent
S Mama Sambo	1-6	**Principl investigator** • Study conceptualization • Study design • Data collection • Formal statistical analysis • Preparation of original draft • Reviewing and editing	
MJ du Plessis	1-6	**PhD promotor** • Study conceptualization • Study design • Supervision • Reviewing and editing	
SA Mohamed	1-6	**PhD co-promotor** • Study conceptualization • Study design • Supervision • Reviewing and editing	
S Ndlela	1-6	**PhD assistant-promotor** • Study conceptualization • Study design • Supervision • Reviewing and editing	
F Obala	1 and 2	**Collaborator** • Study conceptualization • Study design • Reviewing and editing	
GA Azrag	3	**Collaborator** • Formal statistical analysis • Reviewing and editing	
EA Owino	4	**Collaborator** • Data collection • Reviewing and editing	

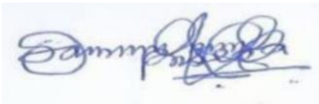
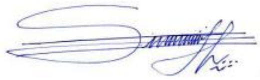
PO Aigbedion-Atalor	5	**Collaborator** • Study conceptualization • Study design • Reviewing and editing	
KS Akutse	5 and 6	**Collaborator** • Study conceptualization • Study design • Supervision • Reviewing and editing	

TABLE OF CONTENT

CHAPTER 1: INTRODUCTION

1.1. Introduction

The invasive tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) invaded areas outside is area of origin since 2006 and is currently spreading rapidly in Europe, Africa and Asia (Desneux *et al.*, 2010; Han *et al.*, 2019). The pest is indigenous to South America, arrived in Spain in 2006 (Desneux *et al.*, 2010) and subsequently spread all over southen Europe getting to northern Africa in 2008 (Abbes *et al.*, 2013; Guenaoui and Ghelamallah, 2008), and East Africa during 2012-2014 (Mansour *et al.*, 2018; Mohamed *et al.*, 2012). Larvae of this multivoltine insect develop in leaves, fruit, flowers, buds, and stems of tomato plants (Guenaoui and Ghelamallah, 2008; Pfeiffer *et al.*, 2019). This pest causes up to 100% yield loss in open-field tomato (El-aassar *et al.*, 2015; Chidege *et al.*, 2016; Ndereyimana *et al.*, 2019) .

Tuta absoluta is generally controlled with insecticides to reduce yield losses in both open-field and greenhouse tomato crops (Aigbedion-Atalor *et al.*, 2019; Rwomushana *et al.*, 2019). Efficacy of synthetic insecticide sprays is, however, hampered by the endophytic feeding behaviour of the larvae. Additionally, resistance to insecticides has been reported from many areas including South-America and invaded regions (Roditakis *et al.*, 2012, 2017; Ayalew, 2015; Grant *et al.*, 2019; 2021). Pesticide residues affect the health of tomato consumers (Mahugija *et al.*, 2017) as well as farmers applying insecticides (Marete *et al.*, 2021). Adverse effects on the environment such as negative effects on parasitoids, predators, pollinators, and microorganisms in the soil, also occur (Brittain and Potts, 2011; Cloyd and Bethke, 2011; Abbes *et al.*, 2015; Mahmood *et al.*, 2016).

Biological control strategies can be used to keep *T. absoluta* under the economic injury level. Native parasitoids and predators formed associations with this pest in invaded areas (Zappalà *et al.*, 2012; Gabarra *et al.*, 2013; Shaltiel-Harpaz *et al.*, 2016; Mansour *et al.*, 2018; Tarusikirwa *et al.*, 2020; Kinyanjui *et al.*, 2021; Seydi *et al*., 2021; Desneux et al., 2022). However, the parasitism rate of parasitoids native to Sub-Saharan Africa is low (Mansour *et al.*, 2018; Kinyanjui *et al.*, 2021; Seydi *et al*., 2021). These are, however, not the only biological control agents known to control *T. absoluta*. Different species and strains of entomopathogenic fungi and nematodes infect *T. absoluta* at different stages and are used in the management of this pest (Gözel and Kasap, 2015; Mahmoud, 2017). *Metarhizium anisopliae* isolates ICIPE 18, ICIPE 20, and ICIPE 665 were reported to be highly pathogenic to *T. absoluta* (Akutse *et al*., 2020), and an increase in temperature did not affect their effectiveness (Agbessenou *et al*., 2021). *Metarhizium anisopliae* and *Beauveria bassiana* strains are already commercialised in many African countries for *T. absoluta* control (Rwomushana *et al.*, 2019; Erasmus *et al.*, 2021; Zekeya *et al.*, 2022). Based on the scarcity of native parasitoids and lack of sufficient control of *T. absoluta*, development of classical biological control projects for

T. absoluta has been initiated in East Africa, by importing the original natural enemy of this pest from its country of origin.

Dolichogenidea gelechiidivoris Marsh (Lepidoptera: Gelechiidae) is the most important parasitoid of *T. absoluta* in its native area, Peru. It has been imported into Africa from the International Potato Center (CIP) in Peru (Aigbedion-Atalor *et al.,* 2020). Parasitism of *T. absoluta* by this parasitoid, was reported to be as high as 90% in a field study in Colombia (Morales *et al*, 2014). This parasitoid prefers first- and second-instar larvae (Aigbedion-Atalor *et al*., 2020), and is optimally efficient at 20 °C (Bajonero *et al.,* 2008). Despite the known importance of *D. gelechiidivoris* as a biocontrol agent of *T. absoluta,* literature on its efficacy of control as well as the conditions or factors that affect its effectiveness in areas outside its native region, is scarce.

The life history parameters and performance of biological control agents in general, are affected by abiotic factors such as temperature and relative humidity (Ahola *et al*., 2004; Mohamed *et al*., 2006; Dannon *et al*., 2010; Martins *et al*., 2016) and also by biotic factors such as inter- and intraspecific competition, host, age and exposure time (Sujii *et al.,* 2002; Farahani, 2013; Chen *et al*., 2017; Rostami *et al.,* 2017). Knowledge on the natural enemy complex already present in the area of introduction, climate and ecological matching are also important (Kenis *et al*., 2019). It is also important to consider intraspecific competition caused by superparasitism in the development of parasitoid mass-rearing and field-release protocols (González *et al.,* 2010; Tunca *et al.,* 2016). Female wasps can occasionally differentiate between non-parasitized hosts and hosts parasitized by conspecific species. Parasitizing the most recently parasitised host, increase the chances of their progeny to be successful in the competition for resources (Bai, 1991; Weisser and Houston, 1993). Mated female parasitoids sometimes regulate the sex ratio of their offspring in response to intraspecific competition (King, 2002; Shuker *et al.,* 2006). Hence, the density of the host and the parasitoid are both crucial factors in a biological control program. Various studies have been conducted on the effect of host density (*T. absoluta* larvae) and the parasitoids of this host , also known as the type of functional response (Sánchez *et al.,* 2009; Savino, Coviella and Luna, 2012; Bodino *et al.,* 2018).

The level of pest control provided by multiple natural enemies inhabiting the same agroecological niche, can be strengthened by the complementarity of these enemies, since each of them might have a preference for a different host stage (Jonsson *et al.,* 2017). However, many interferences in resource sharing might occur (Bográn *et al.,* 2002; Xu *et al.,* 2013; Cusumano *et al.,* 2013, 2016), which can affect the performance of one of the natural enemies (Reitz and Trumble, 2002; Wang *et al.,* 2008; Feng *et al.,* 2015; Tan *et al.,* 2016). The association between parasitoids can enhance *T. absoluta* control, for example, Luna *et al.* (2015) reported high pest control in a field where both

Dineulophus phthorimaeae (De Santis) (Hymenoptera: Eulophidae) and *Pseudapanteles dignus* (Muesebeck) (Hymenoptera: Braconidae) occurred. An additive effect in control of *T. absoluta* was also reported for the co-occurrence of the predators, *Nesidiocoris tenuis* Reuter and *Macrolophus caliginosus* Wagner (both Hemiptera: Miridae) and different parasitoid species (Calvo *et al.,* 2012; Chailleux *et al.,* 2013; Kortam *et al.,* 2014). A similar additive effect was also reported for *D. gelechiidivoris* used in combination with *N. tenuis* (Aigbedion-Atalor *et al*., 2021). However, ecological niche occupation order by a natural enemy can play a role in its performance and domination of the respective competitors (Bokonon-Ganta *et al.,* 2005; Ndlela *et al.,* 2020).

Among the native parasitoids identified in the region where classical biological control of *T. absoluta* have been ititiated, many attack the larval stages. It is therefore important to investigate the possible side effects of the interactions with the introduced *D. gelechiidivoris*. Similarly since *D. gelechiidivoris* is a larval parasitoid of *T. absoluta* (Aigbedion-Atalor *et al.,* 2020), the entomopathogenic fungus, *M. anisopliae* may affect this parasitoid. *Dolichogenidea gelechiidivoris* is susceptible to chemical pesticides (Kroschel and Cañedo, 2009; Mujica and Kroschel, 2013), but the effects of biopesticides are, however, not as severe (Marrone, 2007; Gupta and Dikshit, 2010; Kumar and Singh, 2015).

There is no information available to confirm the effectiveness of *D. gelechiidivoris* outside its native range, when used in combination with biocontrol agents such as *M. anisopliae* ICIPE 20 and native parasitoids already present in the area of release. This study therefore aims to fill these knowledge gaps in the options for biocontrol of *T. absoluta* in invaded regions, with special reference to Kenya.

1.2. Research aims

1.2.1. General objective

To investigate the management of *T. absoluta* with introduced and native biocontrol agents, and to explore the combined use of these biocontrol agents in an integrated pest management strategy for *T. absoluta*.

1.2.2 Specific objectives

i. Estimate the functional response type of *D. gelechiidivoris* females to different densities of *T. absoluta* to improve the rearing protocol of *D. gelechiidivoris*.

ii. Investigate the presence and distribution of native parasitoid species associated with *T. absoluta* in open-field and greenhouse tomato in Kenya and to predict their habitat suitability for use in biocontrol programs.
iii. Investigate competition between parasitoids of *T. absoluta*, *viz*. the native *Stenomesius* sp. near *japonicus* and exotic *D. gelechiidivoris,* and between the native *B. nigricans* and the exotic *D. gelechiidivoris.*
iv. Determine the effect of direct and indirect infection of *D. gelechiidivoris* with *M. anisopliae* ICIPE 20 on the performance of the parasitoid.
v. Evaluate the dispersion of *D. gelechiidivoris* in open-field tomato in central Kenya and its performance in combination with *M. anisopliae* for control of *T. absoluta* under greenhouse conditions.

1.2.3 Hypotheses

i. The host:parasitoid density ratio will optimise mass production of *D.* g*elechiidivoris*.
ii. Both native parasitoids, *B. nigricans* and *Stenomesius* sp. near *japonicus* present in Kenya could be implemented as biocontrol agents in IPM programs for control of *T. absoluta* in Sub-Saharan Africa.
iii. The exotic *D. gelechiidivoris* can be used together with the native *Stenomesius* sp. nr. *japonicus* and *B. nigricans* for control of *T. absoluta.*
iv. The combined use of *D. gelechiidivoris* and *M. anisopliae* ICIPE 20 has an additive effect in biocontrol of *T. absoluta*.
v. The imported endoparasitoid, *D. gelechiidivoris,* will establish in Kenya and could be used with the entomopathogenic fungus, *M. anisopliae* for the biological control of *T. absoluta*.

1.3. References

Abbes, K., Biondi, A., Kurtulus, A., Ricupero, M. and Russo, A. 2015. Combined non-target effects of insecticide and high temperature on the parasitoid *Bracon nigricans*. *PLoS One* 10: 1–14. https://doi.org/10.1371/journal.pone.0138411

Abbes, K., Biondi, A., Zappalà, L. and Chermiti, B. 2013. Fortuitous parasitoids of the invasive tomato leafminer *Tuta absoluta* in Tunisia. *Phytoparasitica* 42: 1–8. https://doi.org/10.1007/s12600-013-0341-x

Agbessenou, A., Akutse, K.S., Yusuf, A.A., Wekesa, S.W. and Khamis, F.M. 2021. Temperature-dependent modelling and spatial prediction reveal suitable geographical areas for deployment of two *Metarhizium anisopliae* isolates for *Tuta absoluta* management. *Sci. Rep*. 11:23346.

https://doi.org/10.1038/s41598-021-02718-w

Ahola, M., Laaksonen, T., Sippola, K., Eeva, T., Rainio, K. and Lehikoinen, E. 2004. Variation in climate warming along the migration route uncouples arrival and breeding dates. *Glob. Chang. Biol*. 10:1610–1617. https://doi.org/10.1111/j.1365-2486.2004.00823.x

Aigbedion-Atalor, P.O., Abuelgasim, S., Hill, M.P., Zalucki, M.P., Azrag, A.G.A., Srinivasan, R. and Ekesi, S. 2020. Host stage preference and performance of *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae), a candidate for classical biological control of *Tuta absoluta* in Africa. *Biol. Control* 144:104215. https://doi.org/10.1016/j.biocontrol.2020.104215

Aigbedion-Atalor, P.O., Hill, M.P., Ayelo, P.M., Ndlela, S., Zalucki, M.P. and Mohamed, S.A. 2021. Can the combined use of the mirid predator *Nesidiocoris tenuis* and a braconid larval endoparasitoid *Dolichogenidea gelechiidivoris* improve the biological control of *Tuta absoluta*? *Insects* 12:1–12. https://doi.org/10.3390/insects12111004

Aigbedion-Atalor, P.O., Hill, M.P., Zalucki, M.P., Obala, F., Idriss, G.E., Midingoyi, S.K., Chidege, M., Ekesi, S. and Mohamed, S.A. 2019. The South America Tomato Leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), spreads its wings in Eastern Africa: Distribution and socioeconomic impacts. *J. Econ. Entomol*. 112:2797–2807. https://doi.org/10.1093/jee/toz220

Akutse, K.S., Subramanian, S., Khamis, F.M., Ekesi, S. and Mohamed, S.A. 2020. Entomopathogenic fungus isolates for adult *Tuta absoluta* (Lepidoptera: Gelechiidae) management and their compatibility with Tuta pheromone. *J. Appl. Entomol*. 144:777–787. https://doi.org/10.1111/jen.12812

Ayalew, G. 2015. Efficacy of selected insecticides against the South American tomato moth, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) on tomato in the Central Rift Valley of Ethiopia . *African Entomol*. 23:410–417. https://doi.org/10.4001/003.023.0205

Bai, B. 1991. Conspecific superparasitism in two parasitoid wasps, *Aphidius ervi* Haliday and *Aphelinus asychis* Walker: reproductive strategies influence host discrimination. *Can. Entomol*. 123:1229–1237. https://doi.org/1229-1237. doi:10.4039/Ent1231229-6

Bajonero, J., Córdoba, N., Cantor, F., Rodríguez, D. and Cure, R. 2008. Biology and life cycle of *Apanteles gelechiidivoris* (Hymenoptera: Braconidae) parasitoid of *Tuta absoluta*. *Agron. Colomb*. 26:417–426.

Bográn, C.E.., Heinz, K.M. and Ciomperlik, M.A. 2002. Interspecific competition among insect parasitoids: field experiments with whiteflies as hosts in Cotton. *Ecol. Soc. Am*. 83:653–668.

Bokonon-Ganta, A.H., Ramadan, M.M., Wang, X. and Messing, R.H. 2005. Biological performance and potential of *Fopius ceratitivorus* (Hymenoptera: Braconidae), an egg – larval parasitoid of tephritid fruit fies newly imported to Hawaii. *Biol. Conserv*. 33:238–247. https://doi.org/10.1016/j.biocontrol.2005.02.015

Brittain, C., and Potts, S.G. 2011. The potential impacts of insecticides on the life-history traits of bees and the consequences for pollination. Basic Appl Ecol. 12: 321–331. https://doi.org/10.1016/j.baae.2010.12.004

Calvo, F.J., Lorente, M.J., Stansly, P.A. and Belda, J.E. 2012. Preplant release of *Nesidiocoris tenuis* and supplementary tactics for control of *Tuta absoluta* and *Bemisa tabaci* in greenhouse tomato. *Entomol. Exp. Appl*. 1:111–119. https://doi.org/10.1111/j.1570-7458.2012.01238.x

Chailleux, A., Bearez, P., Pizzol, J., Amiens-Desneux, E., Ramirez-Romero, R. and Desneux, N. 2013. Potential for combined use of parasitoids and generalist predators for biological control of the key invasive tomato pest *Tuta absoluta. J. Pest Sci*. 56:533–541. https://doi.org/10.1007/s10340-013-0498-6

Chen, X., Triana, M. and Stansly, P.A. 2017. Optimizing production of *Tamarixia radiata* (Hymenoptera: Eulophidae), a parasitoid of the citrus greening disease vector *Diaphorina citri* (Hemiptera: Psylloidea). *Biol. Control* 105:13–18. https://doi.org/10.1016/j.biocontrol.2016.10.010

Cherif, A., Mansour, R., Attia-Barhoumi, S., Zappalà, L. and Grissa-Lebdi, K. 2019. Effectiveness of different release rates of *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae) against *Tuta absoluta* (Lepidoptera: Gelechiidae) in protected and open field tomato crops in Tunisia. *Biocontrol Sci. Technol*. 29:149–161. https://doi.org/10.1080/09583157.2018.1542485

Chidege, M., A-zaildi, S., Hassan, N., Julie, A., Kaaya, E. and Mrogoro, S. 2016. First record of tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Tanzania. *Agric. Food Secur*. 5:1–7. https://doi.org/10.1186/s40066-016-0066-4

Cloyd, R. A., & Bethke, J. A. (2011). Impact of neonicotinoid insecticides on natural enemies in greenhouse and interiorscape environments. Pest Manag. Science. 67: 3–9. https://doi.org/10.1002/ps.2015

Cusumano, A., Peri, E., Amodeo, V., McNeil, J.N. and Colazza, S. 2013. Intraguild interactions between egg parasitoids: window of opportunity and fitness costs for a facultative hyperparasitoid. *PLoS One* 8:1–7. https://doi.org/10.1371/journal.pone.0064768

Cusumano, A., Peri, E. and Colazza, S. 2016. Interspecific competition/facilitation among insect parasitoids. *Curr. Opin. Insect Sci*. 14:12–16. https://doi.org/10.1016/j.cois.2015.11.006

Dannon, E.A., Tamo, M., van Huis, A. and Dicke, M. 2010. Functional response and life history parameters of *Apanteles taragamae*, a larval parasitoid of *Maruca vitrata*. *BioControl* 55:363–378. https://doi.org/10.1007/s10526-009-9263-4

Desneux, N., Han, P., Mansour, R., Arnó, J., Brévault, T., Campos, M.R., Chailleux, A., Guedes, R.N.C., Karimi, J., Konan, K.A.J., Lavoir, A. violette, Luna, M.G., Perez-Hedo, M., Urbaneja, A., Verheggen, F.J., Zappalà, L., Abbes, K., Ali, A., Bayram, Y., Cantor, F., Cuthbertson, A.G.S., De Vis, R., Erler, F., Firake, D.M., Haddi, K., Hajjar, M.J., Ismoilov, K., Jaworski, C.C., Kenis, M., Liu, H. tian, Madadi, H., Martin, T., Mazih, A., J. Messelink, G., Mohamed, S.A., Nofemela, R.S., Oke, A., Ramos, C., Ricupero, M., Roditakis, E., Shashank, P.R., Wan, F.H., Wang, M. hui, Wang, S., Zhang, Y.B. and Biondi, A. 2022. Integrated pest management of *Tuta absoluta*: practical implementations across different world regions. *J. Pest Sci*. 95:17–39. https://doi.org/10.1007/s10340-021-01442-8

El-aassar, M.R., Soliman, M.H.A. and Elaal, A.A.A. 2015. Efficiency of sex pheromone traps and some bio and chemical insecticides against tomato borer larvae, *Tuta absoluta* (Meyrick) and estimate the damages of leaves and fruit tomato plant. *Ann. Agric. Sci.* 60:153–156. https://doi.org/10.1016/j.aoas.2015.05.003

Erasmus, R., Berg, J. Van Den and du Plessis, H. 2021. Susceptibility of *Tuta absoluta* (Lepidoptera: Gelechiidae) pupae to soil applied entomopathogenic fungal biopesticides. *Insect* 12:1–12. https://doi.org/https://doi.org/10.3390/insects12060515

Farahani, K. 2013. Is host age an important factor in the bionomics of *Apanteles myeloenta* (Hymenoptera: Braconidae)? *Eur. J. Entomol.* 110:277–283. https://doi.org/10.14411/eje.2013.041

Feng, Y., Wratten, S., Sandhu, H. and Keller, M. 2015. Interspecific competition between two generalist parasitoids that attack the leafroller *Epiphyas postvittana* (Lepidoptera: Tortricidae). *Bull. Entomol. Res*. 105:426–433. https://doi.org/10.1017/S0007485314000923

Gabarra, R., Arnó, J., Lara, L., Verdú, M.J., Ribes, A., Beitia, F., Urbaneja, A., Téllez, M. del M., Mollá, O. and Riudavets, J. 2013. Native parasitoids associated with *Tuta absoluta* in the tomato production areas of the Spanish Mediterranean Coast. *BioControl* 59:45–54. https://doi.org/10.1007/s10526-013-9545-8

González, P.I., Montoya, P., Pérez-Lachaud, G., Cancino, J. and Liedo, P. 2010. Host discrimination and superparasitism in wild and mass-reared *Diachasmimorpha longicaudata*

(Hym.: Braconidae) females. *Biocontrol Sci. Technol.* 20:137–148. https://doi.org/10.1080/09583150903437266

Gözel, Ç. and Kasap, I. 2015. Efficacy of entomopathogenic nematodes against the Tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in tomato field. *Turkiye Entomoloji Derg.* 39:229–237. https://doi.org/10.16970/ted.84972

Grant, C., Jacobson, R. and Bass, C. 2021. Parthenogenesis in UK field populations of the tomato leaf miner, *Tuta absoluta*, exposed to the mating disruptor Isonet T. *Pest Manag. Sci.* 77:3445–3449. https://doi.org/10.1002/ps.6394

Grant, C., Jacobson, R., Ilias, A., Berger, M., Vasakis, E., Bielza, P., Zimmer, C.T., Williamson, M.S., ffrench-Constant, R.H., Vontas, J., Roditakis, E. and Bass, C. 2019. The evolution of multiple-insecticide resistance in UK populations of tomato leafminer, *Tuta absoluta*. *Pest Manag. Sci.* 75:2079–2085. https://doi.org/10.1002/ps.5381

Guenaoui, Y. and Ghelamallah, A. 2008. *Tuta absoluta* (Meyrick) Lepidoptera: Gelechiidae, un nouveau ravaveur de la tomate en Algerie premieres donnees sur sa biologie en fonction de la temperature, in: AFPP – 8EME Conférence Internationale Sur Les Ravageurs En Agriculture Montpellier – 22 Et 23 Octobre 2008. pp. 266–275.

Gupta, S. and Dikshit, A.K. 2010. Biopesticides: An ecofriendly approach for pest control. J. *Biopestic.* 3:186–188.

Han, P., Bayram, Y., Shaltiel-Harpaz, L., Sohrabi, F., Saji, A., Esenali, U.T., Jalilov, A., Ali, A., Shashank, P.R., Ismoilov, K., Lu, Z. zhi, Wang, S., Zhang, G. fen, Wan, F. hao, Biondi, A. and Desneux, N. 2019. *Tuta absoluta* continues to disperse in Asia: damage, ongoing management and future challenges. *J. Pest Sci.*. 92:1317–1327. https://doi.org/10.1007/s10340-018-1062-1

Jonsson, M., Kaartinen, R. and Straub, C.S. 2017. Relationships between natural enemy diversity and biological control. *Curr. Opin. Insect Sci.* 20:1–6. https://doi.org/10.1016/j.cois.2017.01.001

King, B.H. 2002. Sex ratio response to conspecifics in a parasitoid wasp: Test of a prediction of local mate competition theory and alternative hypotheses. *Behav. Ecol. Sociobiol.* 52:17–24. https://doi.org/10.1007/s00265-002-0492-0

Kinyanjui, G., Khamis, F.M., Ombura, F.L.O., Kenya, E.U., Ekesi, S. and Mohamed, S.A. 2021. Distribution, abundance and natural enemies of the invasive tomato leafminer, *Tuta absoluta* (Meyrick) in Kenya. *Bull. Entomol. Res.* 111:1–16. https://doi.org/10.1017/S0007485321000304

Kortam, M.N., El Arnaouty, S.A., Afifi, A.I. and Heikal, I.H. 2014. Efficacy of different biological methods for controlling the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on tomato in greenhouse in egypt. *Egypt. J. Biol. Pest Control* 24:523–528.

Kroschel, J. and Cañedo, V. 2009. How do insecticides affect potato yield and ecosystem resilience to manage potato pests? An ecological assessment from the central highlands of Peru, in 15th Trienn. Symp. Int. Soc. Trop. Root Crop. 9–22.

Kumar, S. and Singh, A. 2015. Biopesticides: Present Status and the Future Prospects. *J. Biofertilizers Biopestic.* 06. https://doi.org/10.4172/jbfbp.1000e129

Luna, M.G., Pereyra, P.C., Coviella, C.E., Nieves, E., Savino, V., Gervassio, N.G.S., Luft, E., Virla, E. and Sánchez, N.E. 2015. Potential of biological control agents against *Tuta absoluta* (Lepidoptera: Gelechiidae): current knowledge in Argentina. *Florida Entomol.* 98:489–494. https://doi.org/10.1653/024.098.0215

Mahmood, I., Imadi, S.R., Shazadi, K., Gul, A. and Hakeem, K.R. 2016. Effects of Pesticides on Environment, in: Plant, Soil and Microbes: Volume 1: Implications in Crop Science. pp. 1–366. https://doi.org/10.1007/978-3-319-27455-3

Mahmoud, M.F. 2017. Biology and use of entomopathogenic nematodes in insect pests biocontrol, a generic view. *Cercet. Agron. Mold.* 49:85–105. https://doi.org/10.1515/cerce-2016-0039

Mahugija, J.A.M., Khamis, F.A. and Lugwisha, E.H.J. 2017. Assessment of pesticide residues in tomatoes and watermelons (fruits) from markets in Dar es Salaam, Tanzania. *J. Appl. Sci. Environ. Manag.* 21:497-501. https://doi.org/10.4314/jasem.v21i3.10

Mansour, R., Brévault, T., Chailleux, A., Cherif, A., Grissa-Lebdi, K., Haddi, K., Mohamed, S.A., Nofemela, R.S., Oke, A., Sylla, S., Tonnang, H.E.Z., Zappalà, L., Kenis, M., Desneux, N. and Biondi, A. 2018. Occurrence, biology, natural enemies and management of *Tuta absoluta* in Africa. *Entomol. Gen.* 38:83–112. https://doi.org/10.1127/entomologia/2018/0749

Marete, G.M., Lalah, J.O., Mputhia, J. and Wekesa, V.W. 2021. Pesticide usage practices as sources of occupational exposure and health impacts on horticultural farmers in Meru County, Kenya. *Heliyon* 7:e06118. https://doi.org/10.1016/j.heliyon.2021.e06118

Marrone, P.G. 2007. Barriers to adoption of biological control agents and biological pesticides. *CAB Rev.* 2:1-12 https://doi.org/10.1079/PAVSNNR20072051

Martins, J.C., Picanço, M.C., Bacci, L., Guedes, R.N.C., Santana, P.A., Ferreira, D.O. Chediak, M. 2016. Life table determination of thermal requirements of the tomato borer *Tuta absoluta*. J. *Pest Sci.* (2004). 89:897–908. https://doi.org/10.1007/s10340-016-0729-8

Mohamed, E.S.I., Mohamed, M.E. and Gamiel, S.A. 2012. First record of the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Sudan. *Bull. EPPO* 42: 325–327. https://doi.org/10.1111/epp.2578

Mohamed, S.A., Overholt, W.A., Wharton, R.A. and Lux, S.A. 2006. Effect of temperature on developmental time and longevity of *Psyttalia cosyrae* (Hymenoptera: Braconidae). *Biocontrol Sci. Technol.* 16:717–726. https://doi.org/10.1080/09583150600700016

Morales, J., Muñoz, L., Rodríguez, D., and Cantor, F., 2014. Combined action of sex pheromone and wasp *Apanteles gelechiidivoris* in greenhouse tomato crops. *Acta biol. Colomb.*19:175–184.

Mujica, N. and Kroschel, J. 2013. Functional trophic guilds in a subtropical arid agroecosystem: which is the most beneficial?, in: Entomological Society of America Annual Meeting. p. 7.

Ndereyimana, A., Nyalala, S., Murerwa, P. and Gaidashova, S. 2019. Pathogenicity of some commercial formulations of entomopathogenic fungi on the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Egypt. J. Biol. Pest Control* 29:1–5. https://doi.org/https://doi.org/10.1186/s41938-019-0184-y

Ndlela, S., Mohamed, S.A., Azrag, A.G.A., Ndegwa, P.N., Ong'amo, G.O. and Ekesi, S. 2020. Interactions between two parasitoids of tephritidae: *Diachasmimorpha longicaudata* (ashmead) and *Psyttalia cosyrae* (Wilkinson) (Hymenoptera: Braconidae), under laboratory conditions. *Insects* 11:1–16. https://doi.org/10.3390/insects11100671

Pfeiffer, A.D.G., Muniappan, R., Sall, D., Diatta, P., Diongue, A. and Dieng, E.O. 2019. First Record of *Tuta absoluta* (Lepidoptera : Gelechiidae) Senegal. *Florida Entomol.* 96:661–662. https://doi.org/https://doi.org/10.1653/024.096.0241

Reitz, S.R. and Trumble, J.T. 2002. Competitive displacement among insects and arachnids. *Annu. Rev. Entomol.* 47:435– 465.

Roditakis, E., Skarmoutsou, C., Staurakaki, M., Mart, R., Garc, L., Bielza, P., Haddi, K., Rapisarda, and C. Rison, J. 2012. Determination of baseline susceptibility of European populations of *Tuta absoluta* (Meyrick) to indoxacarb and chlorantraniliprole using a novel dip bioassay method. *Pest Manag. Sci.* 69:217-227. https://doi.org/10.1002/ps.3404

Roditakis, E., Steinbach, D., Moritz, G., Vasakis, E., Stavrakaki, M., Ilias, A., García-Vidal, L., Martínez-Aguirre, M. del R., Bielza, P., Morou, E., Silva, J.E., Silva, W.M., Siqueira, H.A.A., Iqbal, S., Troczka, B.J., Williamson, M.S., Bass, C., Tsagkarakou, A., Vontas, J. and Nauen, R. 2017. Ryanodine receptor point mutations confer diamide insecticide resistance in tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae). *Insect Biochem. Mol. Biol.* 80:11–20. https://doi.org/10.1016/j.ibmb.2016.11.003

Rostami, E., Madadi, H., Abbasipour, H., Allahyari, H. and Cuthbertson, A.G.S. 2017. Life table parameters of the tomato leaf miner *Tuta absoluta* (Lepidoptera: Gelechiidae) on different tomato cultivars. *J. Appl. Entomol.* 141:88–96. https://doi.org/10.1111/jen.12319

Rwomushana, I., Day, R., and Gonzalez-Moreno, P., 2019. Evidence Note Tomato leafminer Impacts and coping strategies for Africa. https://doi.org/https://dx.doi.org/10.1079/CABICOMM-62-8100

Sánchez, N.E., Pereyra, P.C. and Luna, M.G. 2009. Spatial patterns of parasitism of the solitary parasitoid *Pseudapanteles dignus* (Hymenoptera: Braconidae) on *Tuta absoluta* (Lepidoptera: Gelechiidae). *Environ. Entomol.* 38:365–374. https://doi.org/10.1603/022.038.0208

Seydi, O., Sylla, S., Diatte, M. and Labou, B. 2021. Recruitment of native parasitoids of the tomato leaf miner *Tuta absoluta* (Meyrick 1917), (Lepidoptera: Gelechiidae) in Senegal. *Int. J. Pest Manag*. https://doi.org/10.1080/09670874.2021.1943047

Shaltiel-Harpaz, L. et al., Gerling, D., Graph, S., Kedoshim, H., Azolay, L., Rozenberg, T., Nachache, Y., Steinberg, S., Allouche, A. and Alon, T. 2016. Control of the tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), in open-field tomatoes by indigenous natural enemies occurring in Israel. *J. Econ. Entomol.* 109:1–12. https://doi.org/10.1093/jee/tov309

Shuker, D.M., Pen, I. and West, S.A. 2006. Sex ratios under asymmetrical local mate competition in the parasitoid wasp *Nasonia vitripennis*. *Behav. Ecol.* 17:345–352. https://doi.org/10.1093/beheco/arj034

Sujii, E.R., Costa, M.L.M., Pires, C.S.S., Colazza, S. and Borges, M. 2002. Inter and intra-guild interactions in egg parasitoid species of the soybean stink bug complex. *Pesqui. Agropecu. Bras.* 37:1541–1549. https://doi.org/10.1590/S0100-204X2002001100004

Tan, X., Hu, N., Zhang, F., Ramirez-Romero, R., Desneux, N., Wang, S. and Ge, F. 2016. Mixed release of two parasitoids and a polyphagous ladybird as a potential strategy to control the tobacco whitefly *Bemisia tabaci*. *Sci. Rep.* 6:28245. https://doi.org/10.1038/srep28245

Tarusikirwa, V.L., Machekan, H., Mutamiswa, R., Chidawanyika, F. and Nyamukondiwa, C. 2020. *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on the "offensive" in Africa: prospects for integrated management initiatives. *Insect* 11:764. https://doi.org/https://doi.org/10.3390/insects11110764

Tropea Garzia, G., Siscaro, G., Biondi, A. and Zappalà, L. 2012. *Tuta absoluta*, a South American pest of tomato now in the EPPO region: Biology, distribution and damage. *EPPO Bull.* 42:205–210. https://doi.org/10.1111/epp.2556

Tscharntke, T., Karp, D.S., Chaplin-Kramer, R., Batáry, P., DeClerck, F., Gratton, C., Hunt, L., Ives, A., Jonsson, M., Larsen, A., Martin, E.A., Martínez-Salinas, A., Meehan, T.D., O'Rourke, M., Poveda, K., Rosenheim, J.A., Rusch, A., Schellhorn, N., Wanger, T.C., Wratten, S. and Zhang, W. 2016. When natural habitat fails to enhance biological pest control – Five hypotheses. *Biol. Conserv.* 204:449–458. https://doi.org/10.1016/j.biocon.2016.10.001

Tunca, H., Buradino, M., Colombel, E.A. and Tabone, E. 2016. Tendency and consequences of superparasitism for the parasitoid *Ooencyrtus pityocampae* (Hymenoptera: Encyrtidae) in parasitizing a new laboratory host, *Philosamia ricini* (Lepidoptera: Saturniidae). *Eur. J. Entomol.* 113:51–59. https://doi.org/10.14411/eje.2016.006

Wang, X.G., Bokonon-Ganta, A.H. and Messing, R.H. 2008. Intrinsic inter-specific competition in a guild of tephritid fruit fly parasitoids: Effect of co-evolutionary history on competitive superiority. *Biol. Control* 44, 312–320. https://doi.org/10.1016/j.biocontrol.2007.10.012

Weisser, A.W.W. and Houston, A.I. 1993. Host discrimination in parasitic wasps: when is it advantageous? *Funct. Ecol.* 7:27–39.

Xu, H., Yang, N. and Wan, F. 2013. Competitive interactions between parasitoids provide new insight into host suppression. *PLoS One* 8:16–21. https://doi.org/10.1371/journal.pone.0082003

Zappalà, L., Bernardo, U., Biondi, A., Cocco, A., Deliperi, S., Delrio, G., Giorgini, M., Pedata, P., Rapisarda, C., Tropea Garzia, G. and Siscaro, G. 2012. Recruitment of native parasitoids by the exotic pest *Tuta absoluta* in southern Italy. *Bull. Insectology* 65:51–61.

Zekeya, N., Dubois, T., Smith, J. and Ramasamy, S. 2022. Field effectiveness of *Metarhizium anisopliae* and pheromone traps against *Phthorimaea absoluta* on tomato in Tanzania. *Crop Prot.* 156:105942. https://doi.org/10.1016/j.cropro.2022.105942

CHAPTER 2: LITERATURE REVIEW

2.1 Biology and economic importance of *Tuta absoluta*

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) was named with several synonyms over the years. It was first identified as *Phthorimaea absoluta* by Meyrick, (1917), as *Gnorimoschema absoluta* by Clarke, (1962), as *Scrobipalpula absoluta* by Povolny, (1964), and *Scrobipalpuloides absoluta* also by Povolny, (1987). Povolny (1994) then placed the pest into the genus *Tuta* (EPPO, 2005; Desneux *et al.*, 2010), but it was again re-classified under its original genus, *Phthorimaea*, It was included in the same monophyletic clade with *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) (Chang & Metz, 2021), the Potato tuber moth and is now known again as *Phthorimaea absoluta*. The scientific name, *Tuta absoluta* is, however, used in this thesis since many chapters were already published before the re-classification.

It is an holometabolic insect with four life stages, *viz*. egg, larva, pupa and adult (EPPO, 2005). The eggs are small (0.36 mm long and 0.22 mm large), cylindrical and creamy white to yellow in colour (EPPO, 2005) (Figure 2.1A). Eggs hatch after 4–5 days into cream coloured first instar larvae, 0.9 mm) with dark heads (Figure 2.1B and C), becoming greenish in the second and changed to light pink in the fourth instar, which is 7.5 mm long at 13–15 days post-oviposition (Figure 2.1D). The mature larvae fall to the ground to transform into greenish pupae (Figure 2.1E), or sometimes, pupation occurs in the leaves (EPPO, 2005). The antennae of the moths are filiform and approximately 10 mm long. The wings are covered with silverish-grey scales, with black spots visible on the anterior wings (Figure 2.1F). A female *T. absoluta* moth can lay up to 260 eggs during her lifetime, and the majority (76%) of these eggs are laid seven days after mating (EPPO, 2005; Harizanova *et al.,* 2009). *Tuta absoluta* is a multivoltine species (EPPO, 2005). The development time of one generation varies depending on environmental conditions (Silva *et al.,* 2015; Mohamed *et al.*, 2022). In a laboratory study, six to 15 generations per year were reported by Mohamed *et al.* (2022) under constant temperatures of 15 and 30 °C, respectively.

The eggs are laid on the underside of the leaves, buds, stems and calyx of unripe fruit. The larvae feed on the leaves, fruit and stems of tomato, thus causing mines and galleries which negatively affects the development of the plant by decreasing its photosynthetic capacity or by damaging the fruit, allowing infestation by other pests (EPPO, 2005; Biondi *et al.*, 2018; Desneux *et al.*, 2010, 2022) .

In the absence of control measures, yield losses caused by *T. absoluta* can reach up to 100% due to direct and indirect damage (Figure 2.2) (Desneux *et al.*, 2010; Moussa *et al.*, 2013; Chidege *et al.*, 2016). Invasions by *T. absoluta* led to an increase in tomato production costs, reduced availability and accessibility of fruit, an increase in tomato price, and it affected food security. Up to 100% losses was reported in Nigeria (Sanda et al., 2018) and Angola (Chidege *et al.*, 2017). In addition, countries where *T. absoluta* does not occur, banned tomato trade from countries already invaded by this pest (Mansour et al., 2018).

Figure 2.1: A: Eggs, B: First and 2nd-instar larvae tunnelling into tomato leaves, C: 3rd-instar larvae, D: 4th-instar larvae, E: Pupa, and F: adult, of *Tuta absoluta* (source: Dr Sevgan Subramanian, *icipe*, Nairobi, Kenya).

Figure 2.2: Greenhouse tomato infested with *Tuta absoluta* at Naivasha, Nakuru county (Source: S. Mama Sambo)

2.2 Ecology

Tuta absoluta is an oligophagous species, with the larvae that tunnel into and feed in the leaves, stems, and fruit of some cultivated and wild solanaceous plants (Portakaldali *et al.,* 2013; Mohamed *et al.,* 2015; Smith *et al.,* 2019). Although *T. absoluta* is known to mine into the leaves of several crops in the Solanaceae family, only tomato fruit are damaged (Smith *et al.,* 2019). Tomato is the most preferred and suitable host plant in terms of oviposition, fecundity and development time compared to potato (Sanchez, 2006; Caparros Megido *et al.,* 2013, 2014). Infestation by *T. absoluta* is more intense in the leaves than the stems, sepals, and fruits (Harizanova *et al.*, 2009). Other invasive Gelechiidae also recorded in Kenya, include *P. operculella*, *Aproaerema simplixella* (Walker), *Sitotroga cerealella* (Olivier) and *Pectinophora gossypiella* (Saunders) (Kinyanjui *et al.,* 2018). Since African agricultural systems are mostly polyculture and crops are rotated, the possibility exists that *T. absoluta* become a pest of legumes in addition to Solanaceae.

The lower- and higher temperature thresholds of 8.0 °C and 37.3 °C, respectively and an optimum temperature of 20-25 °C for survival and development were reported (Cuthbertson *et* *al.*, 2013; Krechemer and Foerster, 2015; Machekano *et al.*, 2018). The pest is currently present in Europe, Africa, and Asia (Figure 2.3). However, with exposure to 0 °C, Van Damme *et al*. (2015) reported that 10% of larvae, pupae and adults remained alive for 23.4, 25.7 and 30.3 days, respectively. The adults therefore survive longer at lower temperatures compared to larvae and pupae, but it does not enter into diapause (Van Damme *et al.*, 2015). The year-round presence of alternative host plants (Guimapi *et al.*, 2016) and the wide climatic adaptation of the pest favour its spread into new regions (Guimapi *et al.*, 2016; Diatte *et al.*, 2017; Desneux *et al.*, 2022; Konan *et al*., 2022). The damage inflicted on tomato is more severe in countries in warmer areas (Desneux *et al.*, 2010), and temperature increases reduce the larval development time of *T. absoluta* (Silva *et al.*, 2015; Mohamed *et al.*, 2022). The tropical regions including those in Africa, are therefore climatically suitable for *T. absoluta*. However, it has been experimentally confirmed that *T. absoluta* can enter a facultative diapause in response to low, non-freezing temperatures, day length and exposure periods (de Campos *et al*., 2021).

The life parameters of *T. absoluta* are also affected by fertilisation. Mahamadi *et al*. (2016) reported a low humic substance used as fertilizer was correlated with the lowest *T. absoluta* infestation. Very high and below optimal doses of nitrogen are unfavourable for development of *T. absoluta* (Han *et al.*, 2014).

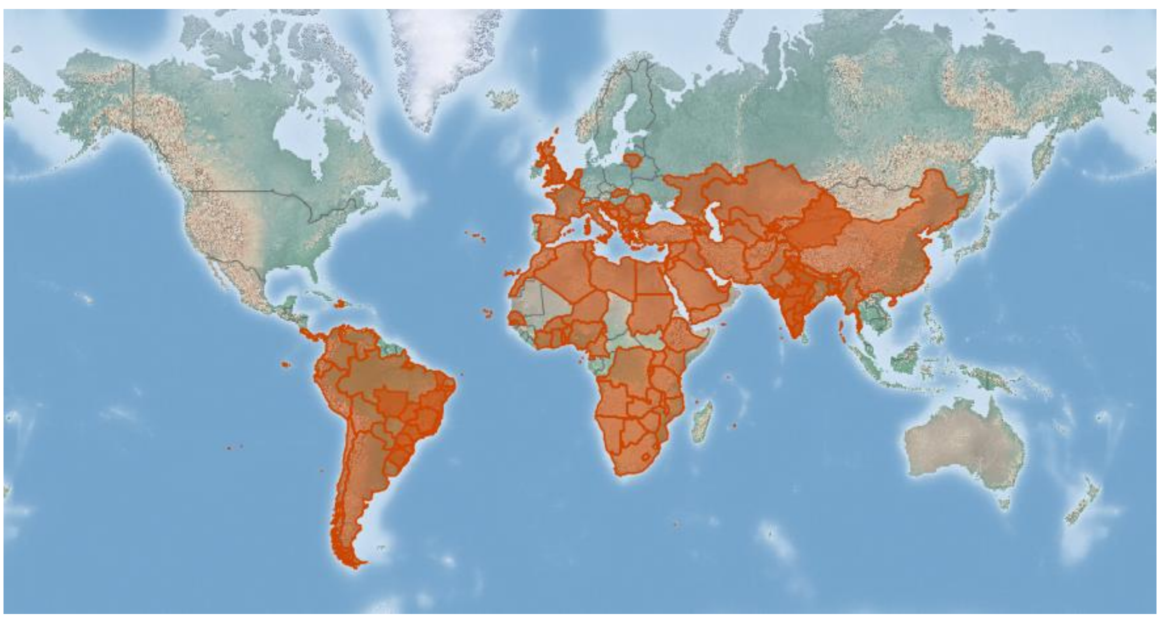


Figure 2.3: Distribution of *T. absoluta* in the world. **Source:** CABI, accessed 28 august 2022 at https://www.cabi.org/isc/datasheet/49260#toDistributionMaps

2.3 Biological control of *Tuta absoluta*

Many different arthropod species (Zappalà *et al.*, 2013; Abbes *et al.*, 2013; Mansour *et al.*, 2018; Ferracini *et al.*, 2019; Salas Gervassio *et al.*, 2019), as well as entomopathogens such as fungi, bacteria, viruses and nematodes (Urbaneja *et al.*, 2012; Sabbour and Singer, 2014; Gözel and Kasap, 2015; Kamali *et al.*, 2018), attack *T. absoluta* in the different geographic areas where it occurs. According to Mansour *et al.* (2018), different biological control approaches, namely augmentative, conservation, classical, as well as natural biological control should be integrated to achieve effective biological control of *T. absoluta*. In March 2017, the most efficient *T. absoluta* parasitoid from the pest's native area, *Dolichogenidea gelechiidivoris* (Marsh) (Hymenoptera: Braconidae), was introduced into Africa by the Centre of Insect Physiology and Ecology (*icipe*). Many laboratory studies on potential control strategies for *T. absoluta* have been conducted in in sub-Saharan Africa (SSA) (Sylla *et al.*, 2016; Zekeya *et al.*, 2019; Agbessenou *et al.*, 2020; Aigbedion-Atalor *et al.*, 2021; Ayelo *et al.*, 2021; Erasmus *et al.*, 2021; Mama Sambo *et al.*, 2022a; 2022b), as well as a few field trials (Kinyanjui *et al.*, 2021; Seydi *et al.*, 2021; Mama Sambo *et al.*, 2022c).

2.3.1 Parasitoids, predators, and Integrated Pest Management (IPM) strategies including parasitoids

Generally native parasitoids of other lepidopteran species established new association with *T. absoluta* in invaded areas. An example of such associations in sub-Saharan Africa, are *Bracon nigricans* Szépligeti (Hymenoptera: Braconidae), and *Dolichogenidea appellator* (Telenga) (Hymenoptera: Braconidae), which are now larval paratisoids of *T. absoluta* also (Idriss *et al.*, 2018). These parasitoids are generalist parasitoids of Lepidoptera species, such as *Lobesia botrana* (Denis & Schif- fermüller) (Lepidoptera: Tortricidae) and *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) (Loni *et al.*, 2016; Becchimanzi *et al.*, 2020; van Noort, 2022).

The two major predators of *T. absoluta*, in the invaded areas are *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* Reuter (Hemiptera Miridae) (Urbaneja *et al.*, 2012; Al-Jboory *et al.*, 2012; Sanchez *et al.*, 2012; Shaltiel-Harpaz *et al.*, 2016; Naselli *et al.*, 2017; Bueno *et al.*, 2019; Kinyanjui *et al.*, 2021; Desneux *et al.*, 2022). These predators were reported to prefer eggs and early larval stages of *T. absoluta* (Guenaoui *et al.*, 2011; Boualem *et al.*, 2012; Zappalà *et* *al.,* 2013; Sylla *et al.,* 2016; Bouagga *et al.,* 2018). *Nesidiocoris tenuis* complements its feeding by preying on other insect pests also, and it feeds on the tomato crop when the *T. absoluta* density is low (Giorgini *et al.,* 2019). Feeding on the plants can negatively affect vegetative growth and fruiting of the plant (Puentes et al., 2018; Chinchilla-ramírez et al., 2021). The performance of these different natural enemies of *T. absoluta* in SSA in single - and combined use is reported in Table 2.1.

Table 2.1: Summary of *Tuta absoluta* parasitoids/predators/IPM documented, and their rate of parasitism in sub-Saharan Africa.

Biocontrol agents	Parasitoid species	Family	Stage parasitised/attacked	Highest parasitism /attack rate (%)	Exposure time	*F/L	Country	References
Native parasitoids	*Chelonus* sp.	Braconidae	Larvae	0.64	N/A	F	Senegal	Seydi *et al.* (2021)
	Apanteles litae (Dixon)	Braconidae	Larvae	0.27				
	Cotesia vestalis (Haliday)	Braconidae	Larvae	0.04				
	Meteorus laphygmarum (Brues)	Braconidae	Larvae	0.04				
	Diadegma insulare (Cresson)	Ichneumonidae	Larvae	0.02				
	Pristomerus pallidus (Kriechbaumer)	Ichneumonidae	Larvae	0.02				
	Hockeria sp.	Chalcididae	Larvae	13				
	Brachymeria sp.	Chalcididae	Larvae	10	N/A	F	Kenya	Kinyanjui *et al.* (2021)
	Bracon sp.	Braconidae	Larvae	6				
	Neochrysocharis formosa (Westwood)	Eulophidae	Larvae	5				
	Goniozus sp.	Bethylidae	Larvae	5				
	Diglyphus isaea(Walker)	Eulophidae	Larvae	4				
	Stenomesius rufescens (Retzius)	Eulophidae	Larvae	4				
	Bracon nigricans (Szépligeti)	Braconidae	Larvae	21	N/A	F	Kenya	Mama Sambo *et al.* (2022c)
	Stenomesius sp. near *japonicus* (Ashmead)	Eulophidae	Larvae	17				

	Dolichogenidea appellator (Telenga)	Braconidae	Larvae	60	24h	L	Sudan	Idriss *et al.* (2018)
	Bracon nigricans	Braconidae	Larvae	54				
Exotic parasitoid	*Dolichogenidea gelechiidivoris* (Marsh)	Braconidae	Larvae	55	24h	L	Kenya	Aigbedion-Atalor *et al.* (2020)
	D. gelechiidivoris	Braconidae	Larvae	59	24h	L	Kenya	Mama Sambo *et al.* (2022b)
Native predators	*Nesidiocoris tenuis* Reuter	Miridae	Eggs	51	24h	L	Senegal	Sylla *et al.* (2016)
	N. tenuis		Larvae	14	24h	L	Senegal	Sylla *et al.* (2016)
	N. tenuis		Eggs and larvae	N/A	N/A	F	Present everywhere in SSA invaded areas	https://www.cabi.org/isc/datasheet/16251#toDistributionMaps assessed on 25 August 2022
	N. tenuis		Eggs and larvae	N/A	N/A	F	Burkina Faso	Sawadogo *et al.* (2022)
	N. tenuis		Eggs	75	24h	L	Burkina Faso	Sawadogo *et al.* (2022)
	Macrolophus pygmaeus	Miridae	Eggs	N/A	N/A	L	Senegal	Sylla *et al.* (2016)
	M. pygmaeus		Eggs and larvae	N/A	N/A	F	Kenya	Kinyanjui *et al.* (2021)
IPM	*D. gelechiidivoris* + *N. tenuis*		Eggs and larvae	83	24h	L	Kenya	Aigbedion-Atalor *et al.* (2021)
	D. gelechiidivoris + *S.* sp. nr. *japonicus*		Larvae	80	24h	L	Kenya	Mama Sambo *et al.* Unpublished data
	D. gelechiidivoris + *M. anisopliae* ICIPE 20		Larvae	78	24h	L	Kenya	Mama Sambo *et al.* (2022b)

*F = Field study, L = laboratory study

2.3.2 Entomopathogens

Different microbial agents such as entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana* can reduce *T. absoluta* infestation (Akutse *et al.*, 2020; Erasmus *et al.*, 2021). Since the active ingredients in entomopathogenic fungi are living organisms, unfavourable conditions including high temperature affect their efficacy negatively (El-Ghany *et al.*, 2018). *Tuta absoluta* is susceptible to *M. anisopliae* ICIPE 18, ICIPE20, and ICIPE 665 at a temperature range of 15 to 30°C, indicating that temperature increases within these limits, did not affect the pathogenicity (Agbessenou *et al.*, 2021). This temperature range is representative of the ambient temperature of many regions in Africa. These *Metarhizium* strains are therefore promising agents for control of *T. absoluta*. Only Kenya, South Africa, Ethiopia and Ghana recorded *Metarhizium* Met69 isolate for commercial use against *T. absoluta* (Rwomushana *et al.*, 2019). Different strains of the nematodes, *Steinernema carpocapsae* (Weiser) and *Heterorhabditis bacteriophora* Poinar (Nematoda: Heterorhabditidae) have been used for *T. absoluta* control in Africa (Ndereyimana *et al.*, 2019; Dlamini *et al.*, 2020). However, the efficacy of *Heterorhabditis bacteriophora* and *S. carpocapsae* can be affected by soil type (Kamali *et al.*, 2018). The efficacy of these entomopathogens for control of *T. absoluta* is summarised in Table 2.2.

Table 2.2: Summary of the entomopathogens tested and success rate in control of *Tuta absoluta* in sub-Saharan Africa.

Types	Species/strains	Trade name	Mode of application	Parameters	Most effective	Highest Mortality	Lethal time* (Days)	Country	Reference
Fungi	ICIPE 07; ICIPE 18; ICIPE 20; ICIPE 30; ICIPE 31; ICIPE 40; ICIPE 41; ICIPE 62; ICIPE 68; ICIPE 78; ICIPE 665 GZP	N/A	Infected soil in laboratory	4^{th}-instar larvae pupation	ICIPE 18 ICIPE 20 ICIPE 665	100% 100% 100%	$LT_{100} = 6$ $LT_{100} = 6$ $LT_{100} = 6$	Kenya	Akutse *et al.* (2020)
	ICIPE 07; ICIPE 18; ICIPE 20; ICIPE 30; ICIPE 31; ICIPE 40; ICIPE 41; ICIPE 62; ICIPE 68; ICIPE 78; ICIPE 665 GZP	N/A	Infected with dry conidia - infection chamber in laboratory	Adult mortality	ICIPE 18 ICIPE 20 ICIPE 665	95% 87.5% 86.3%	LT_{50} = 5.13 LT50 = 3.17 LT_{50} = 2.38	Kenya	Akutse *et al.* (2020)

	ICIPE 18, ICIPE 20 ICIPE 665 at 10, 15, 20,25, and 30°C	N/A	Infected with dry conidia - infection chamber in laboratory	Adult mortality	More effective at 30°C			Kenya	Agbessenou *et al*. (2021)
					ICIPE 18	91%,	LT_{50} = 1.41		
					ICIPE 20	90%	LT_{50} = 1.48		
					ICIPE 665	75%	LT_{50} = 2.92		
	Beauveria bassiana (Bals.) Vuill., strain BB02	Real Beauveria	Infected soil - laboratory	Pupal mortality	Real Beauveria	98%	LT_{50} < 1	South Africa	Erasmus *et al.* (2021)
	B. bassiana (Bals.) Vuill., strain R444	Eco-Bb			Real Metarhizium 69	90%	LT_{50} < 1		
	Metarhizium anisopliae (Metsch.) Sorok., strain ICIPE 69	Real Metarhizium 69							
	M. anisopliae (Metsch.) Sorok., strain E9	Metarril WP E9			Metarril WP E9	89%	LT_{50} = ~5		
	M. anisopliae (Metsch.) Sorok.,	Real Metarhizium 69	Open field experimental	Infested fruit	Real Metarhizium	42% < control	N/A	Tanzania	Zekeya *et al.* (2022)

	strain ICIPE 69		plot spray		69				
	M. anisopliae, Strain FCM Ar 23B3, 5 × 10^9 CFU/g *B. bassiana*, Strain J25, 1 ×10^{10} CFU/g	Metatech®WP Beauvitech® WP	Open field experimental plot spray	Marketable fruits	Both species	92-93%	N/A	Rwanda	Ndereyimana *et al.* (2020)
	Aspergillus oryzae (10^6, 10^7, 10^8) at 30.4°C and 70%RH and 19.5°C and 50%RH	N/A	Infected paper towel in laboratory (petri dish)	Larval mortality	*A. oryzae at* 30.4°C and 70%RH 1.0 × 10^8	77%	LT_{100} = 3.5	Tanzania	Zekeya *et al.* (2019)
Bacteria	*Bacillus thuringiensis* var. kurstaki	BatikWG	Open field - spray	Number of leaflets mined	*Bacillus thuringiensis* var. kurstaki	79% < control	N/A	Senegal	Sarr *et al.* (2021)

Nematodes	*Steinernema* sp. RW14-M-C2a-3, *Steinernema* sp. RW14-M-C2b-1, *S. carpocapsae* RW14-G-R3a- *H. bacteriophora* RW14-N-C4a *S. carpocapsae* All *H. bacteriophora* H06	N/A	Infected paper towel in laboratory (petri dish)	Larval mortality	all	100	$LT_{100} = 4$	Rwanda	Ndereyimana *et al.* (2019)
	Steinernema sp. RW14-M-C2 *Steinernema* sp. RW14-M-C2b Control = Water	N/A	Open field spray	Marketable fruits	Both species	92-93%	N/A	Rwanda	Ndereyimana *et al.* (2020)
	S. yirgalemense *S. jeffreyense* (0, 20, 40, and 60 IJs/insect)		Leaves with mines sprayed in laboratory	Larval mortality	*S. yirgalemense* at 60 IJs/insect	59%	$LT_{100} = 2$	Kingdom of Eswatini	Dlamini *et al.* (2020)

*All studies with no LT indicated, were field studies

2.4 Structure of the thesis

Chapter 1 formulates the rational of the study and presents the aims, objectives, and hypotheses.
Chapter 2 The literature study provides a brief overview of the biology and economic importance of *T. absoluta*, its ecology and biological control of this pest.

Chapter 3 (first article) investigates the functional response type of *D. gelechiidivoris* females to different densities of *T. absoluta* larvae and the effect of *D. gelechiidivoris* densities on their parasitism rate of *T. absoluta*.

Chapter 4 (second article) investigates the occurrence and distribution of native parasitoid species associated with *T. absoluta* in open-field and greenhouse tomato in Kenya and predicts the suitability of habitats for their occurrence and use in biocontrol programs.

Chapter 5 (third article) describes the interaction between two parasitoids of *T. absoluta*: the exotic *D. gelechiidivoris*, imported and released in Kenya, and the indigenous *Stenomesius* sp. near *japonicus* and predicted areas suitable for persistence of *Stenomesius* sp. near *japonicus* in Africa.

Chapter 6 (fourth article) focusses on the Effects of the interaction between two *T. absoluta* parasitoids, *viz*. the exotic *D. glechiidivoris* and the native *B. nigricans* and recommends on release of the exotic parasitoid, taking into consideration the presence of this native parasioid.

Chapter 7 (fifth article) describes the interactions between the entomopathogenic fungus *Metarhizium anisopliae* ICIPE 20 and the endoparasitoid *D. gelechiidivoris*, and implications of their combined use in biocontrol of *T. absoluta*, which provided an additive effect on pest mortality of the target pest.

Chapter 8 (sixth article) investigates the dispersion of *D. gelechiidivoris* in open-field tomato in central Kenya and its performance in combination with *Metarhizium anisopliae* under greenhouse conditions as a means to confirm establishement of the parasitoid in Kenya. It also confirms the success achieved with previous laboratory studies on the combined use of the two biological control agents for control of *T. absoluta*.

Chapter 9 summarizes the key findings of the thesis by discussing the validity of the hypotheses postulated for this study and provides recommendations for future research on this subject.

2.5 References

Abbes, K., Biondi, A., Zappalà, L. and Chermiti, B. 2013. Fortuitous parasitoids of the invasive tomato leafminer *Tuta absoluta* in Tunisia. *Phytoparasitica* 42:1–8. https://doi.org/10.1007/s12600-013-0341-x

Agbessenou, A., Akutse, K.S., Yusuf, A.A., Ekesi, S., Subramanian, S. and Khamis, F.M. 2020. Endophytic fungi protect tomato and nightshade plants against *Tuta absoluta* (Lepidoptera: Gelechiidae) through a hidden friendship and cryptic battle. *Sci. Rep.* 10:22195. https://doi.org/10.1038/s41598-020-78898-8

Agbessenou, A., Akutse, K.S., Yusuf, A.A., Wekesa, S.W. and Khamis, F.M. 2021. Temperature-dependent modelling and spatial prediction reveal suitable geographical areas for deployment of two *Metarhizium anisopliae* isolates for *Tuta absoluta* management. *Sci. Rep.* 11:23346. https://doi.org/10.1038/s41598-021-02718-w

Aigbedion-Atalor, P.O., Mohamed, S.A., Hill, M.P., Zalucki, M.P., Azrag, A.G.A., Srinivasan, R. and Ekesi, S. 2020. Host stage preference and performance of *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae), a candidate for classical biological control of *Tuta absoluta* in Africa. *Biol. Control* 144:104215. https://doi.org/10.1016/j.biocontrol.2020.104215

Aigbedion-Atalor, P.O., Hill, M.P., Ayelo, P.M., Ndlela, S., Zalucki, M.P. and Mohamed, S.A. 2021. Can the combined use of the mirid predator *Nesidiocoris tenuis* and a braconid larval endoparasitoid *Dolichogenidea gelechiidivoris* improve the biological control of *Tuta absoluta*? *Insects* 12:10004. https://doi.org/10.3390/insects12111004

Akutse, K.S., Subramanian, S., Khamis, F.M., Ekesi, S. and Mohamed, S.A. 2020. Entomopathogenic fungus isolates for adult *Tuta absoluta* (Lepidoptera: Gelechiidae) management and their compatibility with Tuta pheromone. *J. Appl. Entomol.* 144:777–787. https://doi.org/10.1111/jen.12812

Al-Jboory, I.J., Katbeh-Bader, A. and Shakir, A.Z. 2012. First observation and identification of some natural enemies collected from heavily infested tomato by *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Jordan. *World Appl. Sci. J.* 17:589–592.

Ayelo, P.M., Mohamed, S.A., Chailleux, A., Yusuf, A.A., Pirk, C.W.W. and Deletre, E. 2021.

The parasitoid *Dolichogenidea gelechiidivoris* eavesdrops on semiochemicals from its host *Tuta absoluta* and tomato. *J. Pest Sci. (2004).* 95:633–652. https://doi.org/https://doi.org/10.1007/s10340-021-01424-w

Becchimanzi, A., Avolio, M., Bostan, H., Colantuono, C., Cozzolino, F., Mancini, D., Chiusano, M.L., Pucci, P., Caccia, S. and Pennacchio, F. 2020. Venomics of the ectoparasitoid wasp *Bracon nigricans*. *BMC Genomics* 21:1–15. https://doi.org/10.1186/s12864-019-6396-4

Biondi, A., Guedes, R., Wan, F. and Desneux, N. 2018. Ecology, worldwide spread, and management of the invasive South American Tomato Pinworm, *Tuta absoluta*: Past, present, and future. *Annu. Rev. Entomol.* 63:239–258. https://doi.org/10.1146/annurev-ento-031616

Bouagga, S., Urbaneja, A. and Pérez-Hedo, M. 2018. Comparative biocontrol potential of three predatory mirids when preying on sweet pepper key pests. *Biol. Control* 121:168–174. https://doi.org/10.1016/j.biocontrol.2018.03.003

Boualem, M., Allaoui, H., Hamadi, R. and Medjahed, M. 2012. Biologie et complexe des ennemis naturels de *Tuta absoluta* a Mostaganem (Algérie). *EPPO Bull.* 42:268–274. https://doi.org/10.1111/epp.2570

Bueno, V.H.P., Lins, J.C., Silva, D.B. and van Lenteren, J.C. 2019. Is predation of *Tuta absoluta* by three Neotropical mirid predators affected by tomato lines with different densities in glandular trichomes? Arthropod. *Plant. Interact.* 13:41–48. https://doi.org/10.1007/s11829-018-9658-1

CABI. Available online: https://www.cabi.org/isc/datasheet/49260#toDistributionMaps (assessed 28 august 2022).

CABI. Available online: https://www.cabi.org/isc/datasheet/16251#toDistributionMaps (assessed 28 august 2022).

Caparros Megido, R., Brostaux, Y., Haubruge, E. and Verheggen, F.J. 2013. Propensity of the Tomato Leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), to Develop on Four Potato Plant Varieties. *Am. J. Potato Res.* 90:255–260. https://doi.org/10.1007/s12230-013-9300-9

Caparros Megido, R., De Backer, L., Ettaïb, R., Brostaux, Y., Fauconnier, M.L., Delaplace, P., Lognay, G., Belkadhi, M.S., Haubruge, E., Francis, F. and Verheggen, F.J. 2014. Role of larval host plant experience and solanaceous plant volatile emissions in *Tuta absoluta* (Lepidoptera: Gelechiidae) host finding behavior. *Arthropod. Plant. Interact.* 8:293–304. https://doi.org/10.1007/s11829-014-9315-2

Chang, P.E.C., and Metz, M.A. 2021. Classification of *Tuta absoluta* (meyrick, 1917) (lepidoptera: Gelechiidae: Gelechiinae: Gnorimoschemini) based on cladistic analysis of morphology. *Proc. Entomol. Soc. Wash.*. 123: 41–54. https://doi.org/10.4289/0013-8797.123.1.41

Chidege, M., Abel, J., Afonso, Z., Tonini, M. and Fernandez, B. 2017. Tomato Leaf Miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) Detected in Namibe Province Angola. *J. Appl. Life Sci. Int.* 12: 1–5. https://doi.org/10.9734/jalsi/2017/33725

Chidege, M., A-zaildi, S., Hassan, N., Julie, A., Kaaya, E. and Mrogoro, S. 2016. First record of tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera : Gelechiidae) in Tanzania. Agric. *Food Secur*. 5:1–7. https://doi.org/10.1186/s40066-016-0066-4

Chinchilla-ramírez, M., Garzo, E., Fereres, A., Gavara-vidal, J., Cindy, J.M., Loon, J.J.A. Van, Urbaneja, A. and Pérez-hedo, M. 2021. Plant feeding by *Nesidiocoris tenuis*: Quantifying its behavioral and mechanical components. *Biol. Control* 152:104402. https://doi.org/10.1016/j.biocontrol.2020.104402

Clarke, J.F. 1962. New species of microlepidoptera from Japan. *Entomol. News* 73.

Cuthbertson, A.G.S., Mathers, J.J., Blackburn, L.F., Korycinska, A., Luo, W., Jacobson, R.J. and Northing, P. 2013. Population development of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under simulated UK glasshouse conditions. *Insects* 4:185–197. https://doi.org/10.3390/insects4020185

Desneux, N., Han, P., Mansour, R., Arnó, J., Brévault, T., Campos, M.R., Chailleux, A., Guedes, R.N.C., Karimi, J., Konan, K.A.J., Lavoir, A. violette, Luna, M.G., Perez-Hedo, M., Urbaneja, A., Verheggen, F.J., Zappalà, L., Abbes, K., Ali, A., Bayram, Y., Cantor, F., Cuthbertson, A.G.S., De Vis, R., Erler, F., Firake, D.M., Haddi, K., Hajjar, M.J., Ismoilov, K., Jaworski, C.C., Kenis, M., Liu, H. tian, Madadi, H., Martin, T., Mazih, A., J. Messelink, G., Mohamed, S.A., Nofemela, R.S., Oke, A., Ramos, C., Ricupero, M., Roditakis, E., Shashank, P.R., Wan, F.H., Wang, M. hui, Wang, S., Zhang, Y.B. and Biondi, A. 2022.

Integrated pest management of *Tuta absoluta*: practical implementations across different world regions. *J. Pest Sci. (2004).* 95:17–39. https://doi.org/10.1007/s10340-021-01442-8

Desneux, N., Wajnberg, E., Kris, W.A.G., Burgio, G., Arpaia, S., Narváez-Vasquez, C.A., González-Cabrera, J., Ruescas, D.C., Tabone, E., Frandon, J., Pizzol, J., Poncet, C., Cabello, T. and Urbaneja, A. 2010. Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, geographic expansion and prospects for biological control. *J. Pest Sci. (2004).* 83:197–215. https://doi.org/10.1007/s10340-010-0321-6

de Campos, M.R., Béarez, P., Amiens-Desneux, E., Ponti, L., Gutierrez, A. P., Biondi, A., Adiga, A. and Desneux, N. 2021. Thermal biology of *Tuta absoluta*: demographic parameters and facultative diapause. *J. Pest Sci.* 94: 829–842. https://doi.org/10.1007/s10340-020-01286-8

Diatte, M., Bal, A.B., Desneux, N., Brévault, T., Chailleux, A., Diarra, K. and Sylla, S. 2017. Rapid spread of the tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), an invasive pest in Sub-Saharan Africa. *Entomol. Gen.* 36:269–283. https://doi.org/10.1127/entomologia/2017/0453

Dlamini, B.E., Dlamini, N., Masarirambi, M.T. and Nxumalo Kwanele, A. 2020. Control of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) larvae in laboratory using entomopathogenic nematodes from subtropical environment. *J. Nematol.* 52: 1–8. https://doi.org/10.21307/jofnem-2020-013

El-Ghany, N.M.A., Abdel-Razek, A.S., Djelouah, K. and Moussa, A. 2018. Efficacy of bio-rational insecticides against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on tomatoes. *Biosci. Res.* 15:28–40.

EPPO, 2005. Data sheets on quarantine pests Fiches informatives sur les organismes de quarantaine, OEPP/EPPO Bulletin. https://doi.org/10.1111/j.1439-0418.1998.tb01459.x

Erasmus, R.; Berg, J.v.d. and du Plessis, H. 2021. Susceptibility of *Tuta absoluta* (Lepidoptera: Gelechiidae) Pupae to Soil Applied Entomopathogenic Fungal Biopesticides. *Insects* 12:515. https://doi.org/https://doi.org/10.3390/insects12060515

Ferracini, C., Bueno, V.H.P., Dindo, M.L., Ingegno, B.L., Luna, M.G., Gervassio, N.G.S., Sánchez, N.E., Lenteren, J.C. Van, Zappalà, L. and Tavella, L. 2019. Natural enemies of

Tuta absoluta in the Mediterranean basin , Europe and South America. *Biocontrol Sci. Technol.* 29:1–32. https://doi.org/10.1080/09583157.2019.1572711

Giorgini, M., Guerrieri, E., Csscone, P. and Gontijo, L. 2019. Current Strategies and Future Outlook for Managing the Neotropical Tomato Pest *Tuta absoluta* (Meyrick) in the Mediterranean Basin. *Neotrop. Entomol.* 48:1–17. https://doi.org/10.1007/s13744-018-0636-1

Gözel, Ç. and Kasap, I. 2015. Efficacy of entomopathogenic nematodes against the Tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in tomato field. Turkiye *Entomoloji Derg.* 39:229–237. https://doi.org/10.16970/ted.84972

Guenaoui, Y.; Bensaad, R. and Ouezzani, K.V.R., 2011. Emerging opportunities to use native entomophagous against *Tuta absoluta* Meyrick (Lepidoptera: Gelechidae) infesting tomato in unheated greenhouse in Northwestern Algeria. in Between benefits and risks. SupAgro, Montpellier, France, 25–27 October 2011:324–33. 9eme Conf. Int. sur les Ravag. en Agric. 25–27 Octobre.

Guimapi, R.Y.A., Mohamed, S.A., Okeyo, G.O., Ndjomatchoua, F.T., Ekesi, S. and Tonnang, H.E.Z. 2016. Modeling the risk of invasion and spread of *Tuta absoluta* in Africa. *Ecol. Complex.* 28:1–17. https://doi.org/10.1016/j.ecocom.2016.08.001

Han, P., Lavoir, A.V., Le Bot, J., Amiens-Desneux, E. and Desneux, N. 2014. Nitrogen and water availability to tomato plants triggers bottom-up effects on the leafminer *Tuta absoluta*. *Sci. Rep.* 4:4455. https://doi.org/10.1038/srep04455

Harizanova, V.B., Stoeva, A. and Mohamedova, M.S. 2009. Tomato leaf miner, *Tuta absoluta* (Povolny) (Lepidoptera: gelechiidae) – first record in Bulgaria. *Agric. Sci. Technol.* 1:95–98.

Idriss, G.E.A., Mohamed, S.A., Khamis, F., Du Plessis, H. and Ekesi, S. 2018. Biology and performance of two indigenous larval parasitoids on *Tuta absoluta* (Lepidoptera: Gelechiidae) in Sudan. *Biocontrol Sci. Technol.* 28:614–628. https://doi.org/10.1080/09583157.2018.1477117

Kamali, S., Karimi, J. and Koppenhöfer, A.M. 2018. New insight into the management of the tomato leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae) with entomopathogenic nematodes. *J. Econ. Entomol.* 111:112–119. https://doi.org/10.1093/jee/tox332

Kenis, M. and Cugala, D. 2006. Prospects for the biological control of the groundnut leaf miner, *Aproaerema modicella*, in Africa. *CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour.* 1:1–9. https://doi.org/10.1079/PAVSNNR20061031

Kinyanjui, G., Khamis, F.M., Ombura, F.L.O., Kenya, E.U., Ekesi, S. and Mohamed, S.A. 2021. Distribution, abundance and natural enemies of the invasive tomato leafminer, *Tuta absoluta* (Meyrick) in Kenya. *Bull. Entomol. Res.* 111:1–16. https://doi.org/10.1017/S0007485321000304

Kinyanjui, G., Khamis, F.M., Ombura, F.L.O., Kenya, E.U., Ekesi, S. and Mohamed, S.A. 2018. Infestation Levels and Molecular Identification Based on Mitochondrial COI Barcode Region of Five Invasive Gelechiidae Pest Species in Kenya. *J. Econ. Entomol.* 112:872–882. https://doi.org/10.1093/jee/toy357

Konan, K.A.J., Ouali-Ngoran, S.W.M., Fondio, L., Ochou, G.O., Koné, D., Desneux, N. and Martin, T. 2022. Geographical distribution and host range status of *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) in Côte d'Ivoire. *Int. J. Trop. Insect Sci.* 42:365–377. https://doi.org/10.1007/s42690-021-00554-z

Krechemer, F. da S. and Foerster, luis amilton, 2015. *Tuta absoluta* (Lepidoptera: Gelechiidae): Thermal requirements and effect of temperature on development , survival , reproduction and longevity. *Eur. J. Entomol.* 112:658–663. https://doi.org/10.14411/eje.2015.103

Loni, A., Samartsev, K.G., Scaramozzino, P.L., Belokobylskij, S.A. and Lucchi, A. 2016. Braconinae parasitoids (Hymenoptera, Braconidae) emerged from larvae of *Lobesia Botrana* (Denis & Schiffermüller) (Lepidoptera, Tortricidae) feeding on *Daphne gnidium* L. Zookeys 587, 125–150. https://doi.org/10.3897/zookeys.587.8478

Machekano, H., Mutamiswa, R. and Nyamukondiwa, C. 2018. Evidence of rapid spread and establishment of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in semi-arid Botswana. *Agric. Food Secur.* 7:1–12. https://doi.org/10.1186/s40066-018-0201-5

Magda, S.M. and Soliman, N. 2014. Evaluations of two *Metarhizium* varieties against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Egypt. *Int. J. Sci. Res.* 3:2067–2073. https://doi.org/10.13140/RG.2.1.3355.3765

Mama Sambo, S., Akutse, K.S., du Plessis, H., Aigbedion-Atalor, P.O., Mohamed, S.A. and Ndlela, S. 2022a. Interactions between the entomopathogenic fungus *Metarhizium anisopliae* ICIPE 20 and the endoparasitoid Dolichogenidea gelechiidivoris, and implications for combined biocontrol of *Tuta absoluta*. *Biology* 11:1323. https://doi.org/https://doi.org/10.3390/biology11091323

Mama Sambo, S., Ndlela, S., du Plessis, H., Obala, F. and Mohamed, S.A. 2022b. Ratio dependence effects of the parasitoid *Dolichogenidea gelechiidivoris* on its associated host *Tuta absoluta*. *Biocontrol Sci. Technol.* 32, 497–510. https://doi.org/10.1080/09583157.2022.2033702

Mama Sambo, S., Ndlela, S., du Plessis, H., Obala, F. and Mohamed, S.A. 2022c. Identification, microhabitat, and ecological niche prediction of two promising native parasitoids of *Tuta absoluta* in Kenya. *Insect* 16:496. https://doi.org/https://doi.org/10.3390/ insects13060496

Mama Sambo, S., Ndlela, S., du Plessis, H., Azrag, A.G.A. and Mohamed, S.A. 2022. Interaction between two parasitoids of *Tuta absoluta*: the introduced *Dolichogenidea gelechiidivoris* and the indigenous *Stenomesius* sp. near *japonicus*. *Biol. Control* (under review).

Mansour, R., Brévault, T., Chailleux, A., Cherif, A., Grissa-Lebdi, K., Haddi, K., Mohamed, S.A., Nofemela, R.S., Oke, A., Sylla, S., Tonnang, H.E.Z., Zappalà, L., Kenis, M., Desneux, N. and Biondi, A. 2018. Occurrence, biology, natural enemies and management of *Tuta absoluta* in Africa. Entomol. Gen. 38, 83–112. https://doi.org/10.1127/entomologia/2018/0749

Meyrick, E. 1917. Descriptions of South American Micro-Lepidoptera. Trans. Ent. Soc. London 1–52.

Mohamed, E.S.I., Mahmoud, M.E.E., Elhaj, M.A.M., Mohamed, S.A. and Ekesi, S. 2015. Host plants record for tomato leaf miner *Tuta absoluta* (Meyrick) in Sudan. *EPPO Bull.* 45:108–111. https://doi.org/10.1111/epp.12178

Mohamed, S.A., Azrag, A.G.A., Obala, F. and Ndlela, S. 2022. Estimating the Demographic Parameters of *Tuta absoluta* Fluctuating Temperature. *Biology* 11:181. https://doi.org/https://doi.org/10.3390/biology11020181

Mohamed, Samira A., Dubois, T., Azrag, A.G.A., Ndlela, S. and Neuenschwander, P. 2022. Classical biological of key horticultural pests in Africa: successes, challenges, and opportunities. *Curr. Opin. Insect Sci.* 53:1–6. https://doi.org/10.1016/j.cois.2022.100945

Moussa, S., Sharma, A., Baiomy, F., Eid-Adl, F.E., 2013. The status of tomato leafminer; *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Egypt and potential effective pesticides. *Acad. J. Entomol.* 6:110–115. https://doi.org/10.5829/idosi.aje.2013.6.3.75130

Naselli, M., Biondi, A., Tropea-Garzia, G., Desneux, N., Russo, A., Siscaro, G., Zappalà, L., Tropea Garzia, G., Desneux, N., Russo, A., Siscaro, G. and Zappalà, L. 2017. Insights into food webs associated with the South American tomato pinworm. *Pest Manag. Sci.* 73:1352–1357. https://doi.org/10.1002/ps.4562

Ndereyimana, A., Nyalala, S., Murerwa, P. and Gaidashova, S. 2020. Field efficacy of entomopathogens and plant extracts on *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) infesting tomato in Rwanda. *Crop Prot.* 134:105183. https://doi.org/10.1016/j.cropro.2020.105183

Ndereyimana, A., Nyalala, S., Murerwa, P. and Gaidashova, S. 2019. Potential of entomopathogenic nematode isolates from Rwanda to control the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Egypt. J. Biol. Pest Control* 29:1–7. https://doi.org/10.1186/s41938-019-0163-3

Portakaldali, M., Öztemiz, S. and Kütük, H. 2013. A new host plant for *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Turkey. *J. Entomol. Res. Soc.* 15:21–24.

Povolny, D. 1994. Gnorimoschemini of South America VI: identification keys checklist of Neotropical taxa and general considerations (Insecta, Lepidoptera, Gelechiidae). *Steenstrupia* 20:1–42.

Povolny, D. 1987. Gnorimoschemini of Southern South America III: the Scrobipalpuloid genera (Insecta, Lepidoptera: Gelechiidae). *Steenstrupia* 13:1–91.

Povolny, D. 1964. Gnorimoschemini Trib. nov.- EineneueTribus der Familie Gelechiidae Bermerkungenzuihrer Taxonomie (Lepidoptera). *Acta. Soc. Ent. Czechoslov* 61:330–359.

Puentes, A., Stephan, J.G. and Björkman, C. 2018. A Systematic Review on the Effects of Plant-Feeding by Omnivorous Arthropods: Time to Catch-Up With the Mirid-Tomato Bias?

Front. Ecol. Evol. 6:218. https://doi.org/10.3389/fevo.2018.00218

Rwomushana, I., Day, R. and Gonzalez-moreno, P. 2019. Evidence Note Tomato leafminer Impacts and coping strategies for Africa. https://doi.org/https://dx.doi.org/10.1079/CABICOMM-62-8100

Salas Gervassio, N.G., Aquino, D., Vallina, C., Biondi, A. and Luna, M.G. 2019. A re-examination of *Tuta absoluta* parasitoids in South America for optimized biological control. *J. Pest Sci. (2004)*. 92:1343–1357. https://doi.org/10.1007/s10340-018-01078-1

Sanchez, J.A., Spina, M. La and Perera, O.P. 2012. Analysis of the population structure of *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) in the Palaearctic region using microsatellite markers. *Ecol. Evol.* 2:3145–3159. https://doi.org/10.1002/ece3.420

Sanda, N., Sunusi, M., Hamisu, H., Wudil, B., Sule, H., and Abdullahi, A. 2018. Biological invasion of tomato teaf miner, *Tuta absoluta* (Meyrick) in Nigeria: problems and management strategies optimization: a review. *Asian J. Agric. Res.* 1: 1–14. https://doi.org/10.9734/ajahr/2018/41959

Sarr, O.M., Bal, A.B., Fossati-Gaschignard, O. and Gauthier, N. 2021. Effectiveness of two biopesticides against the invasive tomato pest *Tuta absoluta*. *Entomol. Exp. Appl.* 169:674–685. https://doi.org/10.1111/eea.13047

Sawadogo, M.W., Mano, E., Régis, B., Somda, I., Nacro, S., Legrève, A. and Verheggen, F.J. 2022. *Nesidiocoris tenuis* in Burkina Faso : distribution , predatory capacity and insecticide sensibility. *Physiol. Entomol.* 47:1–8. https://doi.org/10.1111/phen.12389

Seydi, O., Sylla, S., Diatte, M. and Labou, B. 2021. Recruitment of native parasitoids of the tomato leaf miner *Tuta absoluta* (Meyrick 1917), (Lepidoptera: Gelechiidae) in Senegal. *Int. J. Pest Manag.* 0:1–7. https://doi.org/10.1080/09670874.2021.1943047

Shaltiel-Harpaz, L. et al., Gerling, D., Graph, S., Kedoshim, H., Azolay, L., Rozenberg, T., Nachache, Y., Steinberg, S., Allouche, A. and Alon, T. 2016. Control of the tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), in open-field tomatoes by indigenous natural enemies occurring in Israel. *J. Econ. Entomol.* 109:1–12. https://doi.org/10.1093/jee/tov309

Silva, D.B., Bueno, V.H.P., Caldeira, J.L.J. and Van lenteren, J.C. 2015. Life history data and population growth of *Tuta absoluta* at constant and alternating temperatures on two tomato lines. *EPPO Bull.* 68:223–232.

Smith, J.D., Dubois, T., Mallogo, R., Njau, E.-F., Tua, S. and Srinivasan, R. 2019. Host range of the invasive tomato pest *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) on solanaceous crops and weeds in Tanzania. *Florida Entomol.* 101:573. https://doi.org/10.1653/024.101.0417

Sylla, Serigne, Brévault, T., Diarra, K., Bearez, P. and Desneux, N. 2016. Life-history traits of *Macrolophus pygmaeus* with different prey foods. *PLoS One* 11:1–8. https://doi.org/10.1371/journal.pone.0166610

Sylla, S., Brévault, T., Streito, J. and Diarra, K. 2016. First Record of *Nesidiocoris tenuis* (Reuter) (Heteroptera: Miridae), as a predator of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), in Senegal. *Egypt. J. Biol. Pest Control* 26:851–853.

Urbaneja, A., González-Cabrera, J., Arnó, J. and Gabarra, R. 2012. Prospects for the biological control of *Tuta absoluta* in tomatoes of the Mediterranean basin. *Pest Manag. Sci.* 68:1215–1222. https://doi.org/10.1002/ps.3344

Van Damme, V., Berkvens, N., Moerkens, R., Berckmoes, E., Wittemans, L., De Vis, R., Casteels, H., Tirry, L. and De Clercq, P. 2015. Overwintering potential of the invasive leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) as a pest in greenhouse tomato production in Western Europe. *J. Pest Sci. (2004).* 88:533–541. https://doi.org/10.1007/s10340-014-0636-9

van Noort, S. 2022. Hymenoptera of the Afrotropical region. [WWW Document]. WaspWeb. URL url: www.waspweb.org (accessed 10.30.22).

Zappalà, L., Biondi, A., Alma, A., Al-Jboory, I.J., Arnò, J., Bayram, A., Chailleux, A., El-Arnaouty, A., Gerling, D., Guenaoui, Y., Shaltiel-Harpaz, L., Siscaro, G., Stavrinides, M., Tavella, L., Vercher Aznar, R., Urbaneja, A., Desneux, N., Zappala, L. and Desneux, N. 2013. Natural enemies of the South American moth, *Tuta absoluta*, in Europe, North Africa and Middle East, and their potential use in pest control strategies. *J. Pest Sci. (2004).* 86:635–647. https://doi.org/10.1007/s10340-013-0531-9

Zekeya, N., Dubois, T., Smith, J. and Ramasamy, S. 2022. Field effectiveness of *Metarhizium* *anisopliae* and pheromone traps against *Phthorimaea absoluta* on tomato in Tanzania. *Crop Prot.* 156:105942. https://doi.org/10.1016/j.cropro.2022.105942

Zekeya, N., Mtambo, M., Ramasamy, S., Chacha, M., Ndakidemi, P.A. and Mbega, E.R. 2019. First record of an entomopathogenic fungus of tomato leafminer, *Tuta absoluta* (Meyrick) in Tanzania. *Biocontrol Sci. Technol.* 29:626–637. https://doi.org/10.1080/09583157.2019.1573972

CHAPTER 3

Ratio dependence effects of the parasitoid *Dolichogenidea gelechiidivoris* on its associated host *Tuta absoluta*

Citation:

Mama Sambo, S., Ndlela, S., du Plessis, H., Obala, F. and Mohamed, S.A. 2022. Ratio dependence effects of the parasitoid *Dolichogenidea gelechiidivoris* on its associated host *Tuta absoluta*. Biocontrol Science and Technology, 32:497-510, DOI: 10.1080/09583157.2022.2033702

BIOCONTROL SCIENCE AND TECHNOLOGY
2022, VOL. 32, NO. 4, 497–510
https://doi.org/10.1080/09583157.2022.2033702

RESEARCH ARTICLE

Ratio dependence effects of the parasitoid *Dolichogenidea gelechiidivoris* on its associated host *Tuta absoluta*

Sahadatou Mama Sambo [a,b], Shepard Ndlela [a], Hannalene du Plessis [b], Francis Obala [a] and Samira Abuelgasim Mohamed [a]

[a]International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya; [b]Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa

ABSTRACT
The invasion of Africa by the South American leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) has caused a severe threat to the production of tomato, *Solanum lycopersicum* L. (Solanaceae) crops. The excessive use of pesticides for controlling this pest has increased, together with the associated environmental and human health risks. The parasitoid, *Dolichogenedia gelechiidivoris* Marsh. (Hymenoptera: Braconidae), has been imported into Africa for classical biological control of *T. absoluta*. To facilitate the mass production of the parasitoid and to predict the success under field conditions, its performance under laboratory conditions was investigated at five densities of *T. absoluta* larvae, *viz.* 20, 50, 100, 150, and 200 offered to either a single or group of mated parasitoid females. The findings revealed that *D. gelechiidivoris* exhibited a type II functional response. Host and parasitoid densities affected superparasitism, which was generally low, less than 5%. Parasitoid offspring emergence ranged from 8 to 39 wasps for a single foraging female, and 6-59 wasps, for a group of foraging females. The host larval densities did not affect the male: female ratio. This study showed that *D. gelechiidivoris* acts in a density-dependent manner. Therefore, for optimisation of the mass production of the parasitoid, the maximum number of hosts should be offered to a female. The results suggest that *D. gelechiidivoris* could be effective for classical biological control of *T. absoluta*, albeit field evaluation is required to validate this finding.

ARTICLE HISTORY
Received 3 September 2021
Returned 17 January 2022
Accepted 20 January 2022

KEYWORDS
Functional response; exotic parasitoid; superparasitism; South American tomato leafminer

1. Introduction

The invasion of Africa by *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) has immensely impacted tomato production in the continent (Biondi et al., 2018; Karlsson et al., 2018; Mansour et al., 2018; Tonnang et al., 2015). The pest attacks the aerial plant parts such as the leaves, apical buds, ripe and green fruit, as well as stems (Biondi et al., 2018; Desneux et al., 2010; Proffit et al., 2011), resulting in crop damage and loss in yield. *Tuta absoluta* is responsible for up to 100% yield losses due to direct damage to tomato quality and quantity (Chidege et al., 2016; Mansour et al., 2018;

CONTACT Samira Abuelgasim Mohamed sfaris@icipe.org International Centre of Insect Physiology and Ecology (icipe), P.O. Box 30772-00100, Nairobi, Kenya

Rwomushana et al., 2019). Additionally, the indirect loss due to the pest is a result of quarantine restrictions imposed on exports to lucrative markets (EPPO, 2005).

Tomato growers across Africa respond to this pest by using broad-spectrum synthetic insecticides (Aigbedion-Atalor et al., 2019; Rwomushana et al., 2019). Although insecticides are effective in reducing the pests' infestation levels, they are harmful to non-target species (Abbes et al., 2015; Longley, 1999) and can induce the development of resistance in the pest (Grant et al., 2019, 2021; Guedes et al., 2019), thus exacerbating its infestation levels and impacts. In addition, this approach poses multiple human health hazards such as the intoxication of farmers during application and consumers (Marete et al., 2021; Rwomushana et al., 2019). In that regards, biological control has been reported to play a key role in the control of this pest (Desneux et al., 2010; Gervassio et al., 2019). However, in the early years of invasion by *T. absoluta*, indigenous natural enemies that formed a new association with the pest were not effective in suppressing its population in the field (Abbes et al., 2013; Mansour et al., 2018; Seydi et al., 2021). A study by Kinyanjui et al. (2021) reported a combined parasitism of less than 7%, by several species that formed new associations with the pest in Kenya, East Africa. This low parasitism rate emphasises the need for the introduction of more efficient natural enemies. In 2017, the micrograstrinae koinobiont endoparasitoid, *Dolichogenidea gelechiidivoris* Masch. (Syn.: *Apanteles gelechiidivoris* Marsh) (Hymenoptera: Braconidae) was introduced into Kenya from Peru for classical biological control of this pest in Africa (icipe, 2016). Laboratory studies on the performance of the parasitoid confirmed *D. gelechiidivoris* as a promising control agent, with more than 55% parasitism on the early larval instars of the pest (Aigbedion-Atalor et al., 2020). However, for efficient, and economic field releases, it is vital to know the appropriate parasitoid and host ratios to optimise a protocol for mass production of this parasitoid, and the number of *D. gelechiidivoris* to be released for a significant reduction of *T. absoluta* populations.

Functional response is an approach to shape feeding proportions, to understand the host and natural enemy population dynamics (Holling, 1959). Functional response is affected by abiotic parameters such as temperature and relative humidity (Dannon et al., 2010; Flinn, 1991; Hemptinne & Brodeur, 2012; Kalyebi et al., 2005; Menon et al., 2002) as well as biotic factors such as host plants (Lill et al., 2002). The chemical structure of the natural diet (the plant where the host pest feeds) (Nieminen et al., 2003) or volatiles emitted by the host plant (Liu & Jiang, 2003) and physical structure of the plant parts play a role in how the parasitoid responds (Glas et al., 2012; Norton et al., 2001). The age of the host, exposure time, searching ability, and fecundity of the parasitoid also influence functional response (Chen et al., 2017; Milanez et al., 2018; Nikbin et al., 2014).

Other aspects of the parasitoid such as parasitoid performance in intraspecific conditions can be crucial in implementing parasitoid mass-rearing protocols (Mahmoudi et al., 2010), and in the prediction of the parasitoid's success as a biocontrol agent (Fernández-arhex & Corley, 2003). Mutual interference, defined as the interaction of parasitoids resulting into a reduction of searching efficiency (Visser & Driessen, 1990), destabilises the population dynamics of parasitoids. For instance, more than one female of *Anagyrus* sp. nov. nr. sinope Noyes & Menezes (Hymenoptera: Encyrtidae) significantly reduced the number of parasitised mealybugs, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae), and the number of progenies produced for each parasitoid (Chong & Oetting, 2006). This indicates that both the host and parasitoid density significantly impacts progeny production by the parasitoid and functional response type (Chong & Oetting, 2006).

The functional response for several parasitoid species to *T. absoluta* has been recorded. It includes *Dineulophus phtorimaeae* (de Santis) (Hymenoptera: Eulophidae) (Savino et al., 2012); *Pseudapanteles dignus* (Muesebeck) (Hymenoptera: Braconidae) (Sánchez et al., 2009); *Necremnus tutae* Ribes & Bernardo and *N. cosmopterix* Ribes & Bernardo (Hymenoptera: Eulophidae) (Bodino et al., 2018), *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae) (Guleria et al., 2020) and some *Trichogramma* species (Hymenoptera: Trichogrammatidae) (Manohar et al., 2020). Bajonero et al. (2008) reported the functional response of *D. gelechiidivoris* on third larval instar of *T. absoluta* at different constant temperatures. However, it is more realistic to evaluate the functional responses of a parasitoid using its preferred host stage(s) (Talebi et al., 2021; Wang et al., 2020). Therefore, following the recent findings that *D. gelechiidivoris* prefers first and second instar larvae (Aigbedion-Atalor et al., 2020), the objective of this study was to determine the functional response type of *D. gelechiidivoris* females to different densities of *T. absoluta* larvae and to evaluate the effect of the *D. gelechiidivoris* density on the parasitism rate of *T. absoluta*.

2. Materials and methods

2.1. Tomato plants

Seeds of the tomato variety Moneymaker were planted in 24-cavity plastic nursery trays (50 × 32 cm). Four to five seeds were planted per cavity. Three weeks after planting, seedlings from these trays were individually transplanted into 2 L plastic pots (14 cm upper-diameter, 8.5 cm lower-diameter, and 14 cm height), containing sterilised soil mixed with 100% goat manure. The plants were then maintained for three to four weeks in an insect-proof screen house at the International Centre of Insect Physiology and Ecology-*icipe* in Nairobi.

2.2. Insect colonies

2.2.1. Host colony

A colony was initiated from *T. absoluta* infested tomato leaves collected from open-field farms in Kirinyaga County (0°37’33.3"S 37°21’36.5"E, 1,194 m a.s.l), Kenya. Emerging adults were maintained at ambient laboratory conditions (25 ± 2°C; 65 ± 5% RH; 12hL: 12hD photoperiod). Four-week-old tomato plants were then exposed to *T. absoluta* moths for oviposition in Perspex cages (30 × 40 × 60 cm) and replaced three times a week. Thereafter, the infested plants were transferred into another Perspex cage of the same size for moths’ emergence.

2.2.2. Parasitoid colony

The initial colony of *D. gelechiidivoris* was obtained from the International Potato Center (CIP) and maintained under quarantine conditions in the Animal Rearing and Containment Unit (ARCU) at *icipe* in conditions similar to those described for the rearing of *T. absoluta*. Rearing was done according to the method described by Aigbedion-Atalor et al. (2020). Tomato plants were exposed to *T. absoluta* for oviposition for two days. After hatching, plants with first and second instars *T. absoluta* larvae were exposed to a group of *D. gelechiidivoris* males and females, in a Perspex cage (40 cm × 20 cm × 50 cm), for 48 h. The parasitoid-exposed plants were incubated with the addition of fresh tomato leaves to ensure good development of *T. absoluta* larvae until cocoon formation and wasp emergence. Parasitoids were fed on 80% honey solution, and two-day-old mated females were used in the experiments.

2.3. Bioassay

2.3.1 Effect of host and parasitoid densities on oviposition rate of D. gelechiidivoris female on T. absoluta

A four weeks old potted tomato plant was placed in a Perspex cage (50cm × 50cm × 60cm), containing 50 mature and mated pairs of moths for oviposition. The plants were removed from the cages after 24 h and maintained under the same conditions as described above until the eggs hatched.

The infested tomato plants were used five days after oviposition when the plants had only first instar larvae. These larvae in mines were counted at different densities, *viz*. 20, 50, 100, 150, and 200 *T. absoluta* larvae. The mines on each leaf were examined and the larvae in the mines were counted. All excess larvae and unhatched eggs were removed using fine-tipped forceps and camel hairbrush. The petiole of each test mined leaf was cut from the rachis of the plant and immersed in clean tap water, in a Soda Glass Specimen Test Tube (75mm × 19 mm). The area around the petiole was sealed with cotton wool to prevent parasitoids from drowning. The tubes with the infested leaves were introduced into a Perspex cage (15 × 15 × 15 cm), with the addition of honey droplets on the inside top of the cage, and cotton wool ball moistened with water placed on the floor of the cage. One naive mated *D. gelechiidivoris* couple (1♀:1♂), two days old, was then introduced into each Perspex cage. There were 10 cages (replicates) for each of the larval host densities. The experiment was repeated to investigate the effect of intraspecific competition on the number of eggs laid by *D. gelechiidivoris* females, but 3 couples of parasitoids (3♀:3♂), of the same age, were introduced. The parasitoids in each of these experiments were allowed to oviposit for 24 h. The leaves with larvae were then removed from the cages and kept in separate plastic containers (2 L) at ambient laboratory conditions (25 ± 2°C; 65 ± 5% RH; 12hL: 12hD photoperiod). The parasitised larvae were dissected in 0.9g of Sodium Chloride dissolved in 1 L of distilled water, as the buffer. Dissection was done under a stereomicroscope (Leica EZ4D digital stereomicroscope; Leica Microsystems, Heerbrugg, Switzerland) 24 h later. The number of parasitoid eggs in each host larva was recorded for the respective host densities as well as for both the host-parasitoid combinations. The number of parasitised and superparasitised larvae were recorded.

2.3.2 Effect of host and parasitoid densities on D. gelechiidivoris emergence

This bioassay was conducted using the same methodology as described in section 2.3.1 above, except that the parasitoid exposed larvae were allowed to develop until adult eclosion. Following the 24-hour exposure of *T. absoluta* larvae to *D. gelechiidivoris*, the parasitised host larvae were transferred into 4 L transparent plastic boxes, lined with a paper towel, and kept at ambient laboratory conditions until cocoon formation. Fresh, uninfested tomato leaves were added to the plastic boxes as supplementary food for the larvae. The sex and number of emerged parasitoids were recorded. The experiments were replicated 10 times for each host density and host-parasitoid combination. To determine the proportion of *D. gelechiidivoris* females, the number of females that emerged from each host density treatment, in both experiments, was divided by the total number of parasitoids that emerged.

2.4. Data analysis

The effect of host larval density and parasitoid density on the number of parasitised larvae were arcsine transformed and tested for normality (Shapiro >0.05), and the percentage of superparasitised larvae was log-transformed, before they were subjected to two-way ANOVA. For the effect of the host (*T. absoluta* larvae) and parasitoid densities on the oviposition rate by *D. gelechiidivoris*, the functional response type for each parasitoid density was modelled. The performance of one *D. gelechiidivoris* female in the experiment where intraspecific competition between three foraging females was evaluated as the mean number of eggs recorded per female (one-third of the total number of eggs) for each of the respective host densities provided. The two datasets were analysed separately using the *Frair* package (Pritchard et al., 2017) to determine the functional response Type. The *Frair_test ()* function uses logistic regression of the number of eggs laid by the parasitoid of the initial density was ran, to show the functional response type. *Frair_test ()* output differentiate type II and type III functional based on negative sign and significance of first-order (density) and second-order (density^2) terms respectively (Pritchard et al., 2017). After determining the type of functional response, *frair_fit()* function was used to estimate handling time (h) which is the time spent by *D. gelechiidivoris* to parasitise each *T. absoluta* larvae, and searching efficiency (a) which is the instantaneous *T. absoluta* larvae capture rate of *D. gelechiidivoris* per unit of time. *Frair_compare()* was then used to test whether there are differences between the parameters earlier estimated throughout *Frair_fit()*.

Regarding the effect of host densities on *D. gelechiidivoris* emergence for each parasitoid density, the Shapiro–Wilk test was used to test for normality. When data were not normally distributed, the General Linear Model with Poisson distribution and General Linear model with negative binomial were performed to determine the statistical difference. The model with the lowest Akaike information criterion (AIC) was selected as the best model. Means were separated using Tukey's post hoc range test. The analyses were done using R (R Core Team, 2018).

3. Results

3.1. Effect of host and parasitoid densities on oviposition rate of D. gelechiidivoris on T. absoluta

Both host and parasitoid densities affected the number of parasitised host larvae ($F_{1,96}$ = 48.96, $P < 0.001$) and ($F_{1,96}$ = 55.26, $P < 0.001$), respectively. Similarly, percentage of superparasitised larvae varied with the host density and the parasitoid density ($F_{1,96}$ = 11.16, $P < 0.001$) and ($F_{1,96}$ = 4.40, $P = 0.038$), respectively. The number of host larvae parasitised by both one female and a group of three females increased with the host density, whereas the percentage of superparasitised hosts decreased with increasing host density (Table 1). The highest number of parasitised hosts by single *D. gelechiidivoris* was at the density of 100 *T. absoluta* larvae, while for the group foraging, a positive linear correlation with the number of host larvae exposed was found (Table 1). Frair_test output showed a negative sign and significance of the first-order (density) term in logistic regressions for both scenarios (Table 2). This provided evidence for a type II functional response when a single female was exposed (Figure 1(A)), and in a possible interaction effect (a single female in a group) (Figure 1(B)). The handling time and the searching efficacies by female parasitoids were significantly affected by host density for both single foraging female ($P < 0.001$) and group foraging females ($P < 0.001$) (Table 3). Handling time for group foraging females was lower (0.010 ± 0.001) compared to that of a single foraging female (0.016 ± 0.001) (Table 3). Similarly, the searching efficacy was lower in group foraging females (0.61 ± 0.066) compared to single foraging female (3.034 ± 0.40) (Table 3).

3.2. Effect of host and parasitoid densities on D. gelechiidivoris emergence

The number of *D. gelechiidivoris* offspring ranged from 9±1 to 34±5 and from 8±2 to 51± 8 for single and group foraging parasitoid females, respectively (Table 4). The number of parasitoids that emerged from the host larvae at different densities, differed significantly for single ($F_{1,48} = 3.85$, $P = 0.04$) and group foraging parasitoid females ($F_{1,48} = 10.53$, $P < 0.001$). From the *T. absoluta* larvae exposed to one couple, significantly more parasitoids emerged from 100 larvae than from 20 and 40 exposed larvae. However, host density above 100 larvae did not result in a significant increase in the number of parasitoid progeny (Table 4). Considering the exposure to three parasitoid couples, the highest number of progenies was obtained from exposure to 100 *T. absoluta* larvae, which was significantly more than with 150 and 200 exposed larvae (Table 4). Sex ratio (indicated

Table 1. Mean ± SE of number of parasitised and percentage of superparasitised *T. absoluta* larvae at different host and parasitoid densities.

	Single female foraging (1♀:1♂)		Group of females foraging (3♀:3♂)	
Host density	No parasitised larvae	% Superparasitised larvae	No parasitised larvae	% Superparasitised larvae
20	9.6 ± 1.97 b	2.50 ± 0.83 a	11.17 ± 1.02 c	2.00 ± 0.82 a
50	22.5 ± 2.65 ab	0.80 ± 0.33 b	31.3 ± 2.43 bc	1.60 ± 0.27 ab
100	36.9 ± 4.52 a	0 c	57.8 ± 4.88 ab	0.26 ± 0.10 b
150	18.9 ± 6.63 b	0 c	70.0 ± 9.26 a	0.10 ± 0.10 b
200	21.2 ± 3.32 ab	0.5 ± 0.5 b	74.4 ± 10.45 a	0.10 ± 0.06 b

Note: Means with different letters in columns indicate statistically significant differences between the densities for each parameter (Tukey's HSD, α = 0.5).

Table 2. Type II logistic regression output for the functional response models selection for single foraging female and a female in group foraging.

Parasitoid density	Estimate	Std.Error	z value	Pr(>|z|)
Single female foraging; (1♀:1♂)	−0.0139	0.000632	−21.992	<0.001
A female in group foraging; (1/3 couples)*	−0.00409	0.000666	−6.1495	<0.001

*One female response estimation in group exposure.

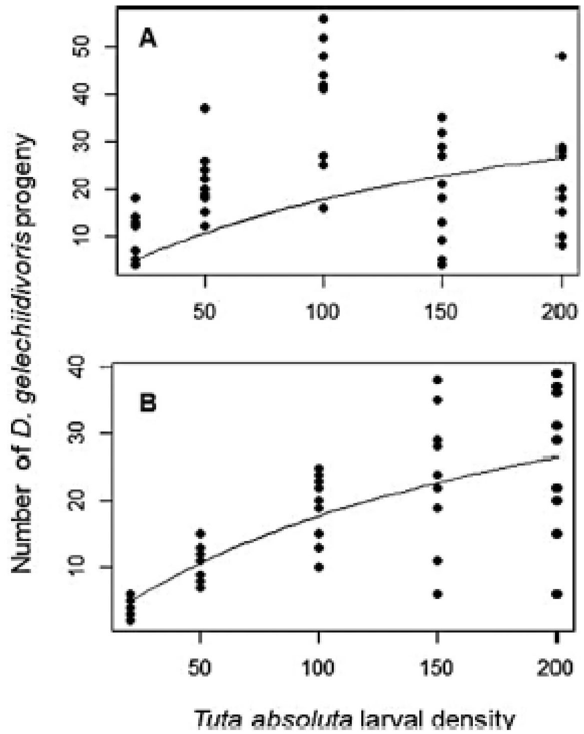


Figure 1. Type II functional response of *D. gelechiidivoris* to *T. absoluta* larval density; (A) Exposure to one couple (single foraging), and (B) Interaction effect (one female response in group of three foraging)

Table 3. Functional responses parameters estimated by equations (Roger's) of *D. gelechiidivoris* to densities of *T. absoluta* larvae at two different parasitoid densities

Parasitoid density	Model	Parameters	Estimate	SE	*P*-value	95% C.I.
Single female foraging (1 couple)	Roger II	a	3.0386	0.3396	<0.001	(1.97-5.00)
		h	0.0162	0.0007	<0.001	(0.01-0.02)
A female in group foraging (1/3 couples)*	Roger II	a	0.6077	0.0663	<0.001	(0.48-0.77)
		h	0.0101	0.0015	<0.001	(0.005-0.02)

*One female response estimation in group foraging, a: searching efficiency/attack rate; h: handling time; C.I.: Confidence intervals.

Table 4. Effect of host and parasitoid density on *D. gelechiidivoris* emergence.

	Single female foraging (1♀:1♂)		Group of females foraging (3♀:3♂)	
Host Densities	Mean No. of emerged parasitoids ± SE	Mean proportion of females ± SE[T]	Mean No. of emerged parasitoids ± SE	Mean proportion of females ± SE[T]
20	9 ± 1 c	0.5 ± 0.06 a	8 ± 2 d	0.5 ± 0.05 a
50	18 ± 3 b	0.3 ± 0.05 a	23 ± 4 c	0.16 ± 0.03 a
100	34 ± 5 a	0.47 ± 0.07 a	51 ± 8 a	0.44 ± 0.05 a
150	24 ± 5 ab	0.5 ± 0.04 a	32 ± 5 bc	0.39 ± 0.06 a
200	20 ± 4 ab	0.5 ± 0.06 a	36 ± 6 b	0.41 ± 0.07 a

Note: Means ± SE with different letters in columns indicate statistically significant difference for the densities for each parameter (Tukey's HSD, α = 0.5).[T] = proportion where the maximum was set as 1.

by proportion of female progeny) was not significantly influenced by larval host density for both single ($F_{1, 48} = 0.43$, $P = 0.05$) and group foraging females ($F_{1, 48} = 0.004$, $P = 0.95$) (Table 4).

4. Discussion

The results of this study showed a functional response type II by *D. gelechiidivoris,* both when exposed singly and in a group. This type of functional response suggests density-dependent parasitism up to a certain level of host density at which the attack rate remains constant regardless of the increase in host density (Holling, 1959). The type II functional response is satisfactory for regulation of pest populations, although type III is found ideal (Fernández-arhex & Corley, 2003; van Lenteren et al., 2016). Other hymenopteran parasitoid species displayed various types of functional response types when tested on the same host (*T. absoluta*). For example, Bodino et al. (2018) reported a similar response (type II), for the larval parasitoid *Necremnus cosmopterix* Ribes & Bernardo (Hymenoptera: Eulophidae), but a type III functional response for the congeneric *Necremnus tutae* Ribes & Bernardo. Whereas a type I functional response was reported for the larval parasitoid *Pseudapanteles dignus* (Muesebeck) (Braconidae) on the same host (Luna et al., 2017).

Differences in the number of eggs laid by a single *D. gelechiidivoris* female in *T. absoluta* larvae were reported in the current study, with an average of 37 eggs per two-days old female on 100 first instar larvae after 24h exposure, while Bajonero et al. (2008) reported a maximum of 12 eggs per 24h at the larval density of 160, and Aigbedion-Atalor et al. (2020) reported 20 eggs from a one-day old female at 26°C and 50–70% relative humidity. The lower number of eggs reported by Bajonero et al. (2008), compared to this study that was conducted under similar temperatures, could be ascribed to the difference in the size of the larvae used between the two studies. Third instar larvae were exposed in the study of Bajonero et al. (2008), while first instar larvae were used in the current study. Although Aigbedion-Atalor et al. (2020), used early instar larvae, similar to those used in this study, their study was conducted at a much higher temperature, which may explain the lower performance of *D. gelechiidivoris* in terms of the number of eggs laid/female, since the optimal temperature for oviposition by this parasitoid, is 20°C (Bajonero et al., 2008). Environmental factors such as temperature and relative humidity have been reported to influence functional responses of parasitoids in host/parasitoid associations (Dannon et al., 2010; Flinn, 1991; Jamshidnia & Sadeghi, 2014; Menon et al., 2002).

The number of eggs laid by one female wasp while foraging in a group, was lower than that laid by a wasp foraging individually, but at higher host densities, the number of eggs laid per female foraging in a group was, however, higher than that for wasps foraging individually. At low host densities, searching time was found to be longer compared to high host density scenarios, and the addition of conspecific competitors forces the female to spend more time searching for non-parasitised larvae (either by conspecific or by the same wasp), before deciding to oviposit. This trend was also reported in other parasitoid-host associations. For example, *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae) females reacted to the presence of conspecifics with a substantial increase in search rate when offered *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs (Mills and Lacan, 2004). Couchoux and Nouhuys (2014) ascribed the longer searching time by *Hyposoter horticola* Gravenhost (Hymenoptera: Ichneumonidae) to the high probability of different female wasps visiting the same host egg in a day, and therefore, females searched for non-parasitised host eggs for longer than when they were alone.

One of the behavioural steps preceding parasitism by parasitoids is the assessment of the quality of the host, including whether the host has already been parasitised. For instance, in solitary larva parasitoids, such as *D. gelechiidivoris* (Fernandez-Triana et al., 2020), only one larva per host completes development in solitary species (Chau & Mackauer, 2001). Thus, deposition of eggs in an already parasitised host, would result in intense intraspecific competition between the progenies (van Alphen & Visser, 1990). Many solitary parasitoids have therefore evolved mechanisms to avoid superparasitism (Chen et al., 2020; Ueno & Tanaka, 1994). Since female parasitoids can distinguish between parasitised and unparasitised hosts (Chen et al., 2020; Gauthier et al., 1996), a decision to avoid or accept the already parasitised host has its effect on the relative profits and costs of oviposition (van Alphen & Visser, 1990). Although superparasitism was recorded in this study, it was very low, and its occurrence was generally at the lowest host densities indicating that *D. gelechiidivoris* may discriminate between parasitised and unparasitised *T. absoluta* larvae. This is a good trait for the success of biological control programmes. The increase in superparasitism with a decrease in host density has also been recorded for the solitary braconid *Aphidius nigripes* Ashmead (Cloutier et al., 1984) and *Lysiphlebus delhiensis* (Subba Rao & Sharma) (Mishra & Singh, 1993) on the aphid *Macrosiphum euphorbiae* (Thomas) and *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae), respectively.

The efficiency of *D. gelechiidivoris* against *T. absoluta*, evident from the type II functional response, is further substantiated by a high number of emerged wasps. Out of the 100 exposed larvae, 34 and 51% yielded *D. gelechiidivoris* wasps, for single and group foraging females, respectively. This outcome indicates that, to maximise adult emergence yield in a mass production protocol, a ratio of one *D. gelechiidivoris* female to 100 first instar larvae of *T. absoluta* is recommended. Although exposure of the same number of larvae to a group of female parasitoids will not limit the parasitisation. This is similar to the findings of Aigbedion-Atalor et al. (2020), who reported 53% of *D. gelechiidivoris* wasps to have emerged from exposed *T. absoluta*. In the latter study, larvae were exposed to a higher parasitoid-host ratio (1:20), compared to this study. The congenic parasitoid, *Dolichogenidea appellator* (Telenga) (Hymenoptera: Braconidae) was reported to have formed a new association with *T. absoluta* in some parts of Africa (Idriss et al., 2018). The highest percentage of emerged wasps reported for *D. appellator* was 30%, with much higher parasitoid-host ratios used (Idriss et al., 2018).

With the exposure of *T. absoluta* larvae to a single or a group of *D. gelechiidivoris* female, no significant sex ratio bias in the parasitoid progeny was observed. It was, however, slightly male-biased when the larvae were exposed to *D. gelechiidivoris* in a group, except at a larval host density of 20. It was in contrast to a female-biased sex ratio reported by Aigbedion-Atalor et al. (2020), for the same parasitoid and host association (originating from the same colonies). Similarly, Bajonero et al. (2008) reported lower male emergence than females at 14°C, 20°C and 26°C with a highest female bias at higher temperatures. The more relatively male-biased sex ratio reported in this study could be due to the fact that the parasitoid was kept for more generations under laboratory conditions compared to those used by Aigbedion-Atalor et al. (2020) and Bajonero et al. (2008), without supplementing the population with wild individuals to enhance their genetic makeup. A more male-biased sex ratio for group foraging *Anagyrus* sp. females was also reported by Chong and Oetting (2006) who found that interference among foraging parasitoids affected the sex ratio of the progeny in favour of males.

In conclusion, results of this study provide information to efficiently rear *D. gelechiidivoris* and to guide field releases of this parasitoid for the control of *T. absoluta*. *Dolichogenidea gelechiidivoris* may be considered as a potential biocontrol agent of *T. absoluta*, as evident from its density-dependent response (type II functional response), high percentage parasitism rate, and the high number of emerged wasps. The response of this parasitoid to other related Gelechiidae, which could be sympatric with the target host, *T. absoluta* needs to be investigated. Several indigenous parasitoid species were found to form new associations with *T. absoluta* in Africa, such as *Bracon sp*., *D. appellator*, *Stenomesius* sp. *Hockeria* sp. and *Necremnus* sp. (Idriss et al., 2018; Kinyanjui et al., 2021). Very low percentage parasitism, by these species, were reported, but the interaction between *D. gelechiidivoris* and these parasitoid species should be investigated for future implementation of Integrated Pest Management.

Acknowledgements

The authors gratefully acknowledge the financial support for this research by the following organizations and agencies: Biovision Foundation grant number BV DPP-012/2019-2022; the Norwegian Agency for Development Cooperation, the section for research, innovation and higher education grant number RAF-3058 KEN-18/0005; the Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); the Federal Democratic Republic of Ethiopia; and the Government of the Republic of Kenya. The first author was supported by the German Academic Exchange Service (DAAD) through the African Regional Postgraduate Programme in Insect Science (ARPPIS) tenable at icipe. The views expressed herein do not necessarily reflect the official opinion of the donors. The authors are also grateful to Esther Awuor Owino, Patrick Kipkorir Koech, and Omar Jimali Ibrahim for their technical support and Dr. Abdelmutalab Gesmalla for assisting in the statistical analysis.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This research received financial support from Biovision Foundation grant number BV DPP-012/2019-2022, Norwegian Agency for Development Cooperation-(NORAD), the section for research, innovation and higher education grant number RAF-3058 KEN-18/0005. The first author was supported by the German Academic Exchange Service (DAAD) through the African Regional Postgraduate Programme in Insect Science (ARPPIS) tenable at *icipe*.

ORCID

Sahadatou Mama Sambo http://orcid.org/0000-0002-4470-5622
Shepard Ndlela http://orcid.org/0000-0003-0015-9946
Hannalene du Plessis http://orcid.org/0000-0003-1163-1468
Francis Obala http://orcid.org/0000-0003-1601-9833
Samira Abuelgasim Mohamed http://orcid.org/0000-0001-6836-7528

References

Abbes, K., Biondi, A., Kurtulus, A., Ricupero, M., & Russo, A. (2015). Combined non-target effects of insecticide and high temperature on the parasitoid *Bracon nigricans*. *PLoS ONE*, *10*(9), e0138411–14. https://doi.org/10.1371/journal.pone.0138411

Abbes, K., Biondi, A., Zappalà, L., & Chermiti, B. (2013). Fortuitous parasitoids of the invasive tomato leafminer *Tuta absoluta* in Tunisia. *Phytoparasitica*, *42*(1), 85–92. https://doi.org/10.1007/s12600-013-0341-x

Aigbedion-Atalor, P. O., Abuelgasim, S., Hill, M. P., Zalucki, M. P., Azrag, A. G. A., Srinivasan, R., & Ekesi, S. (2020). Host stage preference and performance of *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae), a candidate for classical biological control of *Tuta absoluta* in Africa. *Biological Control*, *144*, 104215. https://doi.org/10.1016/j.biocontrol.2020.104215

Aigbedion-Atalor, P. O., Hill, M. P., Zalucki, M. P., Obala, F., Idriss, G. E., Midingoyi, S. K., Chidege, M., Ekesi, S., & Mohamed, S. A. (2019). The South America tomato Leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), spreads its wings in Eastern Africa: Distribution and socioeconomic impacts. *Journal of Economic Entomology*, *112*(6), 2797–2807. https://doi.org/10.1093/jee/toz220

Bajonero, J., Córdoba, N., Cantor, F., Rodríguez, D., & Cure, R. (2008). Biology and life cicle of *Apanteles gelechiidivoris* (Hymenoptera: Braconidae) parasitoid of *Tuta absoluta*. *Agronomía Colombiana*, *26*(3), 417–426. http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0120-99652008000300006&lng=en&tlng=.

Biondi, A., Guedes, R. N. C. R., Wan, F.-H. F., & Desneux, N. (2018). Ecology, worldwide spread, and management of the invasive South American tomato pinworm, *Tuta absoluta*: Past, present, and future. *Annual Review of Entomology*, *63*(1), 239–258. https://doi.org/10.1146/annurev-ento-031616-034933

Bodino, N., Ferracini, C., & Tavella, L. (2018). Functional response and age-specific foraging behaviour of Necremnus tutae and *N. cosmopterix*, native natural enemies of the invasive pest *Tuta absoluta* in Mediterranean area. *Journal of Pest Science*, *92*(4), 1467–1478. https://doi.org/10.1007/s10340-018-1025-6

Chau, A., & Mackauer, M. (2001). Preference of the aphid parasitoid *Monoctonus paulensis* (Hymenoptera: Braconidae, Aphidiinae) for different aphid species: Female choice and offspring survival. *Biological Control*, *20*(1), 30–38. https://doi.org/10.1006/bcon.2000.0881

Chen, W., Vasseur, L., Zhang, S. Q., Zhang, H. F., Mao, J., Liu, T. S., Zhou, X. Y., Wang, X., Zhang, J., You, M. S., & Gurr, G. M. (2020). Mechanism and consequences for avoidance of superparasitism in the solitary parasitoid *Cotesia vestalis*. *Scientific Reports*, *10*(1), 11463. https://doi.org/10.1038/s41598-020-67050-1

Chen, X., Triana, M., & Stansly, P. A. (2017). Optimizing production of *Tamarixia radiata* (Hymenoptera: Eulophidae), a parasitoid of the citrus greening disease vector *Diaphorina citri* (Hemiptera: Psylloidea). *Biological Control*, *105*, 13–18. https://doi.org/10.1016/j.biocontrol.2016.10.010

Chidege, M., A-zaildi, S., Hassan, N., Julie, A., Kaaya, E., & Mrogoro, S. (2016). First record of tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Tanzania. *Agriculture & Food Security*, *5*(17), 1–7. https://doi.org/10.1186/s40066-016-0066-4

Chong, J. H., & Oetting, R. D. (2006). Functional response and progeny production of the Madeira mealybug parasitoid, *Anagyrus* sp. nov. nr. *sinope*: The effects of host and parasitoid densities. *Biological Control*, *39*(3), 320–328. https://doi.org/10.1016/j.biocontrol.2006.08.013

Cloutier, C., Dohse, L. A., & Bauduin, F. (1984). Host discrimination in the aphid parasitoid *Aphidius nigripes*. *Canadian Journal of Zoology*, *62*(7), 1367–1372. https://doi.org/10.1139/z84-196

Couchoux, C., & Nouhuys, S. V. (2014). Effects of intraspecific competition and host-parasitoid developmental timing on foraging behaviour of a parasitoid wasp. *Journal of Insect Behavior*, *27*(3), 283–301. https://doi.org/10.1007/s10905-013-9420-6

Dannon, E. A., Tamo, M., Huis, A. V., & Dicke, M. (2010). Functional response and life history parameters of *Apanteles taragamae*, a larval parasitoid of *Maruca vitrata*. *BioControl*, *55*(3), 363–378. https://doi.org/10.1007/s10526-009-9263-4

Desneux, N., Wajnberg, E., Kris, W. A. G., Burgio, G., Arpaia, S., Narváez-Vasquez, C. A., González-Cabrera, J., Ruescas, D. C., Tabone, E., Frandon, J., Pizzol, J., Poncet, C., Cabello, T., & Urbaneja, A. (2010). Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, geographic expansion and prospects for biological control. *Journal of Pest Science*, *83*(3), 197–215. https://doi.org/10.1007/s10340-010-0321-6

EPPO. (2005). Data sheets on quarantine pests Fiches informatives sur les organismes de quarantaine. *OEPP/EPPO Bulletin*, *35*. https://doi.org/10.1111/j.1439-0418.1998.tb01459.x

Fernández-arhex, V., & Corley, J. C. (2003). The functional response of parasitoids and its implications for biological control. *Biocontrol Science and Technology*, *13*(44), 403–413. https://doi.org/10.1080/0958315031000104523

Fernandez-Triana, J., Shaw, M. R., Boudreault, C., Beaudin, M., & Broad, G. R. (2020). Annotated and illustrated world checklist of Microgastrinae parasitoid wasps (Hymenoptera, Braconidae). *ZooKeys*, *920*, 1–1089. https://doi.org/10.3897/zookeys.920.39128

Flinn, P. W. (1991). Temperature-dependent functional response of the parasitoid *Cephalonomia waterstoni* (Gahan) (Hymenoptera: Bethylidae) attacking rusty grain beetle larvae (Coleoptera: Cucujidae). *Environmental Entomology*, *20*(3), 872–876. https://doi.org/10.1093/ee/20.3.872

Gauthier, N., Monge, J. E., & Huignard, J. (1996). Superparasitism and host discrimination in the solitary ectoparasitoid *Dinarmus basalis*. *Entomologia Experimentalis et Applicata*, *79*(1), 91–99. https://doi.org/10.1111/j.1570-7458.1996.tb00813.x

Gervassio, N. G. S., Aquino, D., Vallina, C., Biondi, A., & Luna, M. (2019). A re-examination of *Tuta absoluta* parasitoids in South America for optimized biological control. *Journal of Pest Science*, *92*(4), 1343–1357. https://doi.org/10.1007/s10340-018-01078-1

Glas, J. J., Schimmel, B. C. J., Alba, J. M., Escobar-Bravo, R., Schuurink, R. C., & Kant, M. R. (2012). Plant glandular trichomes as targets for breeding or engineering of resistance to herbivores. *International Journal of Molecular Sciences*, *13*(12), 17077–17103. https://doi.org/10.3390/ijms131217077

Grant, C., Jacobson, R., & Bass, C. (2021). Parthenogenesis in UK field populations of the tomato leaf miner, *Tuta absoluta*, exposed to the mating disruptor Isonet T. *Pest Management Science*, *77*(7), 3445–3449. https://doi.org/10.1002/ps.6394

Grant, C., Jacobson, R., Ilias, A., Berger, M., Vasakis, E., Bielza, P., Zimmer, C. T., Williamson, M. S., French-Constant, R. H., Vontas, J., Roditakis, E., & Bass, C. (2019). The evolution of multiple-insecticide resistance in UK populations of tomato leafminer, *Tuta absoluta*. *Pest Management Science*, *75*(8), 2079–2085. https://doi.org/10.1002/ps.5381

Guedes, R. N. C., Roditakis, E., Campos, M. R., Haddi, K., Bielza, P., Siqueira, H. A. A., Tsagkarakou, A., Vontas, J., & Nauen, R. (2019). Insecticide resistance in the tomato pinworm *Tuta absoluta*: Patterns, spread, mechanisms, management and outlook. *Journal of Pest Science*, *92*(4), 1329–1342. https://doi.org/10.1007/s10340-019-01086-9

Guleria, P., Sharma, P. L., Verma, S. C., Chandel, R. S., & Sharma, N. (2020). Functional response of *Neochrysocharis formosa* to *Tuta absoluta*. *Biocontrol Science and Technology*, *31*(3), 252–264. https://doi.org/10.1080/09583157.2020.1846163

Hemptinne, A. S. J., & Brodeur, J. (2012). Using functional response modeling to investigate the effect of temperature on predator feeding rate and energetic efficiency. *Oecologia*, *169*(4), 1117–1125. https://doi.org/10.1007/s00442-012-2255-6

Holling, C. S. (1959). Some characteristics of simple types of predation and parasitism. *The Canadian Entomologist*, *91*(5), 317–324. https://doi.org/10.4039/Ent91385-7

Icipe. (2016). *Annual Report*. http://www.icipe.org/publications/annual-reports. https://doi.org/10.5688/ajpe808s9

Idriss, G. E. A., Mohamed, S. A., Khamis, F., Du Plessis, H., & Ekesi, S. (2018). Biology and performance of two indigenous larval parasitoids on *Tuta absoluta* (Lepidoptera: Gelechiidae) in Sudan. *Biocontrol Science and Technology*, *28*(6), 614–628. https://doi.org/10.1080/09583157.2018.1477117

Jamshidnia, A., & Sadeghi, R. (2014). Effect of temperature on the functional response of the egg parasitoid *Telenomus busseolae* (Hymenoptera : Scelionidae) to sugarcane pink borer *Sesamia cretica* (Lepidoptera: Noctuidae) eggs. *International Journal of Tropical Insect Science*, *34*(1), 2–8. https://doi.org/10.1017/S1742758413000398

Kalyebi, A., Overholt, W. A., Schulthess, F., Mueke, J. M., & Hassan, S. A. (2005). Functional response of six indigenous trichogrammatid egg parasitoids (Hymenoptera: Trichogrammatidae) in Kenya: Influence of temperature and relative humidity. *Biological Control*, *32*(1), 164–171. https://doi.org/10.1016/j.biocontrol.2004.09.006

Karlsson, F. M., Rachidatou, S., Sahadatou, M. S., Joseph, Z. A., & Georg, G. (2018). First report of *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) in the Republic of Benin. *BioInvasions Records*, *7*(4), 463–468. https://doi.org/10.3391/bir.2018.7.4.19

Kinyanjui, G., Khamis, F. M., Ombura, F. L. O., Kenya, E. U., Ekesi, S., & Mohamed, S. A. (2021). Distribution, abundance and natural enemies of the invasive tomato leafminer, *Tuta absoluta* (Meyrick) in Kenya. *Bulletin of Entomological Research*, *111*(6), 1–16. https://doi.org/10.1017/S0007485321000304

Lill, J. T., Marquis, R. J., & Ricklefs, R. E. (2002). Host plants influence parasitism of forest caterpillar. *Nature*, *417*(6885), 170–173. https://doi.org/10.1038/417170a

Liu, S., & Jiang, L. (2003). Differential parasitism of *Plutella xylostella* (Lepidoptera: Plutellidae) larvae by the parasitoid *Cotesia plutellae* (Hymenoptera: Braconidae) on two host plant species. *Bulletin of Entomological Research*, *93*(1), 65–72. https://doi.org/10.1079/ber2002208

Longley, M. (1999). A review of pesticide effects upon immature aphid parasitoids within mummified hosts. *International Journal of Pest Management*, *45*(2), 139–145. https://doi.org/10.1080/096708799227932

Luna, M. G., Sánchez, N. E., & Pereyra, P. C. (2017). Parasitism of *Tuta absoluta* (Lepidoptera, Gelechiidae) by *Pseudapanteles dignus* (Hymenoptera, Braconidae) under laboratory conditions. *Behavior*, *36*(4), 887–893. https://doi.org/10.1603/0046-225x(2007)36[887:potalg]2.0.co;2

Mahmoudi, M., Sahragard, A., & Sendi, J. J. (2010). Foraging efficiency of *Lysiphlebus fabarum* Marshall (Hymenoptera: Aphidiidae) parasitizing the black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae), under laboratory conditions. *Journal of Asia-Pacific Entomology*, *13*(2), 111–116. https://doi.org/10.1016/j.aspen.2009.11.007

Manohar, T. N., Sharma, P. L., Verma, S. C., Sharma, K. C., & Chandel, R. S. (2020). Functional response of indigenous *Trichogramma* spp. to invasive tomato leafminer, *Tuta absoluta* (Meyrick) under laboratory conditions. *International Journal of Tropical Insect Science*, *40*(1), 101–107. https://doi.org/10.1007/s42690-019-00057-y

Mansour, R., Brévault, T., Chailleux, A., Cherif, A., Grissa-Lebdi, K., Haddi, K., Mohamed, S. A., Nofemela, R. S., Oke, A., Sylla, S., Tonnang, H. E. Z., Zappalà, L., Kenis, M., Desneux, N., & Biondi, A. (2018). Occurrence, biology, natural enemies and management of *Tuta absoluta* in Africa. *Entomologia Generalis*, *38*(2), 83–112. https://doi.org/10.1127/entomologia/2018/0749

Marete, G. M., Lalah, J. O., Mputhia, J., & Wekesa, V. W. (2021). Pesticide usage practices as sources of occupational exposure and health impacts on horticultural farmers in Meru county, Kenya. *Heliyon*, *7*(2), e06118. https://doi.org/10.1016/j.heliyon.2021.e06118

Menon, A., Flinn, P. W., & Dover, B. A. (2002). Influence of temperature on the functional response of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae), a parasitoid of *Rhyzopertha dominica* (Coleoptera: Bostrichidae). *Journal of Stored Products Research*, *38*(5), 463–469. https://doi.org/10.1016/S0022-474X(01)00050-9

Milanez, A. M., de Carvalho, J. R., Lima, V. L. S., & Pratissoli, D. (2018). Functional response of *Trichogramma pretiosum* on *Trichoplusia ni* eggs at different temperatures and egg densities. *Pesquisa Agropecuária Brasileira*, *53*(5), 641–645. https://doi.org/10.1590/S0100-204X2018000500013

Mills, N. J, & Lacan, I. (2004). Ratio dependence in the functional response of insect parasitoids: Evidence from Trichogramma minutum foraging for eggs in small host patches. *Ecological Entomology*, *29*(2), 208–216. https://doi.org/10.1111/j.0307-6946.2004.00584.x

Mishra, S., & Singh, R. (1993). Factors affecting supernumerary egg deposition by the parasitoid *Lysiphlebus delhiensis* (Subba Rao & Sharma) (Hymenoptera: Aphidiidae) into its host *Rhopalosiphum maidis* (Fitch). *Biological Agriculture and Horticulture*, *10*(1), 39–45. https://doi.org/10.1080/01448765.1993.9754649

Nieminen, M., Suomi, J., Nouhuys, S., Van, S. P., & Riekkola, M.-L. (2003). Effect of iridoid glycoside content on oviposition host plant choice and parasitism in a specialist herbivore. *Journal of Chemical Ecology*, *29*(4), 825–844. https://doi.org/10.1023/a:1022923514534

Nikbin, R., Sahragard, A., & Hosseini, M. (2014). Age-specific functional response of *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) parasitizing different egg densities of *Ephestia kuehniella* (Lepidoptera: Pyralidae). *Journal of Agricultural Science and Technology*, *16*(6), 1205–1216. https://jast.modares.ac.ir/article-23-4409-en.pdf

Norton, A. P., English-Loeb, G., & Belden, E. (2001). Host plant manipulation of natural enemies: Leaf domatia protect beneficial mites from insect predators. *Oecologia*, *126*(4), 535–542. https://doi.org/10.1007/s004420000556

Pritchard, D. W., Paterson, R. A., Bovy, H. C., & Neill, D. B. (2017). FRAIR: An R package for fitting and comparing consumer functional responses. *Methods in Ecology and Evolution*, *8*(11), 1528–1534. https://doi.org/10.1111/2041-210X.12784

Proffit, M., Birgersson, G., Bengtsson, M., Reis, R., Witzgall, P., & Lima, E. (2011). Attraction and oviposition of *Tuta absoluta* females in response to tomato leaf volatiles. *Journal of Chemical Ecology*, *37*(6), 565–574. https://doi.org/10.1007/s10886-011-9961-0

R Core Team. (2018). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. In Vienna, Austria. https://www.R-project.org/

Rwomushana, I., Day, R., & Gonzalez-moreno, P. (2019). *Evidence Note Tomato leafminer impacts and coping strategies for Africa*. https://doi.org/10.1079/CABICOMM-62-8100

Sánchez, N. E., Pereyra, P. C., & Luna, M. G. (2009). Spatial patterns of parasitism of the solitary parasitoid *Pseudapanteles dignus* (Hymenoptera: Braconidae) on *Tuta absoluta* (Lepidoptera: Gelechiidae). *Environmental Entomology*, *38*(2), 365–374. https://doi.org/10.1603/022.038.0208

Savino, V., Coviella, C. E., & Luna, M. G. (2012). Reproductive biology and functional response of *Dineulophus phtorimaeae*, a natural enemy of the tomato moth, *Tuta absoluta*. *Journal of Insect Science*, *12*(153), 1–14. https://doi.org/10.1673/031.012.15301

Seydi, O., Sylla, S., Diatte, M., & Labou, B. (2021). Recruitment of native parasitoids of the tomato leaf miner *Tuta absoluta* (Meyrick 1917), (Lepidoptera: Gelechiidae) in Senegal. *International Journal of Pest Management*, 1–7. https://doi.org/10.1080/09670874.2021.1943047

Talebi, A. A., Kazemi, M., Rezaei, M., Mirhosseini, M. A., & Moharramipour, S. (2021). Host stage preference and temperature-dependent functional response of *Diaeretiella rapae* (Hymenoptera: Braconidae) on *Schizaphis graminum* (Hemiptera: Aphididae). *International Journal of Tropical Insect Science*, 415–424. https://doi.org/10.1007/s42690-021-00558-9

Tonnang, H. E. Z., Mohamed, S. F., Khamis, F., & Ekesi, S. (2015). Identification and risk assessment for worldwide invasion and spread of *Tuta absoluta* with a focus on Sub-saharan Africa : Implications for phytosanitary measures and management. *PLoS ONE*, *10*(8), e0135283–19. https://doi.org/10.1371/journal.pone.0135283

Ueno, T., & Tanaka, T. (1994). Can a female parasitoid recognize rejected host? *Animal Behaviour*, *47*(4), 988–990. https://doi.org/10.1006/anbe.1994.1134

van Alphen, J. J. M., & Visser, M. E. (1990). Superparasitism as an adaptive strategy for insect parasitoids. *Annual Review of Entomology*, *35*(1), 59–79. https://doi.org/10.1146/annurev.en.35.010190.000423

van Lenteren, J. C., Hemerik, L., Lins, J. C., & Bueno, V. H. P. (2016). Functional responses of three neotropical mirid predators to eggs of *Tuta absoluta* on tomato. *Insects*, *7*(34), 34–10. https://doi.org/10.3390/insects7030034

Visser, M. E., & Driessen, G. (1990). Indirect mutual interference in parasitoids. *Netherlands Journal of Zoology*, *41*(2–3), 214–227. https://doi.org/10.1163/156854291X00153

Wang, X., Biondi, A., & Daane, K. M. (2020). Functional responses of three candidate Asian larval parasitoids evaluated for classical biological control of *Drosophila suzukii* (Diptera: Drosophilidae). *Journal of Economic Entomology*, *113*(1), 73–80. https://doi.org/10.1093/jee/toz265

CHAPTER 4

Identification, microhabitat, and ecological niche prediction of two promising native parasitoids of *Tuta absoluta* in Kenya.

Citation:

Mama Sambo, S., Ndlela, S., du Plessis, H., Obala, F., Mohamed, S.A. 2022.Identification, microhabitat, and ecological niche prediction of two promising native parasitoids of *Tuta absoluta* in Kenya. *Insects* 13,496. https://doi.org/10.3390/

insects

Article

Identification, Microhabitat, and Ecological Niche Prediction of Two Promising Native Parasitoids of *Tuta absoluta* in Kenya

Sahadatou Mama Sambo [1,2,*], Shepard Ndlela [1], Hannalene du Plessis [2], Francis Obala [1] and Samira Abuelgasim Mohamed [1,*]

[1] International Centre of Insect Physiology and Ecology (icipe), Nairobi P.O. Box 30772-00100, Kenya; sndlela@icipe.org (S.N.); fobala@icipe.org (F.O.)
[2] Unit for Environmental Sciences and Management, North-West University, Potchefstroom 2520, South Africa; hannalene.duplessis@nwu.ac.za
* Correspondence: smama@icipe.org (S.M.S.); sfaris@icipe.org (S.A.M.)

Simple Summary: Since the arrival of *Tuta absoluta*, a multivoltine insect species whose larvae develop in leaves, fruits, flowers, buds, and stems of tomatoes, producers are facing one of its biggest production challenges. The pest continues to invade new areas, causing heavy losses in the tomato value chain. Sprays of synthetic insecticides have shown very low efficacy on this pest because of its inclination to develop resistance to various insecticide-active ingredients. Biological control is one of the most promising solutions for the management of this pest. In this work, we investigated the most efficient indigenous parasitoids associated with *T. absoluta* in Kenya and their preferable habitat and ecological niche suitability. We identified two species, *Stenomesius* sp. near *japonicus* and *Bracon nigricans*, with up to 17% and 21% parasitism respectively. *Stenomesius* sp. near *japonicus* was more abundant in greenhouses and non-insecticide-treated tomatoes while *B. nigricans* was more abundant in the field tomatoes with a low abundance of *Nesidiocoris tenuis*. The ecological niche of these two species showed that *B. nigricans* was suitable for establishment in sub-Saharan Africa, a big part of South America, and Australia in both current and future scenarios.

Abstract: Associations between the South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), and its native parasitoids need to be updated to increase the implementation of pest control strategies. In this study, *T. absoluta*-infested tomato plants were collected from three regions in Kenya. The emerged parasitoids were identified, and their abundance was correlated with agroecological parameters, *viz.* cropping systems, and the abundance of the predator *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae). The study further conducted a habitat suitability prediction for the identified parasitoids. Two parasitoid species, *Bracon nigricans* (Szépligeti) (Hymenoptera: Braconidae) and *Stenomesius* sp. near *japonicus* (Ashmead) (Hymenoptera: Eulophidae) emerged from *T. absoluta* immature stages, with parasitism rates ranging from 0 to 21% and 0 to 17% respectively. Insecticide application and open field cropping negatively influenced the parasitism by *S.* sp. nr *japonicus*. Low occurrence of *N. tenuis* positively affected *B. nigricans* parasitism. The predicted occurrence of parasitoid species indicated vast suitable areas for *B. nigricans* in sub-Saharan Africa, Australia, and South America. Low suitability was observed for *S.* sp. nr *japonicus* in Africa. Therefore, native parasitoids, especially *B. nigricans* could be considered for implementation as a biocontrol agent in the Integrated Pest Management program of *T. absoluta*.

Keywords: indigenous parasitoids; molecular identification; morphological identification; South American tomato pinworm; parasitism rate; agroecology; habitat suitability

check for updates

Citation: Mama Sambo, S.; Ndlela, S.; du Plessis, H.; Obala, F.; Mohamed, S.A. Identification, Microhabitat, and Ecological Niche Prediction of Two Promising Native Parasitoids of *Tuta absoluta* in Kenya. *Insects* **2022**, *13*, 496. https://doi.org/10.3390/insects13060496

Academic Editor: Antonio Biondi

Received: 16 February 2022
Accepted: 19 May 2022
Published: 25 May 2022

1. Introduction

Invasive species are known to exert undesirable effects on biodiversity and human health [1–3]. They also adversely impact economic activities and food security [4,5]. The spread, establishment, and devastating effects of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) have contributed to the over-reliance on synthetic chemical insecticides for the management of this pest in Africa [6,7]. The preference by farmers for chemical control can be attributed to the observable quick knockdown of pests after application. As a result, the adverse effects associated with the use of synthetic chemicals are therefore ignored [8,9]. Mahugija et al. [10] reported that 50% of tomatoes grown and sold in Tanzania, had pesticide residues exceeding the official maximum residue limits, indicating a higher risk for public health. At the forefront of health risks are tomato farmers who due to the *T. absoluta* menace, apply insecticides regularly, often without any personal protective equipment. For example, up to 60% of Kenyan farmers do not use the necessary protective clothing when applying pesticides and as a result, 26% of them are reported to be suffering from pesticide-related health problems [11]. Due to the high pesticide cost in Kenya, farmers often use pesticides bought from informal markets in neighboring countries (Uganda and Tanzania), which are not registered for use on any specific crop [12]. The use of synthetic insecticides also influences the natural assemblage of parasitoids and predators of the pest [8,13].

Biological control is recommended for the control of *T. absoluta* albeit integrated with other control methods [7,14]. In most cases, invasive pests often arrive in new regions with no co-evolved natural enemies. However, if effective native parasitoids are present in the agroecological system, new associations with the pest may be formed based on multi-level interactions [15]. When the prey/host population levels are low, parasitoids and predators can switch their host and prey preferences depending on the availability of alternatives [16–19]. The use of natural enemies has several advantages for environmental conservation, especially due to their non-effect on non-target organisms [20,21].

Native parasitoids and predators have been used for *T. absoluta* control in several regions around the world. For instance, an estimated 65% predation and 20% parasitism by native species were reported from Israel in open tomato fields where synthetic chemicals were not applied [22]. *Trichogramma* spp. are lucratively used in many countries [23]. For instance, in Spain, the release of 30 *Trichogramma achaeae* Nagaraja and Nagarkatti (Hymenoptera: Trichogrammatidae) adults per plant reduced *T. absoluta* damage by 91.74% under greenhouse conditions [24]. Thus, the identification of effective indigenous parasitoids associated with *T. absoluta* is useful in the implementation of successful biocontrol programs.

Numerous parasitoids attacking *T. absoluta* in Africa have been described. These include the braconids *Cotesia vestalis* (Haliday), *Apanteles litae* Nixon, *Meteorus laphygmarum* Brues, *Chelonus sp*., and *Diadegma insulare* (Cresson), the Ichneumonidae *Pristomerus pallidus* (Kriechbaumer) and the Eulophidae *Elasmus* sp. in Senegal [25]. In North Africa, the Eulophid, *Necrenus artynes* (Walker) and *Neochrysocharis formosa* (Westwood), the Trichrommatid, *Trichrogramma bourarachae* Pintureau & Babault, the Patygastrid, *Telenomus* sp., and the Torymid, *Hemiptarsenus zilahisebessi* Erdös were discovered parasitizing *T. absoluta* under natural conditions [26–28]. Other native parasitoids of *T. absoluta* such as *Bracon nigricans* Szépligeti, *Bracon hebetor* (Say), *Ecdamua cadenat* (Risbec) (Hymenoptera: Torymidae), *Dolichogenidea appellator* (Telenga) (Hymenoptera: Braconidae), and *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae) have been reported in Sudan [29,30]. In the Middle East and North Africa (MENA) region, there have been several efforts of parasitoid releases against *T. absoluta* [31], and this showed a significant reduction in the pest damage [32–36].

The assemblage of native parasitoids in various tomato-producing areas needs to be documented if they are to be incorporated into augmentative and conservative biological control approaches for the management of *T. absoluta*. Furthermore, habitat and climate are significant factors limiting the distribution and abundance of insect pests, as well as their physiology and reproduction [37–42]. Parasitoids and predator distribution on a local scale, are also affected by agronomic practices such as pesticide application [43,44]. It is against this background that the current study sought to assess the presence and distribution of parasitoid species in open-field and greenhouse tomatoes in Kenya and their effectiveness in controlling *T. absoluta* as well as to determine their suitable habitat for perseverance in biological control programs.

2. Materials and Methods

2.1. Parasitoid Collection in the Study Area

The investigation was conducted in Kirinyaga, Nakuru, and Nairobi Counties, Kenya with samples collected 12 times between March 2020 and October 2021. Collections were done six times in Kirinyaga and on three occasions in Nakuru and Nairobi. Sampling sites within the Counties were randomly selected based on the presence of tomato production farms with *T. absoluta* infestations in open fields and greenhouses. For each collection in a site, infested leaves were randomly picked and placed in transparent 4-liter plastic containers and labeled according to GPS coordinates and date of collection. Depending on the availability of infested plants, three to twelve samples were collected. The containers were closed with a mesh-infused lid for aeration and transported to the laboratory at *icipe*. The samples were weighed and incubated separately under ambient laboratory conditions (25 ± 1 °C; 65 ± 5% r.h; 12HL:12HD photoperiod). Un-infested tomato plants grown under standard agronomic practices in a greenhouse at *icipe* were added to the containers regularly as food for the larvae. The plastic containers were monitored daily, and pupae were transferred into Perspex cages (30 cm × 30 cm × 30 cm). The number of moths, parasitoids, and *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) that emerged from each sample were recorded. The parasitoids were grouped based on morphological similarities, counted, and transferred into Perspex cages for initiation of rearing colonies and identification of the respective parasitoids.

2.2. Identification of the Parasitoids

2.2.1. Molecular Identification

Ten female parasitoid wasps obtained from the rearing colony were frozen at −20 °C in Eppendorf tubes. To obtain high DNA quality, the heads of the parasitoids were excised before extraction according to the protocol of Livak, [45]. For the polymerase chain reaction (PCR) amplification, a Master Mix solution was prepared by mixing the barcoding primers LCO1490 (5′GGTCAACAAATCATAAAGATATTGG3′), and HCO2198 (5′TAAACTTCAGGGTGACCAAAAAATCA) for arthropods identification [46], DDH2O and hot start. Each sample was prepared with a 48 µL master mix solution in a PCR tube and 2 µL of DNA was added to the solution. The products were then displayed in Proflex 96-well thermal cycler (Applied Biosystems, Waltham, MA, USA) for PCR running. The PCR products were gel extracted, purified, and sent to Macrogen Europe BV (Meibergreef, Amsterdam, The Netherlands) for sequencing. DNA sequences were manually edited in BioEdit version 7.2.5 [47]. The forward and the reverse sequences were edited pairwise, and the consensus sequences were then generated for each sample. These were then blasted in the National Center for Biotechnology Information (NCBI) and the Barcode of Life Data System (BOLD) databases using nucleotide sequences to determine any similarities with previously described sequences. Nucleic acid sequences from the two samples (herein referred to as first and second samples) were registered in GenBank® as per accession numbers MZ314460, MZ314461, and MZ314460 for the first sample and MZ318061, MZ318062, MZ318063 and MZ318064 for the second sample. Voucher specimens were deposited in the Canadian National Collection of Insects (CNC) at Agriculture and Agri-Food Canada.

2.2.2. Morphological and Ecological Identification

Morphological identification was performed on specimens that did not provide a high match with any species in NCBI and BOLD. Samples of this specific parasitoid were placed in a refrigerator at −20 ± 2 °C for 5 min to incapacitate them for identification under a reflected stereomicroscope (Leica EZ4D digital stereomicroscope; Leica Microsystems, Heerbrugg, Switzerland).

2.3. Parasitoid Species Effectiveness and Distribution in the Field

The geographical coordinates and altitude were recorded for each farm, where samples were collected, using the Global Positioning System (GPS). The percent parasitism of each parasitoid species on the associated host, *T. absoluta*, was estimated as (the number of parasitoids emerging divided by the total number of *T. absoluta* and the parasitoids from a sample) × 100 [44,48], while the level of *T. absoluta* infestation was recorded as the sum of the number of *T. absoluta* recovered from a sample and the number of recovered parasitoids.

2.4. Agroecological Parameters Effect on the Parasitoid Species

In addition to the predators and parasitoids obtained from infested plant material, a survey was conducted to assess the application of pesticides at the tomato farms. Farmers were asked whether they applied pesticides, and if so, the last pesticide application date was recorded. Farms where no pesticide had been applied in the two weeks preceding the survey, were considered as farms with infrequent pesticide application. The tomato production system was also recorded as either an open field or greenhouse/screen house production. Additionally, an average number of *N. tenuis* per kilogram of tomato leaves were estimated and four classes were obtained for this variable 0 = absence of *N. tenuis*, [1–50] = low-level presence of *N. tenuis*, [50–100] = medium level presence of *N. tenuis* and [>100] = high presence level of *N. tenuis*. The different factors were then correlated with the parasitism level of each species.

2.5. Prediction of Habitat Suitability

Occurrence records: A total of 21 georeferenced points for the first specimen (identified as *B. nigricans* and 23 for the second specimen identified as *S.* sp. nr *japonicus* were obtained from different sources to predict the habitat suitability of both parasitoid species. For *B. nigricans* four points were obtained from Biondi et al. and Gabarra et al. [49,50]; five from the Global Biodiversity Information Facility (GBIF) and 12 from points sampled in the present study. Similarly, for the occurrence records of *S.* sp. nr *japonicus*, four points were obtained from GBIF, eight from the Centre for Agriculture and Bioscience International (CABI) [51], and eight were gathered from published articles [49,50,52,53], and three from the current study.

Environmental Variables and modeling: Nineteen bioclimatic variables and elevation data at 2.5-minute spatial resolution were sourced from the WorldClim database [54]. These data points were integrated with occurrence records to predict the habitat suitability of *B. nigricans* and *S.* sp. nr *japonicus* under current climatic conditions. The bioclimatic variables are important in predicting the habitat suitability of the insect species because they reflect diverse characteristics of temperature, precipitation, and seasonality which are factors that affect the distribution and abundance of insects [55]. For future predictions of the distribution of the two parasitoids (i.e., the year 2050), we used simulated bioclimatic variables of representative concentration pathways (RCP 2.6).

2.6. Data Analysis

Mega software 10.2.5 [56] was used to generate the phylogenetic tree. The models with the lowest Bayesian Information Criterion (BIC) scores and the lowest Akaike Information Criterion (AIC) value were used to describe the replacement design. All positions with less than 95% site coverage were eliminated. Parameters with fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option) were used to build the tree.

Tuta absoluta infestation level, as well as parasitoid species abundance, were correlated with the different biotic parameters describing the sites using a general linear model with a *negative binomial distribution*. All analyses were performed in R [57].

For parasitoid species ecological niche prediction, a Pearson test applying a threshold correlation coefficient (r) > 0.7 was run in R to check for collinearity among the environmental variables. Only the variables which were not correlated were used in the prediction models using the maximum entropy algorithm (Maxent) developed by Phillips et al. [58], Maxent is one of the most popular modeling tools that has been widely used for predicting the distribution and ecological niche for many insect species [59]. It uses the correlative approach to correlate species occurrence to environmental layers and performs well even with a small number of occurrence records [60]. QGIS [61] was used to show the parasitoid distribution within the study area as well as in their ecological niche model.

3. Results

3.1. Parasitoid Identification

3.1.1. Molecular Identification

The comparison of the sequence of the first specimen, Accession MZ314460, in NCBI database showed 99.53% genetic similarity (e = 0E00) with the accession MH733585 (Italy) of *Bracon nigricans* Szépligeti (Hymenoptera: Braconidae), 98.28% with MN525195 and 98.12% with MN525197 both unidentified Braconidae from India [53]. Thus, we concluded that the parasitoid species was *B. nigricans* (phylogenetic tree complex is shown in Figure 1).

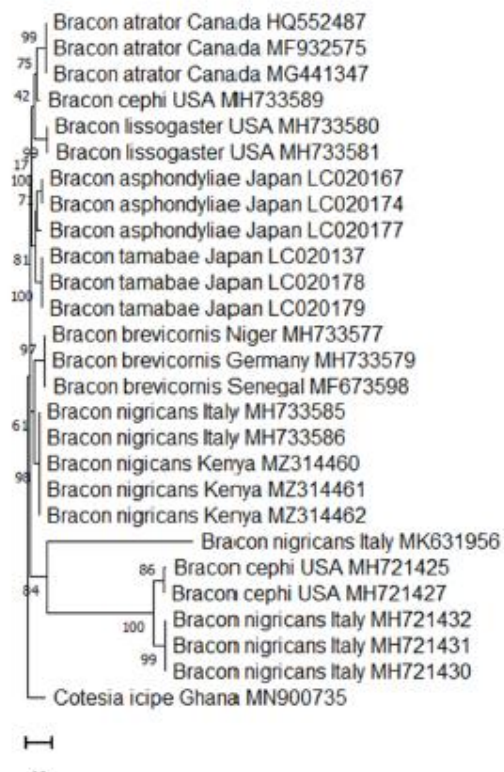


Figure 1. Maximum likelihood phylogenetic tree for the mitochondrial *COI* sequences of *Bracon* species from GenBank together with the specimen *Bracon nigricans* identified in our study.

For the second specimen, the comparison was done both in NCBI and in BOLD. The results from the two databases showed less than 98% similarity; with the highest percentage similarity, 97.66% obtained from sample BIOUG48548-E02 collected in Mpumalanga province, South Africa by Albert Smith. The sequences were also compared with available mitochondrial COI of *Steneomesius* sp. and some closely related sequences in the GenBank. Sequences from specimens of the current study are in the same clade as *Stenomesius Hansson*

(Hymenoptera: Eulophidae) from Costa Rica (Figure 2). We concluded that the parasitoid species was in the genera *Stenomesius* but could not identify it to species level using molecular identification.

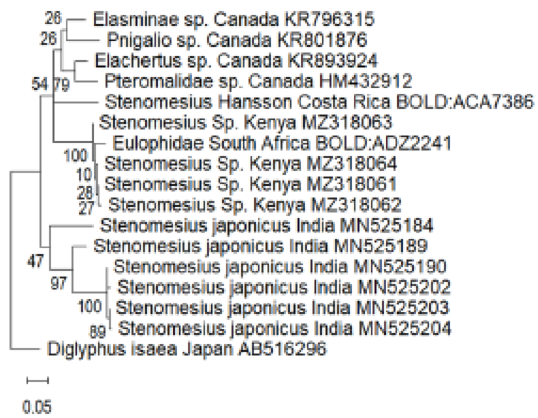


Figure 2. Maximum likelihood phylogenetic tree for the mitochondrial COI sequences of *Stenomesius* species from GenBank and BOLD together with the specimen identified in our study.

3.1.2. Morphological and Ecological Identification

Morphological identification was done for the Eulophidae, *Stenomesius* sp. only, following the description by Reina and La Salle [62]. A comparative analysis of the morphological similarities and differences was done, with the following key features considered: forewing, flagellum, post marginal and stigmal veins, scape, female antennae, mesosoma, setae, and propodeum, as well as the body color. The key features of the *Stenomesius* sp. recovered in the present study include: a scape slightly exceeding the apex of the vertex (Figure 3A), female antennae with a slender scape, and a 4-segmented funicle (Figure 3B). The flagellum has 1–2 anelli (Figure 3B). The forewing has a submarginal vein with more than 4 setae (Figure 3C). The post marginal vein is at least 1.4 times the length of the stigmal vein (Figure 3D). The petiole is not separate (Figure 3E). The propodeum is connected with two submedian carinae in the middle making an H- or X-shaped structure (Figure 3F,G). The complete body is shiny yellowish in color, with a dark spot on the dorsal part of the abdomen and the thorax (Figure 3F,G). These morphological characteristics agree with descriptions for the *Stenomesius* genus.

Three species of the genus *Stenomesius* have been reported in the Afrotropical region, namely *Stenomesius elegantulus* (Risbec) (Hymenoptera: Eulophidae) in Cameroon and Senegal and *S. japonicus* in the Afrotropical, Palaearctic, Indo-Malaya, and Australian regions [63,64] while *Stenomesius rufescens* (Retzius) (Hymenoptera: Eulophidae) was described for the first time in Africa, in Egypt [64], and this species is supposed to be distributed Nearctic and Palaearctic regions. However, the species has been identified in Kenya [65]. *Stenomesius elegantulus* differs from *S. japonicus* mainly by its relatively shorter scutellum abruptly black compared to yellow axillae [52]. The head of *S. rufescens* is mostly black, the mouth and eye rims are reddish yellow, the two edges of the forewing, the dorsum of the mid-ventrum with the shoulder blades and the shield are ochre yellow, and other parts are black [66].

The *Stenomesius* species examined in the current study has a shiny head and body (Figure 3A,F,G). The *S. japonicus* specimens identified by Boucek [52] developed on small size caterpillar hosts, preferentially on herbaceous plants. The parasitoid has been reared on the leaf miner *Stomopteryx nerteria* (Meyrick) (Lepidoptera: Gelechiidae) on groundnut, *Acrocercops* sp. (Lepidoptera: Gracillariidae), and on *Heliothis armigera* (Hübner) (Lepidoptera: Noctuidae). *Stenomesius japonicus* was previously known as a parasitoid of

Liriomyza spp. (Diptera: Agromyzidae) [67]. A few years after the *T. absoluta* invasion of different parts of the world, the parasitoid *S. japonicus* was recovered from field-collected *T. absoluta* larvae in the northeast of Spain [50] and recently in Syria [68]. Based on all these analyses, we identified this *stenomesius* species as *S.* sp. nr *japonicus*.

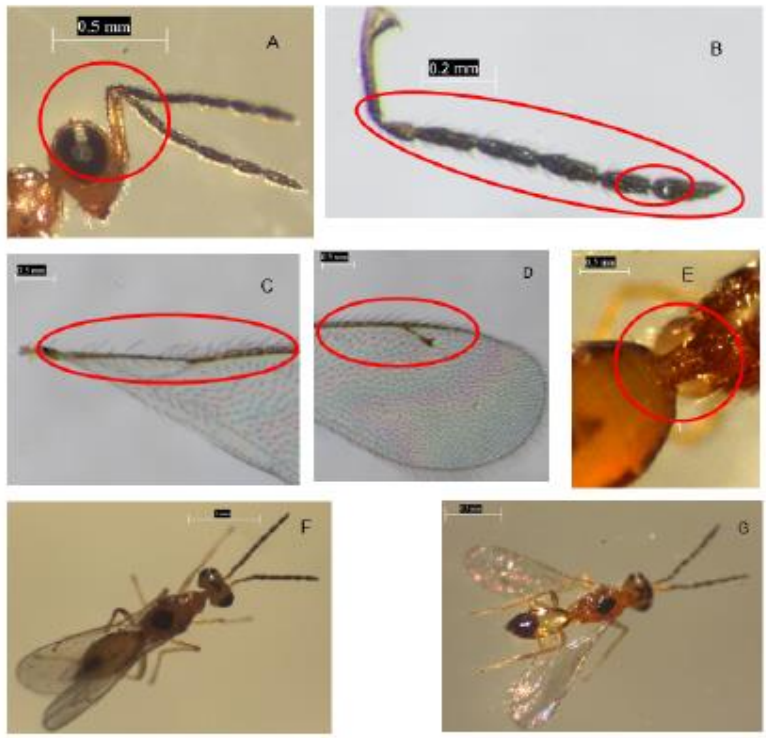


Figure 3. The collected *Stenomesius* sp. near *japonicus* description: (**A**): Scape and vertex, (**B**): Female antenna with scape slender and funicle 4-segmented, (**B**): The flagellum with 1–2 anelli, (**C**): Forewing with submarginal vein with more than 3: setae, (**D**): Postmarginal vein at least 1.5 times the length of the stigmal vein, (**E**): Petiole not separate, (**F**,**G**): Adult with X-shaped structure.

3.2. *Parasitoid Species Effectiveness and Distribution in the Field*

The occurrence and diversity of the parasitoids and parasitism rates significantly varied in the 29 sites sampled. *Bracon nigricans* was recorded only in Kirinyaga county (Figure 4B and Table 1) while *S.* sp. nr *japonicus* was recovered in both Nairobi and Kirinyaga counties (Figure 4C and Table 1). However, none of these parasitoid species were recovered from Nakuru County (Figure 4 and Table 1). Between sites, parasitism rates by *S.* sp. nr *japonicus* and *B. nigricans* ranged between, 0 to 17 % and 0 to 21%, respectively (Figure 4B,C). Whereas the infestation level varied from 52 to 1649 (emerged *T. absoluta* and parasitoids) per Kg of infested leaves (Table 1). in the different counties. Additionally, the highest parasitism rate was observed in June and May respectively for *B. nigricans*, and *S.* sp. nr *japonicus* in 2020 (Table 1).

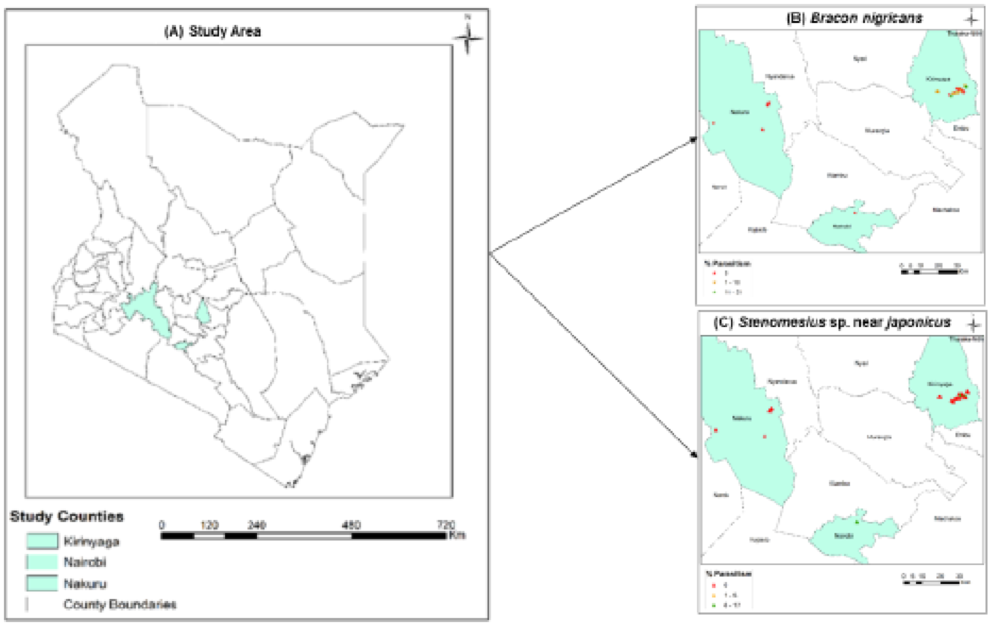


Figure 4. Maps showing (**A**) the study area, (**B**) *Bracon nigricans* parasitism rate, and (**C**) *Stenomesius* sp. nr. *japonicus* parasitism rate, within the sampling sites.

Table 1. Description of *T. absoluta* emergence per kilogram of infested leaves and the corresponding parasitism rate for each species in the 2020 and 2021 collections.

Years	Months	County	No. of Sites	*T. absoluta* Infestation/kg of Leaves (No.)	*B. nigricans* (%)	*S.* sp. nr *japonicus* (%)
2020	March	Kirinyaga	1	290 ± NA	0	0
	May	Nairobi	1	52 ± NA	0	45.16 ± NA
	June	Kirinyaga	2	230 ± 97	12.02 ± 3.77	0
2021	February	Nairobi	2	267 ± 182	0	25.37 ± 11.96
	March	Nairobi	1	1649 ± NA	0	1.10 ± NA
	March	Nakuru	2	948 ± 542	0	0
	May	Kirinyaga	9	825 ± 146	0.73 ± 0.60	1.21 ± 0.80
	August	Nakuru	2	116 ± 99	0	0
	October	Kirinyaga	12	353 ± 69	5.55 ± 1.86	0.03 ± 0.03
	November	Nakuru	1	241 ± NA	0	0

3.3. Effect of Agroecological Parameters on Parasitoid Abundance

The low level of *N. tenuis* was significantly positively correlated (z value = 3.02, $p = 0.002$) with *B. nigricans* abundance (Table 2), while both insecticide application negatively affected *S.* sp. nr *japonicus* population density (z value = −5.56, $p < 0.001$) as well as open-field production (z value = −4.27, $p < 0.001$) (Table 2).

Table 2. Statistical estimates for the effect of agroecological parameters on the parasitoid's abundance.

	Estimate	SE	z Value	Pr(>|z|)
	B. nigricans			
(Intercept)	0.92	0.91	1.02	0.31
Farm with frequent pesticide application	−1.60	0.82	−1.95	0.05
Low level of *N. tenuis*	2.50	0.83	3.02	0.002
Medium level of *N. tenuis*	0.04	1.26	0.03	0.97
High level of *N. tenuis*	1.33	1.23	1.08	0.28
	S. sp. nr *japonicus*			
(Intercept)	3.06	0.24	12.88	<0.001
Farm with frequent pesticide application	−3.46	0.62	−5.56	<0.001
Open-field production	−3.25	0.76	−4.27	<0.001
Low level of *N. tenuis*	−0.27	1.06	−0.25	0.80
Medium level of *N. tenuis*	0.26	0.39	0.65	0.52
High level of *N. tenuis*	−0.88	0.47	−1.85	0.06

3.4. Habitat Suitability Prediction

Evaluation of the model showed high accuracy for predicting the habitat suitability of *B. nigricans* with the area under the curve (AUC) = 0.80. Similarly, the model predicted *S.* sp. nr *japonicus* occurrence well with the area under the curve (AUC) = 0.90. Out of the six variables that have been used to predict the habitat suitability of *B. nigricans*: precipitation of driest month (Bio14), precipitation of coldest quarter (Bio19), mean temperature of the driest quarter (Bio9), mean temperature of the wettest quarter (Bio8), and precipitation seasonality (Bio15) (Table 3). Isothermality (Bio3), mean temperature of the driest quarter (Bio9), mean diurnal range (Bio2), precipitation of wettest month (Bio13), precipitation of warmest quarter (Bio18), elevation, mean temperature of the wettest quarter (Bio8), and precipitation of coldest quarter (Bio19) were the bioclimatic variables that contributed to predicting the occurrence of *S.* sp. nr *japonicus* (Table 4). The model prediction showed that most parts of the world are suitable for *B. nigricans* to thrive under both current and future climatic scenarios (Figures 5 and 6). High to very high suitability for *B. nigricans* occurrence is predicted across Africa under current climatic conditions (Figure 5A). In North Africa, the habitat suitability for this species is moderate under future scenarios (Figure 5B). In the current scenario, *S.* sp. nr *japonicus* showed a high to very high suitability to South America, Australia, and some location in southern Asia and southeast Asia (Figure 6A), with the occurrence probability, greatly reduced in the future climatic scenario (Figure 6B). However, the suitability level for this parasitoid is lower in a major part of Africa in both scenarios (Figure 6).

Table 3. Relative contribution of the various bioclimatic variables for Bracon nigricans ecological niche modeling.

Variables	Percentage Contribution	Permutation Importance
Bio14	40.10	64.00
Bio19	23.26	0
Bio9	14.00	4.10
Bio8	12.70	17.10
Bio15	9.60	14.80
Elevation	0	0

Table 4. Relative contribution of the various bioclimatic variables for Stenomesius sp. near japonicus ecological niche modeling.

Variables	Percentage Contribution	Permutation Importance
Bio3	57.2	51.9
Bio9	18.0	20.5
Bio2	8.1	4.3
Bio13	6.9	13.0
Bio18	3.9	7.3
Elevation	3.3	0
Bio8	2.4	2.6
Bio19	0.2	0.2
Bio15	0	0

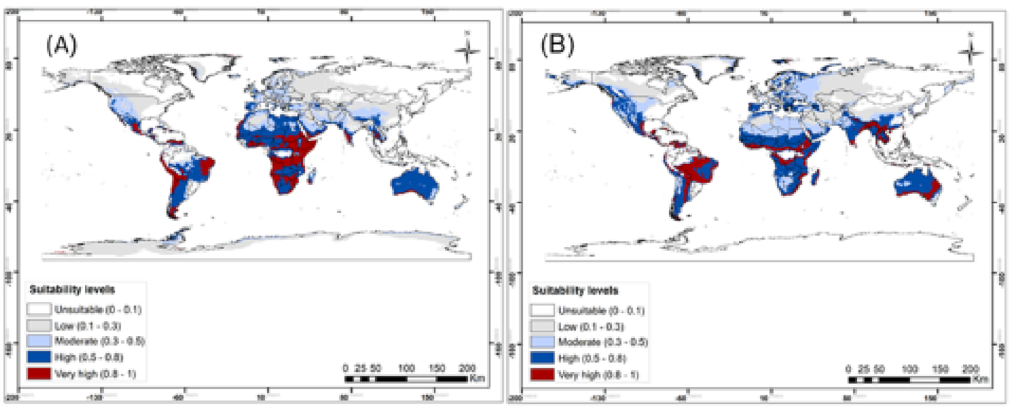


Figure 5. Probable bioclimatic suitability of Bracon nigricans under (**A**) current, and (**B**) representative concentration pathways (RCP2.5) of 2050 climate scenarios.

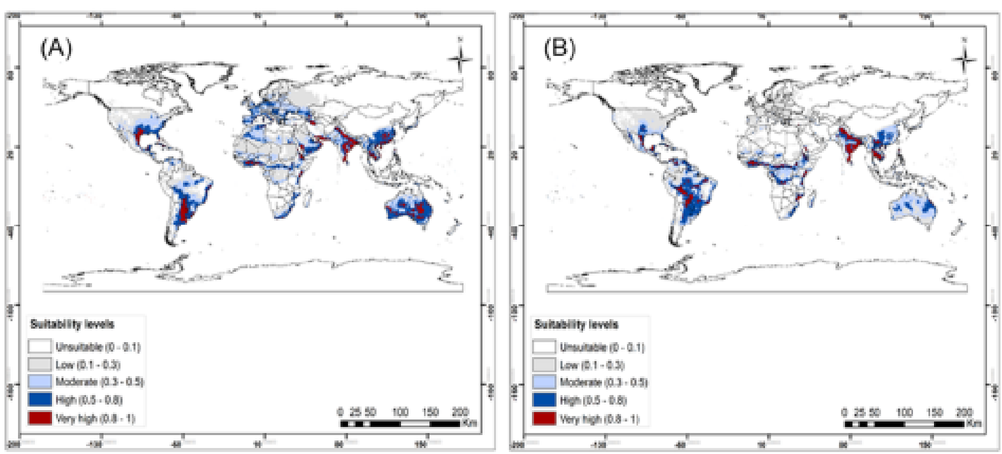


Figure 6. Probable bioclimatic suitability of Stenomesius sp. near japonicus under (**A**) current, and (**B**) representative concentration pathways (RCP2.5) of 2050 climate scenario.

4. Discussion

The current study investigated the presence, distribution, and natural parasitism of promising native parasitoids of *T. absoluta,* six years after its invasion into Kenya. This investigation was conducted to determine their potential contribution to biological control of *T. absoluta* as well as for implementation into IPM strategies. We reported two parasitoid species, *B. nigricans,* and *S.* sp. nr *japonicus.* This is not the first report of these species on the African continent [30,69], but it is the first report of the two species in Kenya, although Kinyanjui et al. [65] reported the presence of a *Bracon* sp. and *S. rufescens.*

Bracon nigricans is a generalist idiobiont parasitoid of different pests such as *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae), *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae) [70,71]. On *T. absoluta*, it has been found in various regions such as Western and Southern Europe, Russia, the Middle East, and China [72–75]. Other *Bracon* species reported on *T. absoluta* are *Bracon lucileae* (Marsh), *B. lulensis,* and *B. tutus* (All) Berta & Colomo [8,72,73]. The maximum *B. nigricans* parasitism rate by *B. nigricans* on *T. absoluta* recorded in this study, was 21%. However, Idriss et al. [30] reported 54% parasitism under laboratory conditions with the highest parasitism obtained on 4th instar larvae compared to only 10% parasitism of 3rd instar larvae. Additionally, a significant pest killing rate, higher than the parasitism rate is observed for this parasitoid species [30,76,77].

Stenomesius spp. are generalist ectoparasitoids from several Lepidoptera families, such as Pyralidae, Noctuidae, Gelechiidae, Tortricidae, Lyonetiidae, Glyphipterygidae [78], but also from Diptera pest [79]. *Stenomesius* sp. nr *japonicus* has been associated with *Aproaerema modicella* Deventer (Lepidoptera: Gelechiidae), the groundnut leafminer [53,80] and with *Phyllonorycter* leafminers (Lepidoptera: Gracillariidae) [81] in India and Thailand, and Japan respectively. In Africa, *Stenomesius* species have been identified as parasitoids of *A. modicella* in Uganda and the DRC [82]. On the same host plant, *S. japonicus* attack *Liriomyza* sp. Mik (Diptera: Agromyzidae) [84]. *Stenomesius* sp. near *japonicus* was also recorded from *T. absoluta*-infested tomato plants in Spain and France [51,72,74]. *Stenomesius* sp. has also been reported on *T. absoluta* in Algeria [26], while *S. rufescens* was previously identified in Kenya on the same host [65]. *Stenomesius* sp. nr *japonicus* has been ranked among the three most frequent native parasitoid species recovered from *T. absoluta* in Spain [50]. It is not surprising that we recovered this parasitoid on *T. absoluta*-infested tomato plants in Kenya.

In the current study, we established up to 17% parasitism rate per site by *S.* sp. nr *japonicus* under field conditions. However, Chailleux et al. [84] found 50% parasitism when offering five hosts to one female of *S.* sp. nr *japonicus,* under laboratory conditions, and 35% field parasitism was reported by Youssef et al. [68]. Additionally, 12 females parasitized around 33% of *T. absoluta* larvae when *S.* sp. nr *japonicus* was exposed alone to *T. absoluta* larvae while only 8% parasitism was recorded when competing with the predator *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) [85]. A possible explanation for the lower rate of parasitism obtained in this study is that it is from open-field tomatoes where plants were infested with different immature stages, and some 4th instar larvae could have already left the plants to pupate in the soil. Parasitism rates under field conditions will most probably always be lower than laboratory conditions since the pest and parasitoids are not kept under confined conditions. Additionally, the level of infestation, i.e., the host-parasitoid ratio could have affected parasitism of *S.* sp. nr *japonicus,* since, in Table 1, 45% parasitism was observed when the infestation was lowest.

Bracon nigricans is a known larval parasitoid of *T. absoluta* [30,76]. The low-level presence of the generalist, omnivorous mirid *N. tenuis* in the field was positively correlated with *B. nigricans* parasitism while no significant effect on *S.* sp. nr *japonicus* was noticed (Table 1). Similarly, the number of progeny and emerged adults of the endoparasitoid of *T. absoluta*, *Dolichogenidea gelechiidivoris* (Marsh) (Hymenoptera: Braconidae) was not affected by the release of this predator [86]. It is, however, known from several studies that the release of *N. tenuis* in tomato fields, considerably reduces the persistence of *T. absoluta* [87,88], although *N. tenuis* prefers eggs to larvae [89]. However, larval ectoparasitoids go through intraguild predation by mirids in natural habitats [84,90]. Bacci et al. [91] estimated that 57% of *T. absoluta* mortality that occurs under field conditions was during the larval stage, with physiological disorders, parasitism, predation, and entomopathogenic agents as the causes. Third and fourth instar larvae are more susceptible to predatory wasps [91]. In a laboratory study, Chailleux et al. [85] reported that *S.* sp. nr *japonicus* increased from a single couple released in a cage to 60 adults within eight weeks, while its combination with a pair of *M. pygmaeus*, *S.* sp. nr *japonicus* adults decreased by 50 adults for the same duration.

Application of insecticides in tomato fields was not found to affect the parasitism by *B. nigricans*; however, there was a negative correlation between insecticide application and parasitism by *S.* sp. nr *japonicus* (Table 1). However, Biondi et al. [92] demonstrated that spinosad caused high pupal and adult mortality of 80% and100%, respectively while other bioinsecticides showed no lethal effect on *B. nigricans* but sublethal ones, especially on adult longevity and female fecundity. The impact varies with the active ingredient of the insecticide, combined with biotic conditions [13]. Further, open field tomato farming was found unfavorable to *S.* sp. nr *japonicus*. Similarly, for the congeneric species, *N. nr artynes* recorded more adult emergence (72) in greenhouse production compare to sentinel plants (23 adults) and open field tomatoes (41adults) in Tunisia [27]. Another Eulophid, *Hemiptarsenus varicornis* (Girault) (Hymenoptera: Eulophidae) was promoted as a good candidate for biocontrol of *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) due to its ability to effectively perform under greenhouse conditions [93].

The generated Maxent models predicted the presence of *S.* sp. nr *japonicus* and *B. nigricans* in many parts of the world (Figure 5). The widespread distribution of *Stenomesius* sp. as predicted by the Maxent model is validated by the occurrence data of the parasitoid already published [49,50,53]. The model predicted low climatic suitability for *S.* sp. nr *japonicus* in most parts of Africa, in both current and future climatic scenarios. For *B. nigricans*, the Palaearctic region was considered the geographical distribution area [30,50,73]. While from our findings, the Afrotropical, Neotropical, Oriental, and Australasian regions showed better suitability than the Palaearctic region. The previous findings of the habitat suitability of *T. absoluta* in Africa, demonstrated proportional habitat suitability for *B. nigricans* [94,95], a result which juxtaposes very lower habitat suitability observed for *S.* sp. nr *japonicus* in our study. This discrepancy could be explained by the difference in tolerance to environmental factors between *T. absoluta* and the identified parasitoid species. Otherwise, major agricultural regions of Africa are highly suitable for *B. nigricans*. In that regard, *B. nigricans* should be considered for importation into the geographic areas indicated to be suitable for its occurrence.

5. Conclusions

Two native parasitoid species, *B. nigricans* and *S.* sp. nr *japonicus* were reported on *T. absoluta* in Kenya. Differences in parasitism rates occurred and varied between the sites and regions of sample collection, where the highest parasitism by *B. nigricans* and *S.* sp. nr *japonicus* was 21% and 17%, respectively. A low abundance of *N. tenuis* was found to be positively correlated to the occurrence of *B. nigricans*, while pesticide application in either greenhouse or open field tomatoes, did not affect the parasitoid. Open-field cropping, as well as insecticide application, were negatively correlated with parasitism by *S.* sp. nr *japonicus*. The ecological niche prediction for the respective parasitoids indicated a high probability that the potential areas for *B. nigricans* occurrence is in almost all African countries. This result can therefore guide future recovery surveys and the implementation of different biological control strategies against *T. absoluta*. With the estimated performance of these parasitoids under field conditions, where many challenges such as exposure to a wide variety of chemical insecticides and other competitors such as predators are realities, an IPM approach integrating the conservation and/or augmentation of these two parasitoids can be very effective. The combination of the different parasitoid species including the introduced *Dolichogenidea gelechiidivoris* (Marsh) (Hymenoptera: Braconidae) [96–98] should also be tested to confirm the theory of diversity of parasitoids for better pest control [99–101]. Moreover, the roles of these parasitoids in conservative biological control should be investigated by simulating agro-ecological conditions, and augmentation biological control with a high number of releases should also be tested.

Author Contributions: Conceptualization, S.M.S., S.N. and S.A.M. methodology and validation, S.M.S., S.N., H.d.P., F.O. and S.A.M.; data collection and curation, S.M.S.; formal statistical analysis, S.M.S.; writing—original draft preparation, S.M.S.; writing—review and editing, S.M.S., S.N., H.d.P., F.O. and S.A.M.; supervision, S.A.M., S.N. and H.d.P.; project administration and funding acquisition, S.A.M. All authors have read and agreed to the published version of the manuscript.

Funding: The authors gratefully acknowledge the financial support for this research by the following organizations and agencies: Biovision Foundation grant number BV DPP-012/ 2019–2022; Norwegian Agency for Development Cooperation-(NORAD), the section for research, innovation, and higher education grant number RAF-3058 KEN-18/0005; Scaling-up Climate-Smart Pest Management Approaches for Enhanced Maize and Tomato Systems Productivity in Eastern Africa (SCLAMP) grant number 81250394; the Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); the Federal Democratic Republic of Ethiopia; and the Government of the Republic of Kenya. The views expressed herein do not necessarily reflect the official opinion of the donors. S.M.S. was sponsored by the German Academic Exchange Services (DAAD) in the African Regional Postgraduate Programme in Insect Science (ARPPIS) hosted by *icipe*.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data presented in this study are available for public used with the URL: https://dmmg.icipe.org/dataportal/dataset/identification-microhabitat-and-ecological-niche-prediction-of-two-promising-native-parasitoids (15 February 2022).

Acknowledgments: We are grateful to John Huber and José L. Fernández-Triana (Canadian National Collection of Insects, CNC) for the invaluable information on the genera reported in this publication. We thank several Scientists at *icipe* for their input in the molecular and morphological identification of the specimens. Furthermore, we are thankful to fellow students Rehemah Gwokyalya and Vivian Hinga for their technical support in insect molecular identification. We are also thankful to Abdelmutalab Gesmalla for his comments and advice on the species distribution modeling. We are grateful to all the *icipe*'s Animal Rearing and Containment Unit (ARCU) *Tuta absoluta* team, especially Esther Awuor Owino, Alex Muthembwa, Patrick Koech Kipkorir, Peterson Nderitu and Omar Jimali Ibrahim for their technical support in the field sample collection and insect monitoring.

Conflicts of Interest: The authors declare that they have no conflict of interest.

References

1. Fessl, B.; Tebbich, S. Philornis downsi—A recently discovered parasite on the Galápagos archipelago—A threat for Darwin's finches? *Ibis* **2002**, *144*, 445–451. [CrossRef]
2. Sileshi, G.W.; Gebeyehu, S.; Mafongoya, P.L. The threat of alien invasive insect and mite species to food security in Africa and the need for a continent-wide response. *Food Secur.* **2019**, *11*, 763–775. [CrossRef]
3. Venette, R.C.; Hutchison, W.D.; Hutchison, W.D. Invasive Insect Species: Global Challenges, Strategies & Opportunities. *Front. Insect Sci.* **2021**, *1*, 650520. [CrossRef]
4. Pimentel, D.; Zuniga, R.; Morrison, D. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol. Econ.* **2005**, *52*, 273–288. [CrossRef]
5. Pyšek, P.; Richardson, D.M. Invasive Species, Environmental Change and Management, and Health. *Annu. Rev. Environ. Resour.* **2010**, *35*, 25–55. [CrossRef]
6. Aigbedion-Atalor, P.O.; Hill, M.P.; Zalucki, M.P.; Obala, F.; Idriss, G.E.; Midingoyi, S.K.; Chidege, M.; Ekesi, S.; Mohamed, S.A. The South America Tomato Leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), Spreads Its Wings in Eastern Africa: Distribution and Socioeconomic Impacts. *J. Econ. Entomol.* **2019**, *112*, 2797–2807. [CrossRef]
7. Mansour, R.; Brévault, T.; Chailleux, A.; Cherif, A.; Grissa-Lebdi, K.; Haddi, K.; Mohamed, S.A.; Nofemela, R.S.; Oke, A.; Sylla, S.; et al. Occurrence, biology, natural enemies and management of *Tuta absoluta* in Africa. *Entomol. Gen.* **2018**, *38*, 83–112. [CrossRef]
8. Biondi, A.; Guedes, R.N.C.R.; Wan, F.-H.F.; Desneux, N. Ecology, Worldwide Spread, and Management of the Invasive South American Tomato Pinworm, *Tuta absoluta*: Past, Present, and Future. *Annu. Rev. Entomol.* **2018**, *63*, 239–258. [CrossRef]
9. Rwomushana, I.; Day, R.; Gonzalez-moreno, P. *Evidence Note Tomato Leafminer Impacts and Coping Strategies for Africa*; CABI: Wallingford, UK, 2019.

10. Mahugija, J.A.M.; Khamis, F.A.; Lugwisha, E.H.J. Assessment of pesticide residues in tomatoes and watermelons (fruits) from markets in Dar es Salaam, Tanzania. *J. Appl. Sci. Environ. Manag.* **2017**, *21*, 497. [CrossRef]
11. Marete, G.M.; Lalah, J.O.; Mputhia, J.; Wekesa, V.W. Pesticide usage practices as sources of occupational exposure and health impacts on horticultural farmers in Meru County, Kenya. *Heliyon* **2021**, *7*, e06118. [CrossRef]
12. Njiraini, J.; Kenya's Crop Protection Industry Fears 'Dire Consequences' of New Pesticides Tax. AgriBusiness Global, 2 November 2018. Available online: https://www.agribusinessglobal.com/agrochemicals/kenyas-crop-protection-industry-fears-dire-consequences-of-new-pesticides-tax/ (accessed on 1 July 2021).
13. Abbes, K.; Biondi, A.; Kurtulus, A.; Ricupero, M.; Russo, A.; Siscaro, G.; Chermiti, B.; Zappalà, L. Combined Non-Target Effects of Insecticide and High Temperature on the Parasitoid *Bracon nigricans*. *PLoS ONE* **2015**, *10*, e0138411. [CrossRef]
14. Desneux, N.; Han, P.; Mansour, R.; Arnó, J.; Brévault, T.; Campos, M.R.; Chailleux, A.; Guedes, R.N.C.; Karimi, J.; Konan, K.A.J.; et al. Integrated pest management of *Tuta absoluta*: Practical implementations across different world regions. *J. Pest Sci.* **2022**, *95*, 17–39. [CrossRef]
15. Yang, Z.Q.; Wei, J.R.; Wang, X.Y. Mass rearing and augmentative releases of the native parasitoid *Chouioia cunea* for biological control of the introduced fall webworm *Hyphantria cunea* in China. *BioControl* **2006**, *51*, 401–418. [CrossRef]
16. Castañé, C.; Arnó, J.; Gabarra, R.; Alomar, O. Plant damage to vegetable crops by zoophytophagous mirid predators. *Biol. Control* **2011**, *59*, 22–29. [CrossRef]
17. Biondi, A.; Zappalà, L.; Di Mauro, A.; Garzia, G.T.; Russo, A.; Desneux, N.; Siscaro, G. Can alternative host plant and prey affect phytophagy and biological control by the zoophytophagous mirid *Nesidiocoris tenuis*? *BioControl* **2016**, *61*, 79–90. [CrossRef]
18. Samková, A.; Raška, J.; Hadrava, J.; Skuhrovec, J. Effect of host switching simulation on the fitness of the gregarious parasitoid *Anaphes flavipes* from a novel two-generation approach. *Sci. Rep.* **2021**, *11*, 19473. [CrossRef]
19. Jones, T.S.; Bilton, A.R.; Mak, L.; Sait, S.M. Host switching in a generalist parasitoid: Contrasting transient and transgenerational costs associated with novel and original host species. *Ecol. Evol.* **2015**, *5*, 459–465. [CrossRef]
20. Barratt, B.I.P.; Howarth, F.G.; Withers, T.M.; Kean, J.M.; Ridley, G.S. Progress in risk assessment for classical biological control. *Biol. Control* **2010**, *52*, 245–254. [CrossRef]
21. Van Lenteren, J.C.; Bale, J.; Bigler, F.; Hokkanen, H.M.T.; Loomans, A.J.M. Assessing risks of releasing exotic biological control agents of arthropod pests. *Annu. Rev. Entomol.* **2006**, *51*, 609–634. [CrossRef]
22. Shaltiel-Harpaz, L.; Gerling, D.; Graph, S.; Kedoshim, H.; Azolay, L.; Rozenberg, T.; Nachache, Y.; Steinberg, S.; Allouche, A.; Alon, T. Control of the tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), in open-field tomatoes by indigenous natural enemies occurring in Israel. *J. Econ. Entomol.* **2016**, *109*, 120–131. [CrossRef]
23. Gervassio, N.G.S.; Aquino, D.; Vallina, C.; Biondi, A.; Luna, M.G. A re-examination of *Tuta absoluta* parasitoids in South America for optimized biological control. *J. Pest Sci.* **2019**, *92*, 1343–1357. [CrossRef]
24. Cabello, T.; Granados, J.R.G.; Vila, A.S.E.; Pérez, M.D.P.; Camero, A.; Hernández-Suarez, E.; Polaszeck, A. Biological control of the South American Tomato Pinworm, *Tuta absoluta* (Lep.: Gelechiidae), with releases of *Trichogramma achaeae* (Hym.: Trichogrammatidae) in tomato greenhouses of Spain. *Integr. Control Prot. Crop. Mediterr. Clim. IOBC/wprs Bull.* **2009**, *49*, 225–230.
25. Seydi, O.; Sylla, S.; Diatte, M.; Labou, B.; Diarra, K. Recruitment of native parasitoids of the tomato leaf miner *Tuta absoluta* (Meyrick 1917), (Lepidoptera: Gelechiidae) in Senegal. *Int. J. Pest Manag.* **2021**, 1–7. [CrossRef]
26. Guenaoui, Y.; Dahliz, A.; Bensaad, R.; Ouezzani, K. Five years after the first record of *Tuta absoluta* (Meyrick) in Algeria, what do we expect from its native natural enemies? In Proceedings of the Fourth International Scientific Symposium "Agrosym 2013", Jahorina, Bosnia and Herzegovina, 3–6 October 2013; pp. 678–682.
27. Abbes, K.; Biondi, A.; Zappalà, L.; Chermiti, B. Fortuitous parasitoids of the invasive tomato leafminer *Tuta absoluta* in Tunisia. *Phytoparasitica* **2014**, *42*, 85–92. [CrossRef]
28. Eman Abdelmaksoud, M.M.; El-Refai, S.A.; Rashwan, S.A.R. Survey of Parasitoids and Predators of Tomato Leaf Miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Egypt. *Arab Univ. J. Agric. Sci.* **2016**, *24*, 547–553. [CrossRef]
29. Mahmoud, M.E.E.; Mohammed, E.S.I.; Mohamed, S.A.; Khamis, F.M.; Ekesi, S. Development and Implementation of a Sustainable IPM and Surveillance Program for the Invasive Tomato Leafminer, *Tuta absoluta* (Meyrick) in Sudan. *Athens J. Sci.* **2020**, *7*, 159–174. [CrossRef]
30. Idriss, G.E.A.; Mohamed, S.A.; Khamis, F.; Du Plessis, H.; Ekesi, S. Biology and performance of two indigenous larval parasitoids on *Tuta absoluta* (Lepidoptera: Gelechiidae) in Sudan. *Biocontrol Sci. Technol.* **2018**, *28*, 614–628. [CrossRef]
31. Mansour, R.; Biondi, A. Releasing natural enemies and applying microbial and botanical pesticides for managing *Tuta absoluta* in the MENA region. *Phytoparasitica* **2021**, *49*, 179–194. [CrossRef]
32. Kortam, M.N.; Arnaouty, S.A.E.; Afifi, A.I.; Heikal, I.H. Efficacy of Different Biological Methods for Controlling the Tomato Leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on Tomato in Greenhouse in Egypt. *Egypt. J. Biol. Pest Control.* **2014**, *24*, 523–528.
33. El-Arnaouty, S.A.; Pizzol, J.; Galal, H.H.; Kortam, M.N.; Afifi, A.I.; Beyssat, V.; Desneux, N.; Biondi, A.; Heikal, I.H. Assessment of two *Trichogramma* species for the control of *Tuta absoluta* in North African tomato greenhouses. *Afr. Entomol.* **2014**, *22*, 801–809. [CrossRef]
34. Cherif, A.; Mansour, R.; Attia-Barhoumi, S.; Zappalà, L.; Grissa-Lebdi, K. Effectiveness of different release rates of *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae) against *Tuta absoluta* (Lepidoptera: Gelechiidae) in protected and open field tomato crops in Tunisia. *Biocontrol Sci. Technol.* **2019**, *29*, 149–161. [CrossRef]

35. Keçeci, M.; Öztop, A. Possibilities for biological control of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) in the western Mediterranean Region of Turkey. *Turkish J. Entomol.* **2017**, *41*, 219–230. [CrossRef]
36. Abdel-Razek, A.S.; Masry, S.H.D.; Sadek, H.E.; Gaafar, N.M. Efficacy of *Trichogramma* wasps for controlling tomato leaf miner *Tuta absoluta*. *Arch. Phytopathol. Plant Prot.* **2019**, *52*, 443–457. [CrossRef]
37. Porter, J.H.; Parry, M.L.; Carter, T.R. The potential effects of climatic change on agricultural insect pests. *Agric. For. Meteorol.* **1991**, *57*, 221–240. [CrossRef]
38. Chichrane, J.; Laugi, G.; Hawlitzky, N. Effects of high temperature shocks of *Trichogramma brassicae* [Hym.: Trichogrammatidae]. *Entomophaga* **1993**, *38*, 185–192. [CrossRef]
39. Fuhrer, J. Agroecosystem responses to combinations of elevated CO_2, ozone, and global climate change. *Agric. Ecosyst. Environ.* **2003**, *97*, 1–20. [CrossRef]
40. Kiritani, K. Different effects of climate change on the population dynamics of insects. *Appl. Entomol. Zool.* **2013**, *48*, 97–104. [CrossRef]
41. Mohamed, S.A.; Wamalwa, M.; Obala, F.; Tonnang, H.E.Z.; Tefera, T.; Calatayud, P.A.; Subramanian, S.; Ekesi, S. A deadly encounter: Alien invasive *Spodoptera frugiperda* in Africa and indigenous natural enemy, *Cotesia icipe* (Hymenoptera, Braconidae). *PLoS ONE* **2021**, *16*, e0253122. [CrossRef]
42. Tepa-yotto, G.T.; Tonnang, H.E.Z.; Goergen, G.; Subramanian, S.; Kimathi, E.; Abdel-rahman, E.M.; Flø, D.; Thunes, K.H.; Fiaboe, K.K.M.; Niassy, S.; et al. Global Habitat Suitability of *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae): Key Parasitoids Considered for Its Biological Control. *Insect* **2021**, *12*, 273. [CrossRef]
43. Wang, X.; Jarjees, E.A.; Mcgraw, B.K.; Bokonon-Ganta, A.H.; Messing, R.H.; Johnson, M.W. Effects of spinosad-based fruit fly bait GF-120 on tephritid fruit fly and aphid parasitoids. *Biol. Control* **2005**, *35*, 155–162. [CrossRef]
44. Mama Sambo, S.; Togbe, D.R.; Sinzogan Antonio, A.C.; Bokonon-Ganta, H.A.; Adomou, A.; Karlsson, M.F. Habitat factors associated with *Fopius caudatus* parasitism and population level of its host, *Ceratitis cosyra*. *Entomol. Exp. Appl.* **2019**, *168*, 1–13. [CrossRef]
45. Livak, K.J. Organization and mapping of a sequence on the drosophila melanogaster X and Y chromosomes that is transcribed during spermatogenesis. *Genetics* **1984**, *107*, 611–634. [CrossRef]
46. Layodé, B.F.R.; Onzo, A.; Karlsson, M.F. Watermelon-infesting Tephritidae fruit fly guild and parasitism by Psyttalia phaeostigma (Hymenoptera: Braconidae). *Int. J. Trop. Insect Sci.* **2020**, *40*, 157–166. [CrossRef]
47. Hall, T.A. BioEdit: A user-friendly biological sequence alignment editor and analysis for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.* **1999**, *41*, 95–98.
48. Sule, H.; Muhamad, R.; Omar, D.; Hee, A.K.W. Parasitism rate, host stage preference and functional response of *Tamarixia radiata* on *Diaphorina citri*. *Int. J. Agric. Biol.* **2014**, *16*, 783–788.
49. Biondi, A.; Chailleux, A.; Lambion, J.; Han, P.; Zappalà, L.; Desneux, N. Indigenous Natural Enemies Attacking *Tuta absoluta* (Lepidoptera: Gelechiidae) in Southern France. *Egypt. J. Biol. Pest Control* **2013**, *23*, 117–121.
50. Gabarra, R.; Arno', J.; Lara, L.; Verdu', M.J.; Ribes, A.; Beitia, F.; Urbaneja, A.; Te'llez, M.d.M.; Molla, O.; Riudavets, J. Native parasitoids associated with *Tuta absoluta* in the tomato production areas of the Spanish Mediterranean Coast. *BioControl* **2013**, *59*, 45–54. [CrossRef]
51. CABI. Stenomesius japonicus. In *Invasive Species Compendium*; CAB Int.: Wallingford, UK, 2021.
52. Boucek, Z. Taxonomic studies on some Eulophidae [Hym.] of economic interest mainly from Africa. *Entomophaga* **1976**, *21*, 401–414. [CrossRef]
53. Murugasridevi, K.; Jeyarani, S.; Nelson, S.J.; Kumar, S.M.; Nakkeeran, S. Assessment of Diversity Indices and DNA Barcoding of Parasitic Fauna Associated with Groundnut Leafminer (GLM), *Aproaerema modicella* Deventer (Lepidoptera: Gelechiidae). *Legum. Res.* **2021**, *1*, 1–9. [CrossRef]
54. Hijmans, R.; Cameron, S.; Parra, J. Worldclim Global Climate Layers Version 1.4; WorldClim Database. Available online: https://www.worldclim.org/data/v1.4/worldclim14.html (accessed on 1 July 2021).
55. O'Donnell, M.S.; Ignizio, D.A. Bioclimatic Predictors for Supporting Ecological Applications in the Conterminous United States. *US Geol. Surv. Data Ser.* **2012**, *691*, 10.
56. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [CrossRef]
57. R Core Team. *R: A language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2018. Available online: https//www.R-project.org/ (accessed on 17 September 2021).
58. Phillips, S.J.; Anderson, R.P.; Schapire, R.E. Maximum entropy modeling of species geographic distributions. *Ecol. Modell.* **2006**, *190*, 231–259. [CrossRef]
59. Merow, C.; Smith, M.J.; Silander, J.A. A practical guide to MaxEnt for modeling species' distributions: What it does, and why inputs and settings matter. *Ecography* **2013**, *36*, 1–12. [CrossRef]
60. Elith, J.; Phillips, S.J.; Hastie, T.; Dudik, M.; Chee, Y.E.; Yates, C.J. A statistical explanation of MaxEnt for ecologists. *Divers. Distrib.* **2011**, *17*, 43–57. [CrossRef]
61. QGIS.org QGIS Geographic Information System. QGIS Association. 2021. Available online: http://www.qgis.org (accessed on 20 September 2021).

62. Reina, P.; Salle, J.L. Key to the World Genera of Eulophidae Parasitoids (Hymenoptera) of Leafmining Agromyzidae (Diptera). Available online: https://keys.lucidcentral.org/keys/v3/eulophidae_parasitoids/eulophidae_parasitoids.html (accessed on 2 May 2021).
63. Van Noort, S. WaspWeb: Hymenoptera of the Afrotropical Region. Available online: www.waspweb.org (accessed on 1 October 2021).
64. Gadallah, N.S.; Yefremova, Z.A.; Yegorenkova, E.N.; Soliman, A.M.; El-Ghiet, U.M.A.; Edmardash, Y.A. A review of the family Eulophidae (Hymenoptera: Chalcidoidea) of Egypt, with thirty three new records. *Zootaxa* **2015**, *4058*, 66–80. [CrossRef]
65. Kinyanjui, G.; Khamis, F.M.; Ombura, F.L.O.; Kenya, E.U.; Ekesi, S.; Mohamed, S.A. Distribution, abundance and natural enemies of the invasive tomato leafminer, *Tuta absoluta* (Meyrick) in Kenya. *Bull. Entomol. Res.* **2021**, *111*, 1–16. [CrossRef]
66. Fazekas, I. *Stenomesius rufescens* (Retzius, 1783), a parasite of *Choreutis nemorana* (Hübner, 1799) in Hungary (Hymenoptera, Lepidoptera). *Microlepidoptera* **2021**, *15*, 21–28. [CrossRef]
67. Murphy, S.; Salle, J.L. Balancing biological control strategies in the IPM of New World invasive *Liriomyza* leafminers in field vegetable crops. *Biocontrol News Inf.* **1999**, *20*, 91–104.
68. Youssef, R.M.; Abo Kaf, N.H.; Aboud, R. *Stenomesius japonicus* (Ashmead) (Hymenoptera: Eulophidae): A new parasitoid of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Syria. *J. Insect Biodivers. Syst.* **2022**, *8*, 219–227. [CrossRef]
69. Kelly, J.; Ranwashe, F. *ARC-PPRI: Chalcidoidea Collection (1900–2010)*; South African National Biodiversity Institute: Brummeria, Pretoria, 2018.
70. Loni, A.; Samartsev, K.G.; Scaramozzino, P.L.; Belokobylskij, S.A.; Lucchi, A. Braconinae parasitoids (Hymenoptera, Braconidae) emerged from larvae of *Lobesia Botrana* (Denis & Schiffermüller) (lepidoptera, tortricidae) feeding on Daphne gnidium L. *Zookeys* **2016**, *587*, 125–150. [CrossRef]
71. Becchimanzi, A.; Avolio, M.; Bostan, H.; Colantuono, C.; Cozzolino, F.; Mancini, D.; Chiusano, M.L.; Pucci, P.; Caccia, S.; Pennacchio, F. Venomics of the ectoparasitoid wasp *Bracon nigricans*. *BMC Genom.* **2020**, *21*, 1–15. [CrossRef] [PubMed]
72. Al-Jboory, I.J.; Katbeh-Bader, A.; Shakir, A.Z. First observation and identification of some natural enemies collected from heavily infested tomato by *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Jordan. *Middle-East J. Sci. Res.* **2012**, *11*, 787–790.
73. Zappalà, L.; Bernardo, U.; Biondi, A.; Cocco, A.; Deliperi, S.; Delrio, G.; Giorgini, M.; Pedata, P.; Rapisarda, C.; Garzia, G.T.; et al. Recruitment of native parasitoids by the exotic pest *Tuta absoluta* in southern Italy. *Bull. Insectology* **2012**, *65*, 51–61.
74. Biondi, A.; Alma, A.; Zappala, L.; Desneux, N. Natural enemies of the South American moth, *Tuta absoluta*, in Europe, North Africa and Middle East, and their potential use in pest control strategies. *J. Pest Sci.* **2013**, *86*, 635–647. [CrossRef]
75. Ghoneim, K. Parasitic insects and mites as potential biocontrol agents for a devastative pest of tomato, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) in the world: A review. *IJRRAS* **2014**, *19*, 36–68.
76. Biondi, A.; Desneux, N.; Amiens-Desneux, E.; Siscaro, G.; Zappalà, L. Biology and Developmental Strategies of the Palaearctic Parasitoid *Bracon nigricans* (Hymenoptera: Braconidae) on the Neotropical Moth *Tuta absoluta* (Lepidoptera: Gelechiidae). *J. Econ. Entomol.* **2013**, *106*, 1638–1647. [CrossRef]
77. van Lenteren, J.C.; Lanzoni, A.; Hemerik, L.; Bueno, V.H.P.; Cuervo, J.G.B.; Biondi, A.; Burgio, G.; Calvo, F.J.; de Jong, P.W.; López, S.N.; et al. The pest kill rate of thirteen natural enemies as aggregate evaluation criterion of their biological control potential of *Tuta absoluta*. *Sci. Rep.* **2021**, *11*, 10756. [CrossRef]
78. Boucek, Z. *Australasian Chalcidoidea (Hymenoptera): A Biosystematic Revision of Genera of Fourteen Families, with a Reclassification of Species*; CAB Int.: Wallingford, UK, 1988.
79. Ridland, P.M.; Umina, P.A.; Pirtle, E.I.; Hoffmann, A.A. Potential for biological control of the vegetable leafminer, *Liriomyza sativae* (Diptera: Agromyzidae), in Australia with parasitoid wasps. *Austral Entomol.* **2020**, *59*, 16–36. [CrossRef]
80. Kenis, M.; Cugala, D. Prospects for the biological control of the groundnut leaf miner, *Aproaerema modicella*, in Africa. *CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour.* **2006**, *1*, 1–9. [CrossRef]
81. Sato, H.; Okabayashi, Y.; Kamijo, K. Structure and Function of Parasitoid Assemblages Associated with *Phyllonorycter* Leafminers (Lepidoptera: Gracillariidae) on Deciduous Oaks in Japan. *Environ. Entomol.* **2002**, *31*, 1052–1061. [CrossRef]
82. Munyuli, T.; Luther, G.; Kyamanywa, S.; Hammond, R. Diversity and distribution of native arthropod parasitoids of groundnut pests in Uganda and Democratic Republic of Congo. *African Crop Sci. Conf. Proc.* **2003**, *6*, 231–237.
83. Martin, A.D.; Stanley-Horn, D.; Hallett, R.H. Adult host preference and larval performance of *Liriomyza huidobrensis* (Diptera: Agromyzidae) on selected hosts. *Environ. Entomol.* **2005**, *34*, 1170–1177. [CrossRef]
84. Chailleux, A.; Desneux, N.; Arnó, J.; Gabarra, R. Biology of two key Palaearctic larval ectoparasitoids when parasitizing the invasive pest *Tuta absoluta*. *J. Pest Sci.* **2014**, *87*, 441–448. [CrossRef]
85. Chailleux, A.; Droui, A.; Bearez, P.; Desneux, N. Survival of a specialist natural enemy experiencing resource competition with an omnivorous predator when sharing the invasive prey *Tuta absoluta*. *Ecol. Evol.* **2017**, *7*, 8329–8337. [CrossRef]
86. Aigbedion-Atalor, P.O.; Hill, M.P.; Ayelo, P.M.; Ndlela, S.; Zalucki, M.P.; Mohamed, S.A. Can the combined use of the mirid predator *Nesidiocoris tenuis* and a braconid larval endoparasitoid *Dolichogenidea gelechiidivoris* improve the biological control of *Tuta absoluta*? *Insects* **2021**, *12*, 1004. [CrossRef]
87. Calvo, F.J.; Lorente, M.J.; Stansly, P.A.; Belda, J.E. Preplant release of *Nesidiocoris tenuis* and supplementary tactics for control of *Tuta absoluta* and *Bemisa tabaci* in greenhouse tomato. *Entomol. Exp. Appl.* **2012**, *143*, 111–119. [CrossRef]
88. Mirhosseini, M.A.; Fathipour, Y.; Soufbaf, M.; Reddy, G.V.P. Implications of using two natural enemies of *Tuta absoluta* (Lepidoptera: Gelechiidae) toward tomato yield enhancement. *Bull. Entomol. Res.* **2019**, *109*, 617–625. [CrossRef]

89. Sylla, S.; Brévault, T.; Streito, J.; Diarra, K. First Record of *Nesidiocoris tenuis* (Reuter) (Heteroptera: Miridae), as a Predator of the Tomato Leaf Miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), in Senegal. *Egypt. J. Biol. Pest Control* **2016**, *26*, 851–853.
90. Naselli, M.; Biondi, A.; Garzia, G.T.; Desneux, N.; Russo, A.; Siscaro, G.; Zappalà, L. Insights into food webs associated with the South American tomato pinworm. *Pest Manag. Sci.* **2017**, *73*, 1352–1357. [CrossRef]
91. Bacci, L.; Silva, É.M.; Silva, G.A.; Silva, L.J.; Rosado, J.F.; Samuels, R.I.; Picanço, M.C. Natural mortality factors of tomato leafminer *Tuta absoluta* in open-field tomato crops in South America. *Pest Manag. Sci.* **2019**, *75*, 736–743. [CrossRef]
92. Biondi, A.; Zappala, L.; Stark, J.D.; Desneux, N. Do Biopesticides Affect the Demographic Traits of a Parasitoid Wasp and Its Biocontrol Services through Sublethal Effects? *PLoS ONE* **2013**, *8*, e76548. [CrossRef]
93. Yano, E. Recent advances in the study of biocontrol with indigenous natural enemies in Japan. *IOBC WPRS Bull. Fr.* **1999**, *22*, 291–294.
94. Tonnang, H.E.Z.; Mohamed, S.F.; Khamis, F.; Ekesi, S. Identification and Risk Assessment for Worldwide Invasion and Spread of *Tuta absoluta* with a Focus on Sub-Saharan Africa: Implications for Phytosanitary Measures and Management. *PLoS ONE* **2015**, *10*, e0135283. [CrossRef]
95. Guimapi, R.Y.A.; Mohamed, S.A.; Okeyo, G.O.; Ndjomatchoua, F.T.; Ekesi, S.; Tonnang, H.E.Z. Modeling the risk of invasion and spread of *Tuta absoluta* in Africa. *Ecol. Complex.* **2016**, *28*, 1–17. [CrossRef]
96. Shiraku, H. ICIPE Makes the First Release of Parasitoids into the Farm in Fight against Tuta absoluta, Infonet-Biovision. Available online: https://infonet-biovision.org/News/ICIPE-Makes-first-release-parasitoids-farm-fight-against-Tuta-Absoluta (accessed on 14 November 2020).
97. Mama Sambo, S.; Ndlela, S.; Plessis, H.; Obala, F.; Mohamed, S.A. Ratio dependence effects of the parasitoid *Dolichogenidea gelechiidivoris* on its associated host *Tuta absoluta*. *Biocontrol Sci. Technol.* **2022**, *32*, 497–510. [CrossRef]
98. Aigbedion-Atalor, P.O.; Abuelgasim, S.; Hill, M.P.; Zalucki, M.P.; Azrag, A.G.A.; Srinivasan, R.; Ekesi, S. Host stage preference and performance of *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae), a candidate for classical biological control of *Tuta absoluta* in Africa. *Biol. Control* **2020**, *144*, 104215. [CrossRef]
99. Tylianakis, J.M.; Romo, C.M. Natural enemy diversity and biological control: Making sense of the context-dependency. *Basic Appl. Ecol.* **2010**, *11*, 657–668. [CrossRef]
100. Jonsson, M.; Wratten, S.D.; Landis, D.A.; Gurr, G.M. Recent advances in conservation biological control of arthropods by arthropods. *Biol. Control* **2008**, *45*, 172–175. [CrossRef]
101. Jonsson, M.; Kaartinen, R.; Straub, C.S. Relationships between natural enemy diversity and biological control. *Curr. Opin. Insect Sci.* **2017**, *20*, 1–6. [CrossRef]

CHAPTER 5

Interaction between two parasitoids of *Tuta absoluta*: the exotic *Dolichogenidea gelechiidivoris* and the indigenous *Stenomesius* sp. near *japonicus*

Citation:

Mama Sambo, S., Ndlela, S., Azrag, A.G.A., du Plessis, H. and Mohamed, S.A. Interaction between two parasitoids of *Tuta absoluta*: the exotic *Dolichogenidea gelechiidivoris* and the indigenous *Stenomesius* sp. near *japonicus.*

Submitted to Biological Control (Elsevier)

Interaction between two parasitoids of *Tuta absoluta*: the exotic *Dolichogenidea gelechiidivoris* and the indigenous *Stenomesius* sp. near *japonicus*

Sahadatou Mama Sambo[a,b,*], Shepard Ndlela[a], Abdelmutalab G. A. Azrag[a], Hannalene du Plessis[b], Samira Abuelgasim Mohamed[a]

[a]International Centre of Insect Physiology and Ecology (*icipe*), Nairobi P.O. Box 30772-00100, Kenya.

[b]Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa.

Corresponding author*: smama@icipe.org P.O. BOX 30772-00100, Nairobi, Kenya.

(+254757420186)

Abstract

The effectiveness of control by native parasitoids of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Africa is low. *Dolichogenidea gelechiidivoris* Marsh (Hymenoptera: Braconidae) was imported into Kenya for implementation as an ecologically friendly management tactic against this pest. The interaction between *D. gelechiidivoris* and native parasitoids is, however, unknown. The aim of this study was to investigate the interactions between *D. gelechiidivoris* and the native parasitoid, *Stenomesius* sp. near *japonicus* (Ashmead) (Hymenoptera: Eulophidae) in single, simultaneous, and sequential releases of the parasitoids. Laboratory experiments were conducted using tomato leaves infested with 1st- and 3rd-instar *T. absoluta* larvae. The probing and oviposition behaviour of the parasitoids, the number of eggs laid and progeny that developed to the adult stage, were recorded. The results showed that *D. gelechiidivoris* was competitively superior to *S.* sp. nr. *japonicus*.

Dolichogenidea gelechiidivoris preferred 1^{st}-instar *T. absoluta* larvae. More eggs were laid in 1^{st}-, compared to 3^{rd}-instar larvae, and more *D. gelechiidivoris* wasps emerged from 1^{st}-instar larvae. *Stenomesius* sp. near *japonicus* laid eggs in both the 1^{st}- and 3^{rd}-larval instars, without any preference, but the highest wasp emergences were recorded from 3^{rd} instar larvae. The combined parasitism by the two parasitoids simultaneously released, resulted in up to 77% pest parasitism. The co-existence of both parasitoid species can therefore significantly contribute to *T. absoluta* control, with minimal effect on each other.

Keywords: Endoparasitoid, ectoparasitoid, native parasitoid, exotic parasitoid, co-existence, South American tomato moth

Introduction

Invasive insect pests often enter into new areas with no associated natural enemies in these areas, which allows for rapid increases in pest populations. The Afro-Eurasian supercontinent invasion by the destructive *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), presents a big challenge for tomato production (Desneux et al., 2010; Tumuhaise et al., 2016; Karlsson et al., 2018, Mansour and Biondi, 2021). It is a cosmopolitan pest, causing huge economic losses in many counties of the world (Biondi et al., 2018; Mansour et al., 2018; Han et al., 2019; Rwomushana et al., 2019). When no control measures are applied, total yield losses due to the direct feeding of the larvae on the leaves, stems and fruit can occur (Chermiti et al., 2009). The main management practice for *T. absoluta*, is the application of synthetic insecticides (Rwomushana et al., 2019; Aigbedion-Atalor et al., 2019; Chepchirchir et al., 2021). However, this pest has developed resistance to various insecticide classes such as pyrethroids, spinosyns and diamides, which presents an additional challenge in its management (Roditakis et al., 2015; Guedes et al., 2019). To improve on the management options currently available for *T. absoluta*, new sustainable management strategies are needed with minimum adverse effects on the environment, users, and consumers.

Biological control using natural enemies is a sustainable management option used in integrated pest management (IPM). Several indigenous parasitoids belonging to various families including Braconidae, Eulophidae, Chalcididae, Torymidae, Bethylidae and Trichogrammatidae have been identified as natural control agents of this pest in invaded areas (Abbes et al., 2013; Mansour et al., 2018; Desneux et al., 2022; van Lenteren et al., 2021). For example, in East Africa, the Braconidae parasitoids, *Bracon nigricans* Szépligeti, *Dolichogenidea appellator* (Telenga), and an unidentified *Bracon* sp. were reported to parasitise different larval stages of *T. absoluta* (Idriss et al., 2018; Kinyanjui et al., 2021). However, these parasitoids are not highly effective and are therefore not able to maintain the damage levels below the economic threshold. In response, the International Centre of Insect Physiology and Ecology (*icipe*), in Kenya introduced the exotic parasitoid *Dolichogenidea gelechiidivoris* Marsh (Hymenoptera: Braconidae) from Peru, for classical biological control of *T. absoluta* in Africa (Shiraku, 2020).

Dolichogenidea gelechiidivoris is a specialist solitary endoparasitoid (Fernandez-Triana et al., 2020; Mama Sambo et al., 2022a) that parasitises all larval stages of *T. absoluta*, but with a preference for 1^{st}- and 2^{nd}-instar larvae (Aigbedion-Atalor et al., 2020). Parasitism levels of approximately 45, 4, 12, and 5% were reported on 1^{st}-, 2^{nd}-, 3^{rd}- and 4^{th}-instar larvae, of *T. absoluta*, respectivelyin a previous study. In its aboriginal area, more than 60% parasitism by *D. gelechiidivoris* was reported in a field study (Morales et al., 2014). However, the establishment and efficiency of an introduced parasitoid is usually influenced by the nature of its competition with the native parasitoids that are associated with the pest (Wang et al., 2008; Cabello et al., 2011). For example, species might seek to exclusively use hosts resulting in fierce competition (Wang et al., 2008). This competition might lead to competitive exclusion with the introduced parasitoid species displacing the native species, hence resulting in biodiversity imbalances (Bennett, 1993; Sorribas et al., 2010).

Following the release of *D. gelechiidivoris* in Kenya, continued efforts had been made to identify new associations between the invasive *T. absoluta*, and its introduced and native parasitoids. During these surveys conducted in Nairobi and Kirinyaga counties in Kenya, a promising native idiobiont ectoparasitoid, *Stenomesius* sp. near *japonicus* (Ashmead) (Hymenoptera: Eulophidae) was found, which caused parasitism of up to 45% in greenhouse tomato production (Mama Sambo et al., 2022b).

Both *D. gelechiidivoris* and *S.* sp. nr. *japonicus* attack *T. absoluta* larvae, resulting in the likelihood of competition for the same host larval instar. Initial parasitisation by either species could also affect the efficiency of the parasitoid targeted for release for biocontrol of the pest. This competition can affect the parasitoids' ability to locate a suitable host for parasitisation and progeny development as well as competition within the host parasitised by different parasitoid species. The aim of this study was to investigate the interspecific competition between parasitoid species in terms of intrinsic and extrinsic competition between the native and exotic parasitoids of *T. absoluta*.

2. Materials and methods

2.1. Host plants

Tomato seedlings (cv Moneymaker) were transplanted into 1.67 L plastic pots containing soil composted with goat manure and kept inside an insect-proof screenhouse at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi. These seedlings were used in bioassays after 3-4 weeks.

2.2. Insects

2.2.1. Tuta absoluta

Tuta absoluta-infested tomato plants were sampled from Naivasha (36° 12' 52.1" E, 0° 46' 22.5" S), Nairobi (36°53'48.1" E, 1°13'18.3" S), and Kirinyaga County (037°22'299" E, 00°36'992" S), Kenya to initiate a rearing colony. The colony was kept in an insectary at ambient conditions of 25 ± 2 °C; 70 ± 5% Relative humidity (RH); 12hL:12hD photoperiod and reared following the protocol described by Mama Sambo et al. (2022a). The moths used in the current study were from the F_5-generation after field collections.

2.2.2. Dolichogenidea gelechiidivoris

Approximately 200 *D. gelechiidivoris* cocoons were imported from the International Potato Center (CIP) Lima, Peru. These cocoons were received in the quarantine facility of *icipe* for pre-release evaluation. After wasp emergence in the quarantine, the colony was maintained according to the procedure described by Mama Sambo et al. (2022). The colony was reared for approximately 73 generations before the onset of the experiment.

2.2.3. Stenomesius sp. near japonicus

The ectoparasitoid *S.* sp. nr. *japonicus* colony originated from samples of *T. absoluta*-infested tomato collected from an experimental screenhouses at *icipe*, Nairobi (1°13'18.3" S, 36°53'48.1" E, and altitude of 1604 m). For laboratory rearing of *S.* sp. nr. *japonicus*, potted tomato plants were exposed to *T. absoluta* in a perspex cage (50×40×40 cm) for two days. Thereafter, the plants were removed and kept at ambient temperature (25 ± 2 °C), 70 ± 5% RH and 12hL:12hD photoperiod for nine days, until the larvae reached the 3rd-instar. The plants were then introduced into a cage (50×40×40 cm) containing 100 *S.* sp. nr. *japonicus* pairs. The plants were left in the cage for two days, to allow the parasitoids to oviposit, whereafter the plants were removed. All the leaves with larvae were incubated in a plastic lunch box (21 x 15 x 16 cm), covered with a sheet of paper towel. The larvae were regularly supplied with uninfested tomato leaflets until the wasps emerged. These wasps were transferred daily to a clean cage containing *T. absoluta*-infested plants and kept under laboratory conditions. Droplets of 80% honey solution were applied on the inside of the top of the cages with a camel-hair brush to serve as food for the wasps. The parasitoids were reared for seven generations before the onset of the experiment.

2.3. Interaction between D. gelechiidivoris and S. sp. nr. japonicus

The interaction between *D. gelechiidivoris* and *S.* sp. nr. *japonicus* was evaluated in laboratory experiments using 1^{st}- and 3^{rd}-instar *T. absoluta* larvae. The experiment consisted of five scenarios (Table 1). The *T. absoluta* larvae that hatched from eggs that were laid six days prior to the experiment, were regarded as 1^{st}-instar larvae, while those that hatch from eggs laid 12 days prior to the experiment, as 3^{rd}-instar larvae. For each of the scenarios, leaves containing 50 *T. absoluta* larvae inside mines were used. Each scenario was replicated eight times for both 1^{st}- and 3^{rd}- instar *T. absoluta* larvae. The unhatched eggs and extra larvae were removed. The petioles of leaves used in these tests, were placed into a glass test tube (75 x 19 mm) with water and sealed around the petiole with cotton wool. These tubes containing the *T. absoluta*-infested leaves, were placed in a perspex cage (20×14×14 cm), with 80% honey solution droplets applied as described above. The extrinsic competition between *D. gelechiidivoris* and *S.* sp. nr. *japonicus* was assessed at 21 ± 2 °C and 70 ± 5% RH.

Table 1 Experimental scenarios used to evaluate the interaction between *D. gelechiidivoris* and *S.* sp. nr. *japonicus.*

Treatments	Description
Dg/Sj: *D. gelechiidivoris* alone or *S.* sp. nr. *japonicus* alone	Either one mated naïve *D. gelechiidivoris* or one mated naïve female of *S.* sp. nr. *japonicus* female was released in a perspex cage containing 50 1^{st}- or 3^{rd}-instar *T. absoluta* larvae and allowed to forage and oviposit for 24 h.
Dg & Sj: *D. gelechiidivoris & S.* sp. nr. *japonicus* released simultaneously	One mated naïve *D. gelechiidivoris* female and one mated naïve *S.* sp. nr. *japonicus* female were released simultaneously in a perspex cage containing 50 1^{st}- or 3^{rd}-instar *T. absoluta* larvae and allowed to oviposit for 24 h.
DgSj: *S.* sp. nr. *japonicus* (Sj) first followed by *D. gelechiidivoris*	One mated naïve *D. gelechiidivoris* female was released in a perspex cage containing 50 1^{st}- or 3^{rd}-instar *T. absoluta* larvae and allowed to oviposit for 24h. It was then removed and one mated naïve female *S.* sp. nr. *japonicus* was released and allowed to oviposit for 24h.
SjDg: *D. gelechiidivoris* released first followed by *S.* sp. nr. *japonicus*	One mated naïve *S.* sp. nr. *japonicus* female was released in a perspex cage containing 50 1^{st}-or 3^{rd}-instar *T. absoluta* larvae and allowed to oviposit for 24h. It was then removed and one mated naïve female *D. gelechiidivoris* was introduced and allowed to oviposit for 24h.

2.3.1. Extrinsic competition

For each treatment, behavioural activities such as probing and oviposition in the host larvae were observed for 5 mins, every hour, for a period of six hours. Twenty-four hours after exposure, half the number of larvae (25) from each treatment were dissected under a Leica EZ4D digital stereomicroscope (Leica Microsystems, Heerbrugg, Switzerland) to count the number of eggs laid by each parasitoid species. Multiparasitism was investigated by counting the eggs of *D. gelechiidivoris* and *S.* sp. nr. *japonicus* laid in the same larvae. The eggs were distinguished by means of dissimilarity in their shape, color and size, described by Bajonero et al. (2008) for *D. gelechiidivoris* and Youssef et al. (2022) for *S. sp. nr. japonicus*. The percentage of multiparasitised hosts was calculated as: (number of multiparasitised lavae/ the number of dissected larvae) x100.

2.3.2. Intrinsic competition

Intrinsic competition was observed on the remaining 25 larvae from each treatment, which were transferred to plastic containers with a net-inserted lid and kept at ambient laboratory conditions. Uninfested tomato leaflets were added into the plastic containers every three days to maintain larval feeding. The number and sex of parasitoids that eclosed per species and the number of *T. absoluta* moths that emerged were recorded daily to assess the developmental time of each parasitoid species under the different treatments. The percentage parasitism by each the parasitoid species was calculated as: (total number of wasps per species emerging from each sample)/(total number of moths + total number of parasitoids) x 100. The combined parasitism by both species was also calculated.

2.5. Data analysis

A two-way repeated measures Analysis of variance (ANOVA) was used to analyse the number of probing and oviposition events executed on *T. absoluta* larvae for each larval stage and for the respective parasitoid treatment combinations. A Generalized Linear Model (GLM) with negative binomial distribution was used to assess the effect of larval instar and parasitoid combinations on the number of eggs laid by each parasitoid species, percentage of multi-parasitised host larvae, percentage parasitism by each species, percentage parasitism by both species, and percentage of female wasps that eclosed. The developmental times for each parasitoid species in the respective treatment combinations in 1^{st} and 3^{rd}-instar *T. absoluta* larvae were analysed using GLM with gamma distribution. All tests were followed by Tukey's multiple comparison of means post hoc test, whenever a significant difference was detected. Data were analysed with R software (R Core Team, 2018).

3. Results

3.1. Extrinsic competition

The parasitoid combinations did not significantly affect probing ($F_{2, 284} = 0.77$, $P = 0.46$) and oviposition ($F_{2, 284} = 0.90$, $P = 0.40$) by *D, gelechiidivoris*. However, host larval instars significantly influenced *D. gelechiidivoris* probing ($F_{1, 284} = 23.18$, $P < 0.001$) and oviposition ($F_{1, 284} = 28.02$, $P < 0.001$). Significantly more probing and oviposition of 1^{st}-instar larvae occurred compared to 3^{rd}-instar *T. absoluta* larvae (Table 2). Neither parasitoid combinations, nor *T. absoluta* larval instars had an effect on the frequency of probing (parasitoid combinations: $F_{2, 284} = 0.3$, $P = 0.74$; larval stages: $F_{1, 284} = 0$, $P = 1$), and oviposition (parasitoid combinations: $F_{2, 284} = 2.85$, $P = 0.06$; larval stages: $F_{1, 284} = 2.85$, $P = 0.09$) by *S.* sp. nr. *japonicus*. Overall, *D. gelechiidivoris* was more active than *S.* sp. nr. *japonicus* regardless of parasitoid combinations or host larval instar exposed (Table 2).

The number of eggs laid by *D. gelechiidivoris* was significantly affected by *T. absoluta* larval instars ($F_{1, 59} = 81.41$, $P < 0.001$), but not by the parasitoid combinations ($F_{3, 59} = 2.28$, $P =$

0.09). The highest number of eggs laid by *D. gelechiidivoris* was in 1st-instar larvae, compared to 3rd- instar larvae (Fig. 1). However, the number of eggs laid by *S.* sp. nr. *japonicus* was not affected by the larval instars ($F_{1, 59}$ = 2.01, *P* = 0.16) as well as by the parasitoid combinations ($F_{3, 59}$ = 1.10, *P* = 0.36). The highest percentage multiparasitism of 5 ± 3% on1st-instar, and 3 ± 1% on 3rd-instar *T. absoluta* larvae were recorded when *S.* sp. nr. *japonicus* was released first for parasitism of *T. absoluta* larvae, followed by release of *D. gelechiidivoris*. However, no multiparasitism was recorded for the scenario where *D. gelechiidivoris* was released before *S.* sp. nr. *japonicus* for both larval instars. When both parasitoid species were released simultaneously, 1% of 1st-instar larvae, but no 3rd-instar larvae were multiparasitised.

Table 2 Probing and oviposition by *D. gelechiidivoris* and *S.* sp. nr. *japonicus* in 1st- and 3rd-instar *Tuta absoluta* larvae under a single release (Dg/Sj), simultaneous release (Dg & Sj), and sequential releases either of the parasitoids, *D. gelechiidivoris* (DgSj) or *S.* sp. nr. *japonicus* released first (SjDg).

T. absoluta larval instar	Parasitoid combinations	Mean no. of probes in 5 min		Mean no. of eggs in 5 min	
		D. gelechiidivoris	*S. sp. nr. japonicus*	*D. gelechiidivoris*	*S. sp. nr. japonicus*
1st	Dg/Sj	2.77 ± 0.60 Aa	0.06 ± 0.05 Aa	0.92 ± 0.30 Aa	0.10 ± 0.06 Aa
	Dg & Sj	3.88 ± 0.64 Aa	0 Aa	1.69 ± 0.35 Aa	0 Aa
	DgSj	NA	0 Aa	NA	0 Aa
	SjDg	4.08 ± 0.86 Aa	NA	0.94 ± 0.21 Aa	NA
3rd	Dg/Sj	1.44 ± 0.26 Ba	0 Aa	0.44 ± 0.14 Ba	0 Aa
	Dg & Sj	1.46 ± 0.30 Ba	0.02 ± 0.02 Aa	0.042 ± 0.029 Ba	0 Aa
	DgSj	NA	0.04 ± 0.04	NA	0 Aa
	SjDg	1.35 ± 0.37 Ba	NA	0.21 ± 0.08 Ba	NA

Means within the column followed by the same uppercase letters are not significantly different for the larval instars tested per parasitoid species, while the means within the column followed by the same lowercase letters are not significantly different for tested parasitoid combinations per parasitoid species (Tukey's HSD test, α = 0.05). NA= data not recorded in the bioassay.

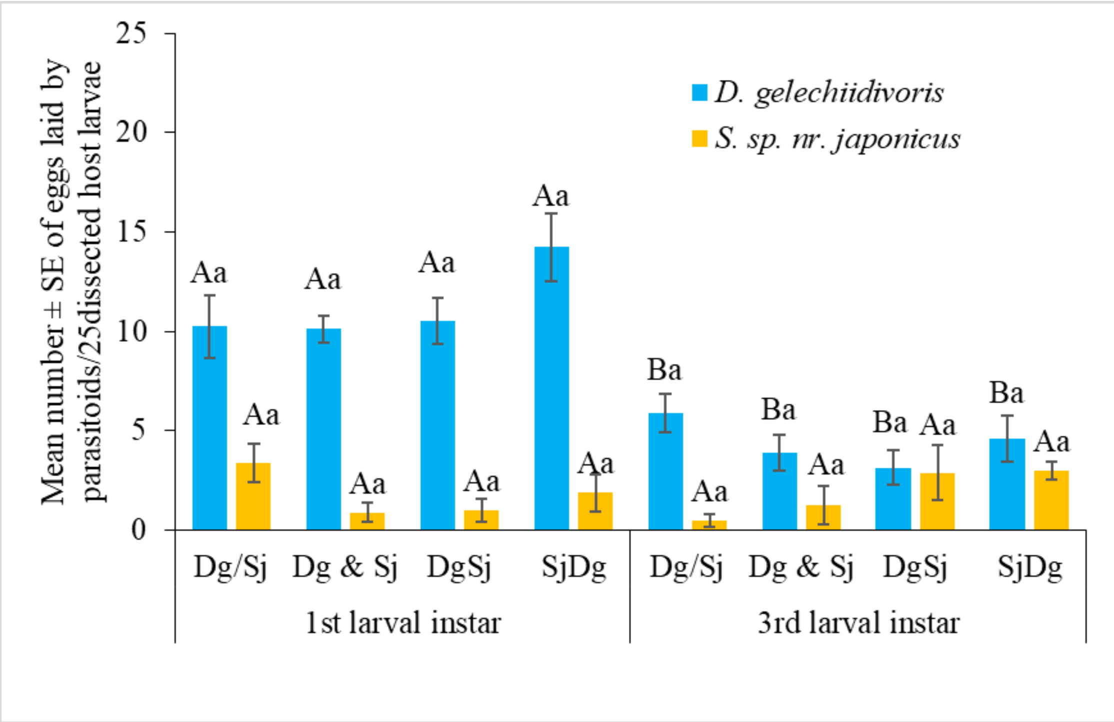


Fig. 1. Mean number of eggs laid by *D. gelechiidivoris* (Dg) and *S.* sp. nr. *japonicus* (Sj) in 1^{st}- and 3^{rd}-instar *T. absoluta* larvae following a single release (Dg/Sj), simultaneous release (Dg & Sj), and sequential releases, with either *D. gelechiidivoris* (DgSj) or *S.* sp. nr. *japonicus* released first (SjDg). Means with different uppercase letters indicate significant differences in the number of eggs laid by the respective parasitoid species in 1^{st}- and 3^{rd}-instar larvae. Means with different lowercase letters indicate significant differences in the number of eggs laid by the respective parasitoids released in different combinations for 1^{st}- and 3^{rd}-instar *T. absoluta* larvae (Tukey's HSD test, $\alpha = 0.05$).

3.2. Intrinsic competition

3.2.1. Percentage parasitism by the respective parasitoid species

Dolichogenidea gelechiidivoris parasitism of *T. absoluta* larvae varied significantly between the larval instars ($F_{1, 59} = 37.41$, $P < 0.001$) as well as between the parasitoid combinations ($F_{3, 59} = 4.95$, $P = 0.004$). The highest parasitism by *D. gelechiidivoris* was reported on 1st-instar larvae (Fig. 2). Parasitism by *S.* sp. nr. *japonicus* was also significantly affected by host larval instars ($F_{1, 59} = 6.19$, $P = 0.02$), but not by the parasitoid combinations ($F_{3, 59} = 1.17$, $P = 0.34$). The 3rd-instar larvae were the most parasitised by *S.* sp. nr. *japonicus* (Fig. 2). The proportion of *D. gelechiidivoris* females in the progeny was neither affected by host larval instars ($F_{1, 50} = 0.15$, $P = 0.70$), nor by parasitoid combinations ($F_{3, 50} = 0.55$, $P = 0.65$). However, the proportion of *S.* sp. nr. *japonicus* females that emerged, was affected by host larval instars ($F_{1, 14} = 11.15$, $P = 0.004$), as well as by parasitoid combinations ($F_{3, 10} = 4.34$, $P = 0.03$). Overall, *D. gelechiidivoris* that emerged from 3rd-instar larvae, was male biased in all the scenarios where the two species were combined either simultaneously or in sequential release (Table 3).

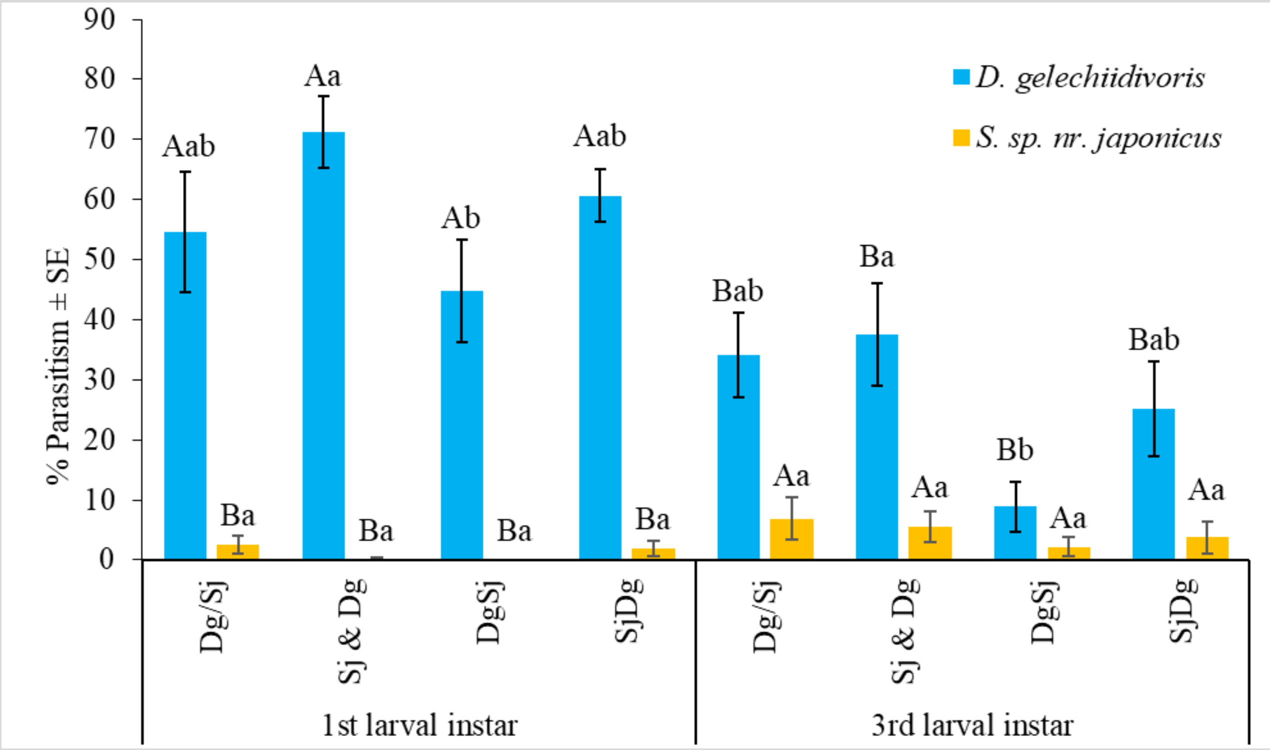


Fig. 2. Percentage parasitism by *D. gelechiidivoris* (Dg) and *S.* sp. nr. *japonicus* (Sj) in 1^{st}- and 3^{rd}-instar *T. absoluta* larvae with a single release (Dg/Sj), simultaneous release (Dg & Sj), and sequential releases of either of the parasitoid species, *D. gelechiidivoris* (DgSj) or *S.* sp. nr. *japonicus* released first (SjDg). Means with different uppercase letters indicate significant differences in parasitism between1^{st}- and 3^{rd}-instar larvae per parasitoid species, released in different combinations. Means with different lowercase letters indicate significant differences in parasitism by the respective parasitoids released in different combinations for 1^{st} and 3^{rd}-instar *T. absoluta* larvae (Tukey's HSD test, $\alpha = 0.05$).

Table 3 The effect of the interaction between *D. gelechiidivoris* and *S.* sp. nr. *japonicus* on the sex ratio of the progeny that emerged from 1st- and 3rd-instar *T. absoluta* larvae following a single release (Dg/Sj), simultaneous release (Dg & Sj), and sequential releases of either of the parasitoid species, *D. gelechiidivoris* (DgSj) or *S.* sp. nr. *japonicus* (SjDg) released first.

T. absoluta larval instar	Parasitoid combinations	% *D. gelechiidivoris* females	% *S.* sp. nr. *japonicus* females
1st	Dg/Sj	34.98 ± 9.16 Aa	11.11 ± 11.11 Bb
	Dg & Sj	49.06 ± 10.33 Aa	*
	DgSj	56.09 ± 15.54 Aa	*
	SjDg	38.80 ± 6.79 Aa	0
3rd	Dg/Sj	58.07 ± 14.14 Aa	20.61 ± 8.43 Ab
	Dg & Sj	42.80 ± 12.12 Aa	83.75 ± 9.87 Aa
	DgSj	30.21 ± 20.65 Aa	50 ± 50 Aa
	SjDg	26.23 ± 12.30 Aa	100 ± 0 Aa

Means with different uppercase letters indicate significant differences between larval stages per parasitoid species and means with different lowercase letters indicate significant differences between parasitoid combinations per parasitoid species (Tukey's HSD test, $\alpha = 0.05$). *= no parasitoid emergence was recorded.

3.2.2. *Parasitoid development time*

Development time of *D. gelechiidivoris* males and females varied significantly according to the instar of the host larvae being parasitised (males: $F_{1, 124} = 48.20$, $P < 0.001$ and females: $F_{1, 100} = 33.01$, $P < 0.001$), as well as for the parasitoid combinations used (males: $F_{3, 124} = 8.91$, $P < 0.001$ and females: $F_{3, 100} = 8.20$, $P < 0.001$). For *S.* sp. nr. *japonicus*, host larval stages affected female and male developmental time ($F_{1, 13} = 11.74$, $P = 0.004$) and ($F_{1, 13} = 52.08$, $P < 0.001$) respectively, while parasitoid combinations affected only female developmental time ($F_{3, 13} = 5.63$, $P = 0.01$) but not the male development time ($F_{1, 13} = 2.86$, $P = 0.08$). Developmental time of *S.* sp. nr. *japonicus* was shorter on 3^{rd}-instar larvae (Table 4).

Table 4. Effect of the interaction between *D. gelechiidivoris* and *S.* sp. nr. *japonicus* on the developmental time from egg to adult (days) of the respective parasitoids from *T. absoluta* larvae parasitised while in the 1st- and 3rd-instars following a single release (Dg/Sj), simultaneous release (Dg & Sj), and sequential releases with either of the parasitoid species, *D. gelechiidivoris* (DgSj) or *S.* sp. nr. *japonicus* (SjDg), released first.

T. absoluta larval instar	Parasitoid combinations	*D. gelechiidivoris*		*S.* sp. nr. *japonicus*	
		♂	♀	♂	♀
1st	Dg/Sj	21 ± 0.25 Ac	21 ± 0.31 Ac	15 ± 1.00 Aa	17 ± NA Aa
	Dg & Sj	23 ± 0.69 Ab	23 ± 0.67 Ab	*	*
	DgSj	25 ± 0.33 Aa	26 ± 0.37 Aa	*	*
	SjDg	18 ± 1.01 Ad	16 ± 1.43 Ad	12 ± 1.00 Aa	*
3rd	Dg/Sj	18 ± 0.24 Bc	18 ± 0.29 Bc	11 ± 0.29 Ba	12 ± 0.58 Ba
	Dg & Sj	18 ± 0.27 Bb	18 ± 0.22 Bb	12 ± 1.53 Ba	11 ± 0.20 Bb
	DgSj	16 ± 2.25 Ba	17 ± 0.71 Ba	13 ± NA Ba	10 ± NA Bbc
	SjDg	18 ± 0.44 Bd	19 ± 0.32 Bd	*	9 ± 0.29 Bc

Means with different uppercase letters indicate a significant difference between larval instars per parasitoid species and means with different lowercase letters indicate significant differences between parasitoid combinations per parasitoid species (Tukey's HSD test, α = 0.05). *= no parasitoid emergence was recorded.

Parasitism of T. absoluta larvae by D. gelechiidivoris and S. sp. nr. japonicus used in combination

The larval instar affected the total number of *T. absoluta* larvae parasitised by *D. gelechiidivoris and S.* sp. nr. *japonicus* ($F_{1, 74} = 24.97$, $P < 0.001$). Similarly, parasitoid combinations affected the total parasitism of *T. absoluta* larvae ($F_{4, 74} = 16.59$, $P < 0.001$). The lowest percentage parasitism was reported on 1st larval instars, and the host exposed to only *S.* sp. nr *japonicus* (Fig. 3).

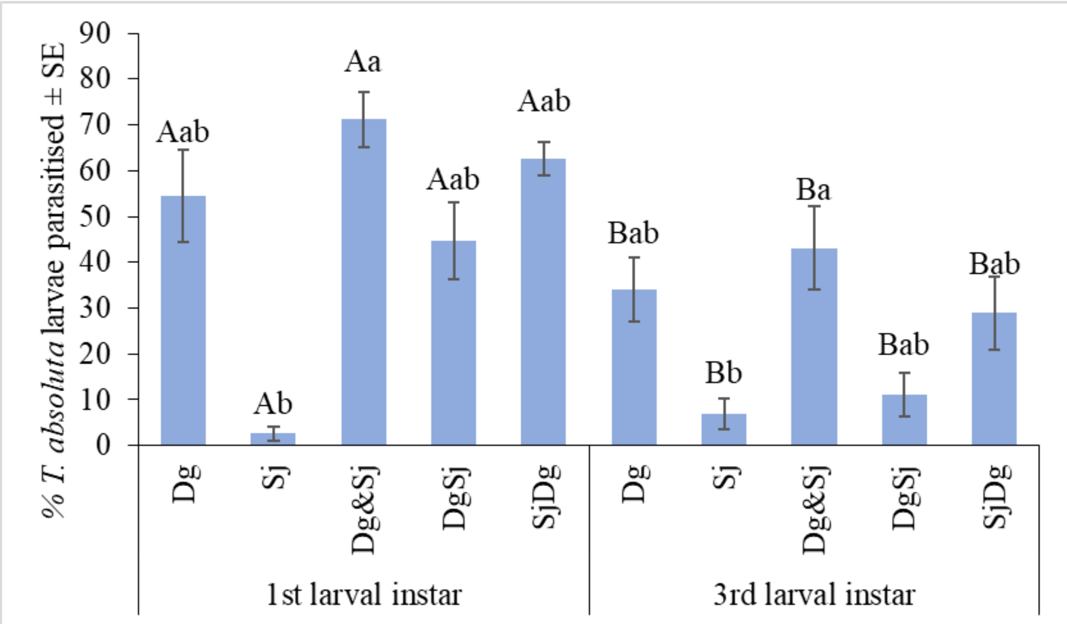


Fig. 3. Percentage *T. absoluta* larvae parasitised by *D. gelechiidivoris* (Dg) and *S.* sp. nr. *japonicus* (Sj), when the parasitoids were released simultaneously (Dg & Sj), in sequence with either of the parasitoid species, *D. gelechiidivoris* release first (DgSj) or *S.* sp. nr. *japonicus* (SjDg), released first. Means with different uppercase letters indicate significant differences in parasitism between 1st and 3rd instar *T. absoluta* larvae. Means with different lowercase letters indicate significant differencesin parasitism by the respective parasitoids released either alone, or in combinations (Tukey's HSD test, $\alpha = 0.05$).

Discussion

The introduction of a parasitoid species that can be useful for classical biocontrol may have a negative impact on other native species (Kenis et al., 2019). *Stenomesius* sp. nr. *japonicus* is a native ectoparasitoid, which attacks the different larval instars of *T. absoluta* with a high killing rate in addition to parasitism (Chailleux et al., 2014). *Dolichogenidae gelechiidivoris* was released in Kenya, where a native parasitoid, *S.* sp. nr. *japonicus* widely occurs and where it has formed a new association with the invasive *T. absoluta* (Mama Sambo et al., 2022b; Shiraku, 2020).

4.1. Extrinsic competition

The combined release of both the exotic parasitoid, *D. gelechiidivoris* and the native parasitoid, *S.* sp. nr. *japonicus* did not significantly affect the probing and oviposition of *D. gelechiidivoris* in *T. absoluta* larvae. However, exposure of *D. gelechiidivoris* wasps to different host larval instars, resulted in significant differences in probing and oviposition by the parasitoid. This is in accordance with the findings of Savino et al. (2016) who reported that the interaction between other parasitoids of *T. absoluta*, the endoparasitoid *Pseudapanteles dignus* (Muesebeck) (Hymenoptera: Braconidae) and the ectoparasitoid *Dineulophus phthorimaeae* De Santis (Hymenoptera: Eulophidae), did not affect the searching activity of the former, but that of the ectoparasitoid was affected when in the presence of the endoparasitoid. *Dolichogenidea gelechiidivoris* exhibit more probing and oviposition behaviour and laid more eggs than *S.* sp. nr. *japonicus* in the presence and absence of *S.* sp. nr. *japonicus*, but oviposition of both parasitoid species was affected by the instar of the host larvae present. This suggests that the egg load and maturation of *D. gelechiidivoris* is higher than that of *S.* sp. nr. *japonicus*. Aigbedion-Atalor et al. (2020) reported *D. gelechiidivoris* to lay between 15-20 eggs per day from the first- to the third day after emergence, while Mama Sambo et al. (2022a) reported up to 37 eggs laid per day when

100 first-instar larvae were offered to the same parasitoid. A much lower number of two to five progenies per day was recorded by Chailleux et al. (2014) for *S.* sp. nr. *japonicus*. The number of eggs laid by *D. gelechiidivoris* was the highest in 1st-instar larvae, which is also its preferred host stage (Aigbedion-Atalor et al., 2020).

Stenomesius sp. nr. *japonicus* discriminated against already parasitised larvae during oviposition, since multiparasitism only occurred when this parasitoid was released before *D. gelechiidivoris*, or when the *T. absoluta* larvae were exposed to the two species simultaneously. Similarly, Savino et al. (2016) also reported the highest searching time for *T. absoluta* larvae by the ectoparasitoid *D. phthorimaeae*, when the endoparasitoid *P. dignus* was present. However, it differs from other larval parasitoids, for example, the ectoparasitoid, *Spathius agrili* Yang (Hymenoptera: Braconidae) that parasitised *Agrilus planipennis* (Fairmaire) (Coleoptera: Buprestidae) larvae, which were previously parasitised by the endoparasitoid, *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) (Ulyshen et al., 2010). The *S. agrili* progeny did, however, not develop on hosts previously parasitised by *T. planipennisi* (Ulyshen et al., 2010). On the same pest, the endoparsitoid, *Dinarmus basalis* (Rond.) (Hymenoptera: Pteromalidae), also discriminated against pulse beetle larvae earlier parasitised by the ectoparasitoid, *Eupelmus vuilleti* Crawford (Hymenoptera: Eupelmidae) (van Alebeek et al., 1993).

Intrinsic competition

The percentage parasitism by *D. gelechiidivoris* was significantly affected by *T. absoluta* larval instar. It confirmed the results of Aigbedion-Atalor et al. (2020), who reported that the highest number of *D. gelechiidivoris* cocoons formed in earlier instar larvae (1st- and 2nd-instar), compared to late instar *T. absoluta* larvae (3rd- and 4th-instar). The percentage parasitism by *D. gelechiidivoris* was, however, significantly lower when *S.* sp. nr. *japonicus* was released after *D. gelechiidivoris* has already been present. The killing ability of the ectoparasitoid *S.* sp. nr. *japonicus* released after *D. gelechiidivoris*, slightly affected the emergence of the endoparasitoid also. Chailleux et al. (2014) estimated about two larvae killed per day per female by this ectoparasitoid. Although the percentage parasitism of *S.* sp. nr. *japonicus* was higher in 3rd-instar larvae as demonstrated by Chailleux et al. (2014). The highest parasitism by this parasitoid occurred when it operated alone, in the absence of the exotic parasitoid. The exotic *D. gelechiidivoris* therefore dominated the native *S.* sp. nr. *japonicus*.

Developmental time of *D. gelechiidivoris* and *S.* sp. nr. *japonicus* was found to be host larval instar dependent in this study. It is in contrast to the findings of Aigbedion-Atalor et al. (2020) who reported no difference in developmental time of *D. gelechiidivoris* in the four host larval instars. These authors also reported no difference in the proportion of male and female parasitoids that eclosed, which is also in contrast to the findings of this study. The sex ratio of *D. gelechiidivoris* progeny was male biased when it co-occurred with *S.* sp. nr. *japonicus* on 3rd-instar larvae. Similarly, Mama Sambo et al. (2022a) also reported male biased progeny for this parasitoid, while Aigbedion-Atalor et al. (2020) the progeny to be female biased. However, abiotic factors affect parasitoid performance, including development time and sex of the parasitoids (Bajonero et al., 2008; Dannon et al., 2010), which may explain the differences found between the respective studies. The study of Aigbedion-Atalor et al. (2020) was done at 26 ± 4 °C and 60 ± 5% RH, while this study was conducted at 21 ± 2 °C and 70 ± 5% RH. Developmental time of *D. gelechiidivoris* was longer than that of *S.* sp. nr. *japonicus*, and almost similar to the developmental time of *D. gelechiidivoris* reported by Bajonero et al. (2008) and Aigbedion-Atalor et al. (2020), as well as the developmental time of *S.* sp. nr. *japonicus* reported by Chailleux et al. (2014). If only the shorter duration of the life cycle of *S.* sp. nr. *japonicus* is considered, this parasitoid can be regarded as the more preferred species, compared to *D. gelechiidivoris* (Chailleux et al., 2014).

The parasitism of *T. absoluta* larvae by both *D. gelechiidivoris* and *S.* sp. nr. *japonicus* varied depending on the larval instar been parasitised and the combinations in which the two parasitoids were used, with more than 75% parasitism when both species were released simultaneously. This is in agreement with approximately 70% *T. absoluta* parasitism reported for *D. gelechiidivoris* by Morales et al. (2014) and 75% that was reported by Bajonero et al. (2008). Several parameters such as the host-parasitoid ratio, parasitoid age and temperature during exposure, have an effect on the parasitim efficacy of *D. gelechiidivoris* (Bajonero et al., 2008; Aigbedion-Atalor et al., 2020; Mama Sambo et al., 2022a). To the best of our knowledge this is the first report on parasitism by *D. gelechiidivoris* in combination with another parasitoid, but combinations of this parasitoid with the predator *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) was highly effective (Aigbedion-Atalor et al., 2021). Up to 90% *T. absoluta* mortality was recorded, which was higher than *T. absoluta* mortality caused by either the parasitoid or the predator alone. Although parasitism by *S.* sp. nr. *japonicus* was low on 3rd-instar larvae, it still contributed towards control of the pest in this life stage. A low efficacy in control of *T. absoluta* by a related species, *Stenomesius rufescens* (Retzius) (Hymenoptera: Eulophidae), also a native parasitoid of *T. absoluta* has also been reported by Kinyanjui et al. (2021). However, Chailleux et al. (2017) reported similar control by *S.* sp. nr. *japonicus* and *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) from the fourth week after release of the parasitoid.

Conclusion

Dolichogenidea gelechiidivoris performed better than *S.* sp. nr. *japonicus* in terms of percentage parasitism. Although both species oviposit in the two *T. absoluta* larval instars, *D. gelechiidivoris* preferred 1st-instar larvae, while *S.* sp. nr. *japonicus* preferred 3rd-instars. It is possible that this preference may become more delineated with time, if the two species segregate their niches as a survival mechanism. Developmental time and sex ratio of the progenies of both species were not affected by their interspecific interaction. To deepen the understanding of this interaction, chemical cues released by the host when parasitised by a specific species should be investigated. Additionally, further studies should assess the combined use of these two parasitoids species in commercial greenhouses and in open-field conditions with conventional practices used by farmers.

Funding: The authors gratefully acknowledge the financial support for this research by the following organizations and agencies: Biovision Foundation grant number BV DPP-012/ 2019-2022, Norwegian Agency for Development Cooperation-(NORAD), the section for research, innovation and higher education grant number RAF-3058 KEN-18/0005, Scaling-up Climate-Smart Pest Management Approaches for Enhanced Maize and Tomato Systems Productivity in Eastern Africa (SCLAMP) grant number 81250394, the Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); the Federal Democratic Republic of Ethiopia; and the Government of the Republic of Kenya. The first author was supported by the German Academic Exchange Service (DAAD) through the African Regional Postgraduate Programme in Insect Science (ARPPIS) tenable at *icipe*. The views expressed herein do not necessarily reflect the official opinion of the donors.

Acknowledgements: The authors recognize the technical assistance of Esther Awuor Owino, Alex Muthembwa, Patrick Kipkorir Koech, Francis Obala, and Omar Jimali Ibrahim.

Sahadatou Mama Sambo: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing - original draft; Writing - review &editing. Shepard Ndlela: Conceptualization; Data curation; Formal analysis; Funding acquisition; Methodology; Project administration; Resources; Supervision; Validation; Visualization;

Writing - review & editing. Abdelmutalab G. A. Azrag: Data curation; Formal analysis; Methodology; Validation; Visualization; Writing - original draft; Writing - review &editing. Hannalene du Plessis: Conceptualization; Data curation; Formal analysis; Supervision; Validation; Visualization; Writing - review & editing. Samira Abuelgasim Mohamed: Conceptualization; Data curation; Formal analysis; Funding acquisition; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing - review & editing.

References

Abbes, K., Biondi, A., Zappalà, L., Chermiti, B., Abbes K, Biondi A, Zappalà L, C.B., 2013. Fortuitous parasitoids of the invasive tomato leafminer *Tuta absoluta* in Tunisia. Phytoparasitica 42, 85–92. https://doi.org/10.1007/s12600-013-0341-x

Aigbedion-Atalor, P.O., Abuelgasim, S., Hill, M.P., Zalucki, M.P., Azrag, A.G.A., Srinivasan, R., Ekesi, S., 2020. Host stage preference and performance of *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae), a candidate for classical biological control of *Tuta absoluta* in Africa. Biol. Control 144, 104215. https://doi.org/10.1016/j.biocontrol.2020.104215

Aigbedion-Atalor, P.O., Hill, M.P., Ayelo, P.M., Ndlela, S., Zalucki, M.P., Mohamed, S.A., 2021. Can the combined use of the mirid predator *Nesidiocoris tenuis* and a braconid larval endoparasitoid *Dolichogenidea gelechiidivoris* improve the biological control of *Tuta absoluta*? Insects 12, 1–12. https://doi.org/10.3390/insects12111004

Aigbedion-Atalor, P.O., Hill, M.P., Zalucki, M.P., Obala, F., Idriss, G.E., Midingoyi, S.K., Chidege, M., Ekesi, S., Mohamed, S.A., 2019. The South America tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), spreads its wings in Eastern Africa: distribution and socioeconomic impacts. J. Econ. Entomol. 112, 2797–2807. https://doi.org/10.1093/jee/toz220

Bajonero, J., Córdoba, N., Cantor, F., Rodríguez, D., Cure, R., 2008. Biology and life cicle of *Apanteles gelechiidivoris* (Hymenoptera: Braconidae) parasitoid of *Tuta absoluta*. Agron. Colomb. 26, 417–426.

Bennett, F.D., 1993. Do introduced parasitoids displace native ones? Florida Entomol. 76, 54–63.

Biondi, A., Guedes, R., Wan, F., Desneux, N., 2018. Ecology, worldwide spread, and management of the invasive South American Tomato Pinworm, *Tuta absolut*a: Past, present, and future. Annu. Rev. Entomol. 63, 239–258. https://doi.org/10.1146/annurev-ento-031616Cabello, T., Gámez, M., Torres, A., Garay, J., 2011. Possible effects of inter-specific competition on the coexistence of two parasitoid species: *Trichogramma brassicae* Bezdenko and *Chelonus oculator* (F.) (Hymenoptera: Trichogrammatidae, Braconidae). Community Ecol. 12, 78–88. https://doi.org/10.1556/ComEc.12.2011.1.10

Chailleux, A., Desneux, N., Arnó, J., Gabarra, R., 2014. Biology of two key Palaearctic larval ectoparasitoids when parasitizing the invasive pest *Tuta absoluta*. J. Pest Sci. (2004). 87, 441–448. https://doi.org/10.1007/s10340-014-0557-7

Chailleux, A., Droui, A., Bearez, P., Desneux, N., 2017. Survival of a specialist natural enemy experiencing resource competition with an omnivorous predator when sharing the invasive prey *Tuta absoluta*. Ecol. Evol. 7, 8329–8337. https://doi.org/10.1002/ece3.3396

Chepchirchir, F., Muriithi, B.W., Langat, J., Mohamed, S.A., Ndlela, S., Khamis, F.M., 2021. Knowledge, attitude, and practices on tomato leaf miner, *Tuta absoluta* on tomato and potential demand for integrated pest management among smallholder farmers in Kenya and Uganda. Agric. 11, 1242. https://doi.org/10.3390/agriculture11121242

Chermiti, B., Abbes, K., Aoun, M., Ben, S., Ouhibi, M., Gamoon, W., Kacem, S., 2009. First estimate of the damage of *Tuta absoluta* (Povolny) (Lepidoptera: Gelechiidae) and evaluation of the efficiency of sex pheromone traps in greenhouses of tomato crops in the Bekalta Region, Tunisia. African J. Plant Sci. Biotechnol. 3, 49–52.

Dannon, E.A., Tamo, M., Huis, A. van, Dicke, M., 2010. Functional response and life history parameters of *Apanteles taragamae*, a larval parasitoid of *Maruca vitrata*. BioControl 55, 363–378. https://doi.org/10.1007/s10526-009-9263-4

Desneux, N., Wajnberg, E., Kris, W.A.G., Burgio, G., Arpaia, S., Narváez-Vasquez, C.A., González-Cabrera, J., Ruescas, D.C., Tabone, E., Frandon, J., Pizzol, J., Poncet, C., Cabello, T., Urbaneja, A., 2010. Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, geographic expansion and prospects for biological control. J. Pest Sci. (2004). 83, 197–215. https://doi.org/10.1007/s10340-010-0321-6

Desneux, N., Han, P., Mansour, R., Arnó, J., Brévault, T., Campos, M.R., Chailleux, A., Guedes, R.N.C., Karimi, J., Konan, K.A.J., Lavoir, A. violette, Luna, M.G., Perez-Hedo, M., Urbaneja, A., Verheggen, F.J., Zappalà, L., Abbes, K., Ali, A., Bayram, Y., Cantor, F., Cuthbertson, A.G.S., De Vis, R., Erler, F., Firake, D.M., Haddi, K., Hajjar, M.J., Ismoilov, K., Jaworski, C.C., Kenis, M., Liu, H. tian, Madadi, H., Martin, T., Mazih, A., J. Messelink, G., Mohamed, S.A., Nofemela, R.S., Oke, A., Ramos, C., Ricupero, M., Roditakis, E., Shashank, P.R., Wan, F.H., Wang, M. hui, Wang, S., Zhang, Y.B., Biondi, A., 2022. Integrated pest management of *Tuta absoluta*: practical implementations across different world regions. J. Pest Sci. (2004). 95, 17–39. https://doi.org/10.1007/s10340-021-01442-8

Fernandez-Triana, J., Shaw, M.R., Boudreault, C., Beaudin, M., Broad, G.R., 2020. Annotated and illustrated world checklist of Microgastrinae parasitoid wasps (Hymenoptera, Braconidae). Zookeys 920, 1–1089. https://doi.org/10.3897/zookeys.920.39128

Guedes, R.N.C., Roditakis, E., Campos, M.R., Haddi, K., Bielza, P., Siqueira, H.A.A., Tsagkarakou, A., Vontas, J., Nauen, R., 2019. Insecticide resistance in the tomato pinworm *Tuta absoluta*: patterns, spread, mechanisms, management and outlook. J. Pest Sci. (2004). 92, 1329–1342. https://doi.org/10.1007/s10340-019-01086-9

Han, P., Bayram, Y., Shaltiel-Harpaz, L., Sohrabi, F., Saji, A., Esenali, U.T., Jalilov, A., Ali, A., Shashank, P.R., Ismoilov, K., Lu, Z. zhi, Wang, S., Zhang, G. fen, Wan, F. hao, Biondi, A., Desneux, N., 2019. *Tuta absoluta* continues to disperse in Asia: damage, ongoing management and future challenges. J. Pest Sci. (2004). 92, 1317–1327. https://doi.org/10.1007/s10340-018-1062-1

Idriss, G.E.A., Mohamed, S.A., Khamis, F., Du Plessis, H., Ekesi, S., 2018. Biology and performance of two indigenous larval parasitoids on *Tuta absoluta* (Lepidoptera: Gelechiidae) in Sudan. Biocontrol Sci. Technol. 28, 614–628. https://doi.org/10.1080/09583157.2018.1477117

Karlsson, F.M., Rachidatou, S., Sahadatou, M.S., Joseph, Z.A., Georg, G., 2018. First report of *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) in the Republic of Benin. BioInvasions Rec. 7, 463–468. https://doi.org/https://doi.org/10.3391/bir.2018.7.4.19

Kenis, M., Hurley, B.P., Colombari, F., Lawson, S., Sun, J., Wilcken, C., Weeks, R., Sathyapala, S., 2019. Guide to the classical biological control of insect pests in planted and natural forests, FAO Forestry Paper No. 182. Rome, FAO.

Kinyanjui, G., Khamis, F.M., Ombura, F.L.O., Kenya, E.U., Ekesi, S., Mohamed, S.A., 2021. Distribution, abundance and natural enemies of the invasive tomato leafminer, *Tuta absoluta* (Meyrick) in Kenya. Bull. Entomol. Res. 111, 1–16. https://doi.org/10.1017/S0007485321000304

Mama Sambo, S., Ndlela, S., du Plessis, H., Obala, F., Mohamed, S.A., 2022a. Ratio dependence effects of the parasitoid *Dolichogenidea gelechiidivoris* on its associated host *Tuta absoluta*. Biocontrol Sci. Technol. 32, 497–510. https://doi.org/10.1080/09583157.2022.2033702

Mama Sambo, S., Ndlela, S., du Plessis, H., Obala, F., Mohamed, S.A., 2022b. Identification, microhabitat, and ecological niche prediction of two promising native parasitoids of *Tuta absoluta* in Kenya. Insects 16, 496. https://doi.org/https://doi.org/10.3390/ insects13060496

Mansour, R., Brévault, T., Chailleux, A., Cherif, A., Grissa-Lebdi, K., Haddi, K., Mohamed, S.A., Nofemela, R.S., Oke, A., Sylla, S., Tonnang, H.E.Z., Zappalà, L., Kenis, M., Desneux, N., Biondi, A., 2018. Occurrence, biology, natural enemies and management of *Tuta absoluta* in Africa. Entomol. Gen. 38, 83–112. https://doi.org/10.1127/entomologia/2018/0749

Mansour, R., Biondi, A., 2021. Releasing natural enemies and applying microbial and botanical pesticides for managing *Tuta absoluta* in the MENA region. Phytoparasitica 49, 179–194. https://doi.org/10.1007/s12600-020-00849-w

Morales, J., Muñoz, L., Rodríguez, D., Cantor, F., 2014. Combined Action of Sex Pheromone and Wasp *Apanteles gelechiidivori*s in Greenhouse Tomato Crops. Acta Biol. Colomb. 19, 175–184.

R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. URL https//www.R-project.org/.

Roditakis, E., Vasakis, E., Grispou, M., Stavrakaki, M., Nauen, R., Gravouil, M., Bassi, A., 2015. First report of *Tuta absoluta* resistance to diamide insecticides. J. Pest Sci. (2004). 88, 9–16. https://doi.org/10.1007/s10340-015-0643-5

Rwomushana, I., Day, R., Gonzalez-Moreno, P., 2019. Evidence note Tomato leafminer impacts and coping strategies for Africa. https://doi.org/https://dx.doi.org/10.1079/CABICOMM-62-8100

Savino, V., Luna, M.G., Gervassio, N.G.S., Coviella, C.E., 2016. Interspecific interactions between two *Tuta absoluta* (Lepidoptera: Gelechiidae) larval parasitoids with contrasting life histories. Bull. Entomol. Res. 107, 32–38. https://doi.org/10.1017/S0007485316000547

Shiraku, H., 2020. ICIPE Makes the first release of parasitoids into the farm in fight against *Tuta absoluta*. Infonet-Biovision. https://infonet-biovision.org/News/ICIPE-Makes-first-release-parasitoids-farm-fight-against-Tuta-Absoluta

Sorribas, J., Rodríguez, R., Garcia-Mari, F., 2010. Parasitoid competitive displacement and coexistence in citrus agroecosystems: linking species distribution with climate. Ecol. Appl. 20, 1101–1113.

Tumuhaise, V., Khamis, F.M., Agona, A., Sseruwu, G., Mohamed, S.A., 2016. First record of *Tuta absoluta* (Lepidoptera: Gelechiidae) in Uganda. Int. J. Trop. Insect Sci. 36, 135–139. https://doi.org/10.1017/S1742758416000035

Ulyshen, M.D., Duan, J.J., Bauer, L.S., 2010. Interactions between *Spathius agrili* (Hymenoptera: Braconidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), larval parasitoids of *Agrilus planipennis* (Coleoptera: Buprestidae). Biol. Control 52, 188–193. https://doi.org/10.1016/j.biocontrol.2009.10.017

van Alebeek, F.A.N., Rojas-Rousse, D., Leveque, L., 1993. Interspecific competition between *Eupelmus vuilleti* and *Dinarmus basalis*, two solitary ectoparasitoids of Bruchidae larvae and pupae. Entomol. Exp. Appl. 69, 21–31. https://doi.org/10.1111/j.1570-7458.1993.tb01724.x van Lenteren, J.C., Lanzoni, A., Hemerik, L., Bueno, V.H.P., Bajonero Cuervo, J.G., Biondi, A., Burgio, G., Calvo, F.J., de Jong, P.W., López, S.N., Luna, M.G., Montes, F.C., Nieves, E.L., Aigbedion-Atalor, P.O., Riquelme Virgala, M.B., Sánchez, N.E., Urbaneja, A., 2021. The pest kill rate of thirteen natural enemies as aggregate evaluation criterion of their biological control potential of *Tuta absoluta*. Sci. Rep. 11, 1–13. https://doi.org/10.1038/s41598-021-90034-8

Wang, X.G., Bokonon-Ganta, A.H., Messing, R.H., 2008. Intrinsic inter-specific competition in a guild of tephritid fruit fly parasitoids: Effect of co-evolutionary history on competitive superiority. Biol. Control 44, 312–320. https://doi.org/10.1016/j.biocontrol.2007.10.012

Youssef, R.M., Abo Kaf, N.H., Aboud, R., 2022. *Stenomesius japonicus* (Ashmead) (Hymenoptera: Eulophidae): a new parasitoid of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Syria. J. Insect Biodivers. Syst. 8, 219–227. https://doi.org/10.52547/jibs.8.2.219

CHAPTER 6

Effects of the interaction between *Tuta absoluta* parasitoids: the exotic *Dolichogenidea gelechiidivoris* and the native *Bracon nigricans*

Citation:

Sahadatou Mama Sambo, S., Ndlela, S., du Plessis, H., Owino, E.A. and Mohamed, S.A. Effects of the interaction between *Tuta absoluta* parasitoids: the exotic *Dolichogenidea gelechiidivoris* and the native *Bracon nigricans*.

Submitted to International Journal of Tropical Insect Science (Springer)

Effects of the interaction between *Tuta absoluta* parasitoids: the exotic *Dolichogenidea gelechiidivoris* and the native *Bracon nigricans*

Sahadatou Mama Sambo[1,2*], Shepard Ndlela[1], Hannalene du Plessis[2], Esther Awuor Owino[1], Samira Abuelgasim Mohamed[1*]

[1]International Centre of Insect Physiology and Ecology (*icipe*), Nairobi P.O. Box 30772-00100, Kenya.

[2]Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa.

Corresponding author*: smama@icipe.org P.O. BOX 30772-00100, Nairobi, Kenya.

Authors ORCID numbers

Sahadatou Mama Sambo: 0000-0002-4470-5622

Shepard Ndlela: 0000-0003-0015-9946

Hannalene Du Plessis: 0000-0003-1163-1468

Samira Abuelgasim Mohamed: 0000-0001-6836-7528

Abstract

The coexistence and efficiency in pest control of introduced and native parasitoids can be challenging. Continuous observations of cohabitation of parasitoid species could confirm the persistence of the introduced parasitoid in the ecosystem under co-existence scenarios. This study provides an example of such a co-existence for biocontrol of the invasive pest, *Tuta* *absoluta* (Merick) (Lepidoptera: Gelechiidae). Two parasitoids, the introduced endoparasitoid *Dolichogenidea gelechiidivoris* (Marsh) (Hymenoptera: Braconidae) and the native ectoparasitoid *Bracon nigricans* Szépligeti (all Hymenoptera: Braconidae) were released in cages containing a tomato plant infested with *T. absoluta*. Parasitism and killing rate of *T. absoluta* by both parasitoid species, and the parasitoid and *T. absoluta* population were monitored weekly. The parasitoid species coexisted for seven weeks in the experimental units. Parasitism by *D. gelechiidivoris* was significantly affected by the presence of *B. nigricans*, with 73% and 22% parasitism in the absence and presence of *B. nigricans*, respectively. Parasitism by *B. nigricans* was not affected by its co-existence with *D. gelechiidivoris*. The number of *D. gelechiidivoris* adults increased to 156 in five weeks in the absence of *B. nigricans*, while only eight adults were present in co-existence with *B. nigricans*. The *T. absoluta* infestation declined from the fifth week to ~ 15 mines where the pest was exposed to *D. gelechiidivoris* and *B. nigricans* as standalone treatments, as well as in combination. Since *B. nigricans* negatively affected *D. gelechiidivoris* population growth, releases of this introduced parasitoid should be considered with caution in areas where *B. nigricans* occurs.

Keywords population dynamic, co-existence, ectoparasitoid, endoparasitoid, pest control

Competing Interests We declare that this study has no conflict of interest.

Fundings This research was supported by the following organizations: Biovision Foundation grant number BV DPP-012/2019-2022, Norwegian Agency for Development Cooperation-(NORAD), the section for research, innovation and higher education grant number RAF-3058 KEN-18/0005, Scaling-up Climate-Smart Pest Management Approaches for Enhanced Maize and Tomato Systems Productivity in Eastern Africa (SCLAMP) grant number 81250394, the Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); the Federal Democratic Republic of Ethiopia; and the

Government of the Republic of Kenya. The first author was supported by the German Academic Exchange Service (DAAD) through the African Regional Postgraduate Programme in Insect Science (ARPPIS) tenable at *icipe*. The views expressed herein do not necessarily reflect the official opinion of the donors.

Acknowledgments The authors acknowledge Alex Muthembwa, Omar Jimali Ibrahim, Peterson Nderitu and all the African Fruit Fly Program (AFFP) colleagues for their technical support

Introduction

Interaction between species occurs when more than one species attack the same host species, affecting the species distribution and population dynamics (Xu et al. 2013; Holt and Bonsall 2017). Interactions between species arise during adult host searching or during development of the immature stages of the parasitoids inside the same host (Bográn et al. 2002; Cusumano et al. 2013, 2016; Xu et al. 2013). The outcomes of such an interference can affect the ecosystem in different ways such as the reduction or extinction of a specific species, which affects pest control in terms of the depletion, displacement or extinction of natural enemies (Reitz and Trumble 2002; Wang et al. 2008; Feng et al. 2015; Tan et al. 2016). For example, in a situation where the host is attacked by an ectoparasitoid and is also parasitized by an endoparasitoid wasp, the progeny of the endoparasitoid wasp could subsequently be killed with the hosts (Feng et al. 2015). It is therefore important to understand the possible effects that different parasitoids can have, when planning a biological control program that involves more than one parasitoid species (Mills 1992; Cusumano et al. 2016).

Since the invasion of *T. absoluta*, numerous parasitoids associated with this pest, were discovered in invaded countries (Tropea Garzia et al. 2012; Zappalà et al. 2012, 2013; Abbes et al. 2013; Naselli et al. 2017; Mansour et al. 2018; Ferracini et al. 2019; Salas Gervassio et al. 2019). Competition between natural enemies for control of this pest has been reported in various studies. Chailleux et al. (2017) demonstrated improved control of *T. absoluta* when *Stenomesius japonicus* Ashamed (Hymenoptera: Eulophidae) was released together with *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae). The efficacy of *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) as a predator of *T. absoluta* was reported by Mirhosseini et al. (2019) who found the highest tomato yield in a trial where *N. tenuis* was present alone or in combination with the egg parasitoid, *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae). When tested in combination, females of both the ectoparasitoid, *Dineulophus phthorimaeae* De Santis (Hymenoptera: Eulophidae) and the endoparasitoid, *Pseudapanteles dignus* (Muesebeck) (Hymenoptera: Braconidae) changed their searching behaviour for *T. absoluta* in the presence of the competing species. The efficacy of *P. dignus* was, however, not affected by the presence of the ectoparasitoid and it achieved the same parasitism rate whether the competitor was present or absent (Savino et al. 2016).

Bracon nigricans Szépligeti (Hymenoptera: Braconidae) is a gregarious, generalist idiobiont larval ectoparasitoid (Yu and Actherberg 2010) recorded in *T. absoluta* in several invaded areas. For example, its association with *T. absoluta* was recorded in Italy, Jordan, Spain and Sudan (Al-Jboory et al. 2012; Zappalà et al. 2012; Gabarra et al. 2013; Idriss et al. 2018). This parasitoid was amongst the three most abundant parasitoids recorded in Italy. It was reported to co-occur with other larval parasitoid species such as *Diadegma pulchripes* (Hymenoptera: Ichneumonidae), *Elachertus inunctus*, *Necremnus* sp., *Neochrysocharis formosa* (Hymenoptera: Eulophidae) and *Elasmus* sp. (Hymenoptera: Elasmidae). In Spain, *B. nigricans* was reported to co-occur with *Hockeria unicolor* Walker (Hymenoptera: Chalcididae), *Pnigalio cristatus* (Ratzeburg) and *Neochrysocharis formosa* (Westwood) (Hymenoptera: Braconidae), while a *Cotesia* sp., and *Dolichogenidea litae* (Nixon, 1972)

(Hymenoptera: Braconidae) has also been recorded in a nearby locality during a previous year (Gabarra et al. 2013). *Bracon nigricans* has also recently been recorded in Kenya, and several areas in Africa are suitable for persistence of this parasitoid (Mama Sambo et al. 2022b). Approximately 21% parasitism of *T. absoulta* by *B. nigricans* was reported from open field tomato in central Kirinyaga county, Kenya where the exotic *D. gelechiiidivoris* was released (Shiraku 2020; Mama Sambo et al. 2022b).

Dolichogenidea gelechiidivoris is a specialist solitary endoparasitoid of some Gelechiidae species, including *T. absoluta* (Bajonero et al. 2008; Mujica and Kroschel 2017; Aigbedion-Atalor et al. 2020; Mama Sambo et al. 2022c). The parasitism rate by this parasitoid under laboratory conditions varied between 55% and 87% (Bajonero et al. 2008; Aigbedion-Atalor et al. 2020; Mama Sambo et al. 2022a,c), depending on the host and parasitoid density, as well as *T. absoluta* larval stage. In open-field tomato, *Tuta absoluta* parasitism rates of 59% to 77% by this parasitoid, was reported in Colombia (Valencia and Penaloza, 1990; Agudelo and Kaimowitz, 1997; Vallejo, 1999). In the central Coast of Peru, *D. gelechiidivoris* was reported as the most widespread parasitoid of *T. absoluta*, with parasitism rates of 41% and 57% reported by Palacios and Cisneros (1995) for tomato production with and without chemical pesticides use, respectively. *Dolichogenidea gelechiidivoris* established in Chile 10 years after its release (Desneux et al. 2010). In Kenya, 7% and less than 5% parasitism of *T. absoluta* larvae were recorded respectively in greenhouses and open-fields, five months after the release of *D. gelechiidivoris* (Mama Sambo et al. unpublished data). However, *D. gelechiidivoris* has also been recovered from non-release areas in Europe and Africa (Krache et al. 2021; Denis et al. 2022).

Bracon nigricans has a very high ability to kill its host (Biondi et al. 2013; Becchimanzi et al. 2017, 2020). This parasitoid prefers 4th-instar *T. absoluta* larvae (Idriss et al. 2018), while *D. gelechiidivoris* prefers 1st and 2nd-instar larvae of this host (Aigbedion-Atalor et al. 2020).

Parasitism by *D. gelechiidivoris* in Kenya was reported to be very low in the release area, where *B. nigricans* was recovered (Shiraku 2020; Mama Sambo et al. 2022b). The aim of this study was to investigate the population dynamics of *T. absoluta* and two of its parasitoid species, *B. nigricans* and *D. gelechiidivoris* in laboratory bioassays, when present alone or in combination.

Materials and methods

Host plants

Tomato (cv. Moneymaker) seedlings were planted in plastic pots (14 × 14 × 9 cm) containing soil mixed with goat manure. The seedlings were grown in a greenhouse following the procedures described by Mama Sambo et al. (2022c).

Insects rearing

Tuta absoluta

Tomato plants with *T. absoluta* larvae and eggs were collected from farmers' plots in the Kirinyaga County (00°37'196" S, 37°22'615" E, Elevation ≈ 1200 msl and 00°37'922" S, 37°22'794" E, Elevation ≈ 1200), Kenya to initiate a colony. The infested plants were incubated and *T. absoluta* obtained from these plants was reared on healthy tomato plants following the procedure described by Mama Sambo et al. (2022c) (see chapter 3).

Dolichogenidea gelechiidivoris

Dolichogenidea gelechiidivoris cocoons were kept in a Perspex cage (40cm x 20cm x 40cm) with 80% honey solution droplets provided on the inside of the top of the cage for the emerged parasitoids to feed on. The adults were kept isolated in the Animal Rearing and Containment Unit (ARCU) at *icipe* and reared on *T. absoluta* larvae according to the protocol described by Mama Sambo et al. (2022c) (see chapter 3).

Bracon nigricans

The *B. nigricans* colony was initiated from parasitoids that emerged from infested tomato plant material collected from Mwea (00°37'196" S, 37°22'615" E, Elevation ≈ 1200 msl and 00°37'922" S, 37°22'794" E, Elevation ≈ 1200 msl), Kirinyaga, Kenya. The infested leaves were kept in a transparent plastic lunch box. Upon adult parasitoid emergence, insects were aspirated into a clean Perspex cage (40cm x 20cm x 50cm) and provided with 80% honey solution droplets applied on the top of the cage. The insects were maintained under ambient laboratory conditions (25 ± 1°C, 70 ± 5 RH, and 12:12 L:D of photoperiod) in the Animal Rearing and Containment Unit (ARCU) at *icipe*. Third instar *T. absoluta* larvae in tomato plant leaves were exposed to *B. nigricans* adults for 48h. Thereafter, the tomato leaves were transferred to another Perspex cage until *B. nigricans* and *T. absoluta* emerged. Two days-old females (F3) were used in this experiment.

Experimental setup

The interaction between *D. gelechiidivoris* and *B. nigricans* was investigated under laboratory conditions (25 ± 1°C, 70 ± 5 RH, and 12:12 L:D of photoperiod). The experiment was conducted in cages (30 cm (W) × 30 cm (H) × 30 cm (W)) and consisted of four treatments. Initially one uninfested potted tomato plant (3-weeks old) was placed in each cage. Ten newly emerged naïve *T. absoluta* male-female moth pairs were released per cage. Drops of an 80% honey solution were streaked onto the inner top part of the cage as food for the moths. Four treatments were applied: 1) release of 10 *D. gelechiidivoris* pairs into a cage where *T. absoluta* moths were released five days previously; 2) release of 10 *B. nigricans* pairs into a cage where *T. absoluta* moths were released 10 days previously; 3) release of five pairs of *D. gelechiidivoris* pairs into a cage where *T. absoluta* moths were released five days previously, followed by the release of five *B. nigricans* pairs into the same cage, five days after release of *D. gelechiidivoris*; 4) a control treatment with a cage containing only *T.*

absoluta with no parasitoids released into the cage. Each treatment was replicated 10 times. For each treatment, an additional potted plant was placed into each cage at three-day intervals for the entire duration of the study and supplemented daily with fresh tomato leaves to ensure that enough food was available for *T. absoluta* larvae.

Data collection

Parasitism by the two species

To determine the level of parasitism, 10 *T. absoluta* larvae were removed from plants in each cage, two weeks after release of *B. nigricans* and dissected under a stereomicroscope (Leica EZ4D digital stereomicroscope; Leica Microsystems, Heerbrugg, Switzerland). Additionally, dead *T. absoluta* larvae inside mines were recorded in each of the treatments.

Population dynamics of the different insect species

The number of *T. absoluta* mines and eggs were determined by counting the number of eggs and mines in each leaflet on the plant every week from the third week of monitoring. In addition, the number of *D. gelechiidivoris* and *B. nigricans* parasitoids that emerged were counted. The experiment was conducted over a period of 10 weeks.

Data analysis

A Generalised Linear Model (GLM) with negative binomial was used to determine the difference in percentage parasitism by *D. gelechiidivoris* and *B. nigricans* of dissected larvae, as well as the number of larvae killed by *B. nigricans*. The numbers of *B. nigricans* and *D. gelechiidivoris* adults and mines per treatment were analysed using a mixed effects model with repeated measures, using *lmer* function under the lme4 package. All the data was analysed in R (R Core Team 2018).

Results

Parasitism by the two species

The level of *D. gelechiidivoris* parasitism varied significantly, depending on the presence or the absence of *B. nigricans* ($F_{1, 18} = 26.69$, $P < 0.001$). Significantly more larvae were parasitised by *D. gelechiidivoris,* when it was present alone, compared to the level of parasitism when *T. absoluta* larvae were exposed to both *D. gelechiidivoris* and *B. nigricans* (Fig. 1a). However, no difference in parasitism by *B. nigricans* was observed regardless of the presence or absence of *D. gelechiidivoris* ($F_{1, 18} = 1$, $P = 0.33$). Similarly, release of *B. nigricans* after *D. gelechiidivoris* was already present, did not increase *T. absoluta* larval mortality ($F_{1, 18} = 0.35$, $P = 0.56$). Although the maximum parasitism by *B. nigricans* (10%) (Fig. 1b) was lower than that by *D. gelechiidivoris* (73%) (Fig. 1a), its parasitism was not affected by the presence of *D. gelechiidivoris* (Fig. 1b).

Population dynamics of the different insect species

The treatments with an absolute t-value >2 affected the parameters significantly (Table 1). The *D. gelechiidivoris* population (t = 5.70) was negatively affected by the association with *B. nigricans* while no significant effect on the *B. nigricans* population was observed by the co-occurrence of the two parasitoids species (Table 1). Where the *D. gelechiidivoris* population was kept separate, the number of adults present was high, with an average of 156 parasitoids five weeks after introduction. In co-occurrence with *B. nigricans* only 8 *D. gelechiidivoris* individuals survived at week 5 (Fig. 2). The number of *T. absoluta* progeny expressed as the number of mines and eggs, was significantly higher compared to all other treatments with parasitoids present in the respective combinations, five weeks after release of the *T. absoluta* moths (t = 2.72) (Table 1). At week 4, the pest level significantly decreased where both *B. nigricans* occurred alone and also where the combination of *B. nigricans* and *D. gelechiidivoris* occurred (Fig. 3). From week 5, significantly lower pest levels occurred in all treatments, except for the control (Fig. 3).

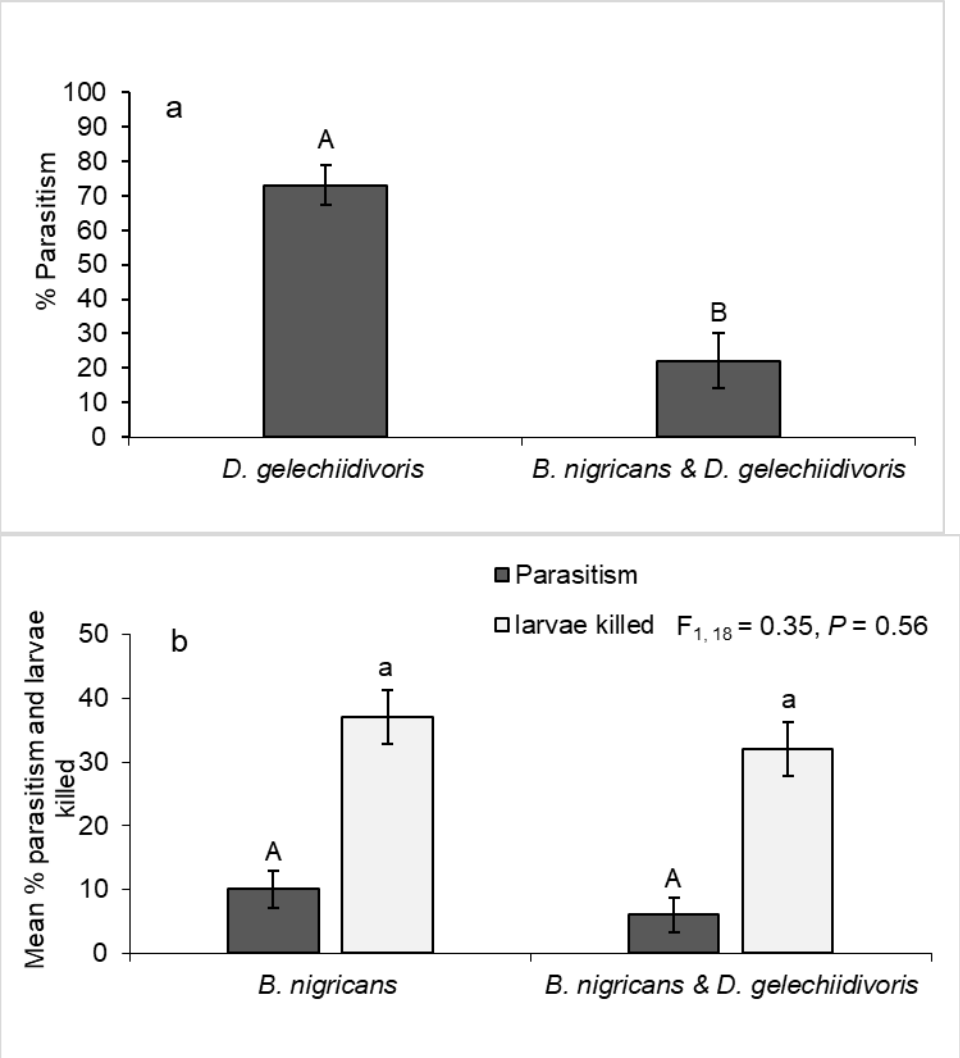


Fig. 1 (a) Mean percentage (±SE) *T. absoluta* larvae parasitised by *D. gelechiidivoris* and (b) Mean percentage *T. absoluta* larvae parasitised and killed by *B. nigricans* when released as a standalone treatment as well as in combination with *D. gelechiidivoris*. Bars capped with the same upper or lower letters are not significantly different (*Tukey's HSD*, $p < 0.05$).

Table 1 Parameter estimates for the effect of parasitoid combinations in terms of *D. gelechiidivoris* and *B. nigricans* population growth and *T. absoluta* infestation.

		Estimate	Std. Error	t-value
Number of *D. gelechiidivoris*	(Intercept)	5.08	14.91	0.34
	D. gelechiidivoris	41.62	7.31	5.70
Number of *B. nigricans*	(Intercept)	6.36	3.26	1.95
	B. nigricans and *D. gelechiidivoris*	3.46	1.94	1.79
Number of *T. absoluta* progenies (eggs and larvae)	(Intercept)	41.80	28.47	1.47
	B. nigricans and *D. gelechiidivoris*	-8.66	32.45	-0.27
	D. gelechiidivoris	23.44	34.72	0.67
	Control (no parasitoids)	482.66	177.39	2.72

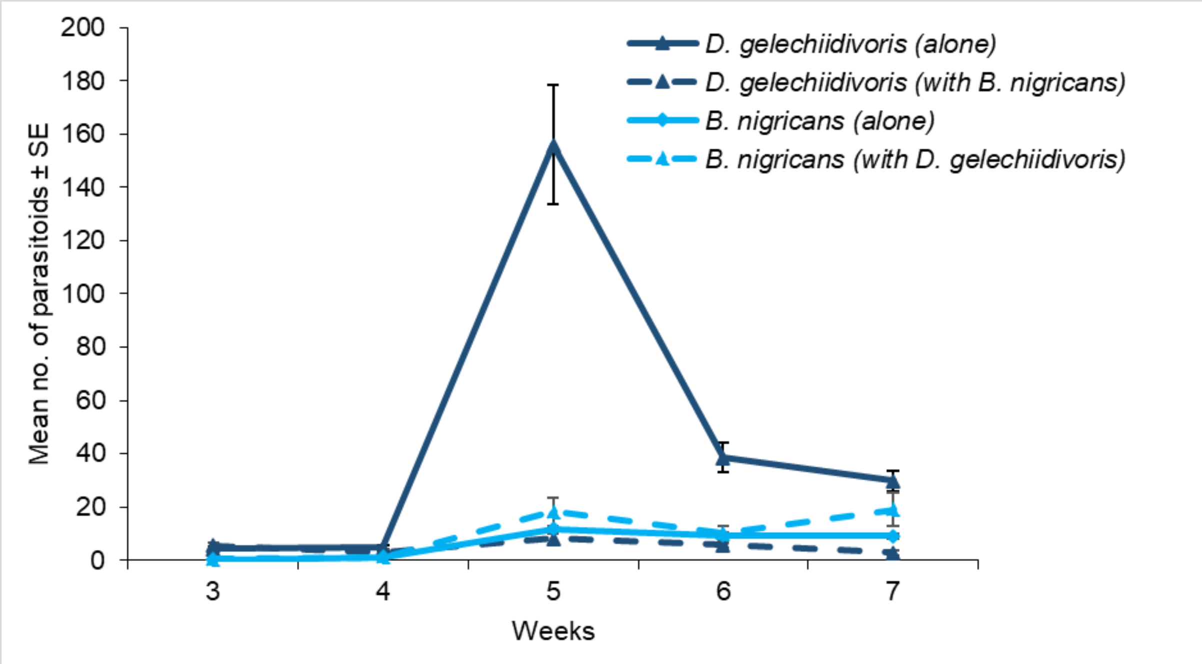


Fig. 2 Mean number (±SE) of *D. gelechiidivoris* and *B. nigricans* adults, per week in the respective treatments over time.

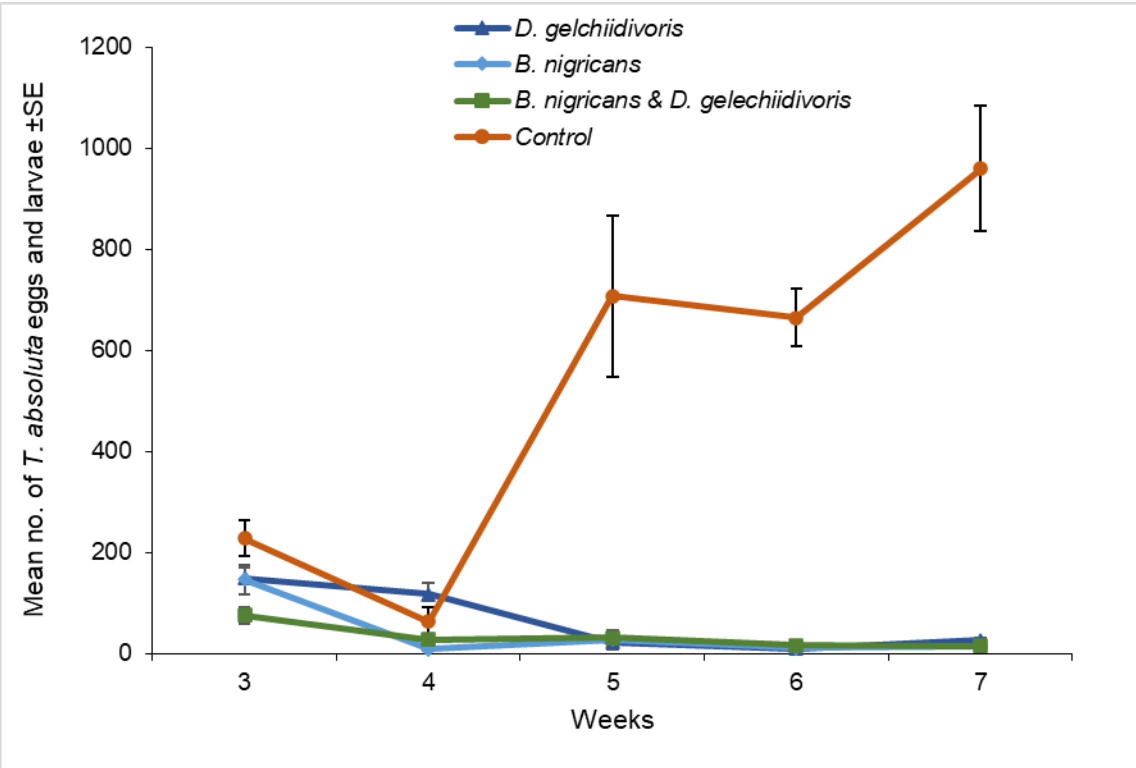


Fig. 3 Mean number (±SE) of *T. absoluta* progeny per week in the respective treatments over time.

Discussion

The efficacy of control of *T. absoluta* by two parasitoids, *viz*. the imported endoparasitoid, *D. gelechiidivoris* and the endogenous ectoparasitoid, *B. nigricans* was confirmed in this laboratory study. The negative impact of co-occurrence of these two species on *D. gelechiidivoris* was also demonstrated. Multiple parasitism by parasitoid species frequently occur in the absence of interspecific host discrimination (van Alphen and Visser 1990; Hassell and Godfray 1994), and causes competition by the offspring of parasitoid species within a host. It also affects the population growth of a specific parasitoid if, as in most cases with ectoparasitoids, the female kills the host (Biondi et al. 2013; Chailleux et al. 2014; Idriss et al. 2018). Consequently, the chances of survival of a host is low when initially parasitized by an endoparasitoid and subsequently by an ectoparasitic, resulting in the larvae of the endoparasitoid being instantly killed together with the host (Rosenheim et al. 1995). This might be the case with *B. nigricans*, an ectoparasitoid (Biondi et al. 2013; Becchimanzi et al. 2017) when present in co-occurrence with the endoparasitoid, *D. gelechiidivoris* (Fernandez-Triana et al. 2020). *Bracon nigricans* is abundant in Kenya (Mama Sambo et al. 2022b), where *D. gelechiidivoris* was also released (Shiraku 2020). The level of parasitism by *D. gelechiidivoris* in the area investigated in this study, was low in comparison to parasitism levels when released in a greenhouse in the absence of *B. nigricans* (Mama Sambo et al. Unpublished data).

Tuta absoluta larvae were effectively controlled by *D. gelechiidivoris* when exposed to this species alone (parasitism level of 73%). It was in contrast to the level of parasitism by *B. nigricans* of only 10%, when exposed under the same conditions. Previously, parasitism levels of 87% (Mama Sambo et al. 2022a), 58% (Mama Sambo et al. 2022c), and 55% (Aigbedion-atalor et al. 2020), were reported when1st-instar *T. absoluta* larvae were exposed to female *D. gelechiidivoris* for 24 hours. Biondi *et al*. (2013) and Idriss et al. (2018) reported that *B. nigricans* parasitised 4th-instar *T. absoluta* larvae, and that only one or two parasitoids emerged from these larvae per day. Parasitism by *B. nigricans*, therefore, appears to be insignificant compared to parasitism by *D. gelechiidivoris*. However, Idriss et al. (2018), reported 54% parasitism of 4th instar larvae under laboratory conditions, and Mama Sambo et al. (2022b), up to 21% parasitism of *T. absoluta* larvae by this parasitoid species in a recent field studies. It emphasises the effect of other factors such as the host:parasitoid ratio and host stage available that can significantly affect the level of parasitism. A reduction of approximately 50% in *D. gelechiidivoris* parasitism was recorded in this study when this species co-occurred with *B. nigricans.* A highly negative impact on the population growth and resultant parasitism level and control of *T. absoluta* larvae by *D. gelechiidivoris* can therefore be expected in areas where *B. nigricans* is abundant.

Bracon nigricans did not discriminate against the larvae already parasitised by the endoparasitoid, *D. gelechiidivoris*. Ectoparasitoids are in general also better competitors than endoparasitoids, and their female's venom often paralyze the immature stages of the endoparasitoid already present in the host, as well as the host itself (Harvey et al. 2013). However, Savino et al. (2016) reported that the ectoparasitoid, *D. phthorimaeae* spent more time in general host searching of *T. absoluta* when in competition with the endoparasitoid *P. dignus*, while the presence of the ectoparasite did not have any effect in this regard on *P. dignus*. From the perspective of insect biological control, a superior parasitoid species must have a shorter developmental time, greater searching capability, high host specificity, a positive correlation with host density, good synchronization between the populations of the host and parasitoid as well as a good dispersal ability (Haye et al. 2008). The approximate female developmental time and longevity of *B. nigricans* is 12 days and 43 days, respectively at 24 ± 1 °C, 60 ± 10% relative humidity (RH), and a photoperiod of 14 L:10D (Biondi et al. 2013) and at 25 ± 0.5 °C and 16D:8L photoperiod (Idriss et al. 2018). The developmental time of *D. gelechiidivoris* is approximately 19 days and its longevity, 5 days at 26 °C (Bajonero et al. 2008). Aigbedion-Atalor et al. (2020) reported female *D. gelechiidivoris* developmental time of 25 days and longevity of 9 days at 26 ± 4 °C, 50–70% RH. The development time of *B. nigricans* is therefore shorter than that of *D. gelechiidivoris,* but it lives longer than *D. gelechiidivoris*. The number of *B. nigricans* progeny per day was reported to be fewer than five per female (Biondi et al. 2013; Idriss et al. 2018), while the number of *D. gelechiidivoris* progeny was reported to be host-density dependent (Mama Sambo et al. 2022c). *Bracon nigricans* is a generalist parasitoid, known to attack different lepidopteran families (Loni et al. 2016; Becchimanzi et al. 2017; Aigbedion-Atalor et al. 2019; Mama Sambo et al. 2022b), while the only family known to be attacked by *D. gelechiidivoris* to date, is Gelechiidae (Bajonero et al. 2008; Mujica and Kroschel 2017; Aigbedion-Atalor et al. 2020, 2021; Mama Sambo et al. 2022c).

It should, however, be noted that 32% killing of host larvae could have interfered with the population growth of a competing parasitoid as well as with that of the pest population. Several studies documented the performance of *B. nigricans* on host larval killing. For example, killing of approximately 50% of mature *T. absoluta* larvae by *B. nigricans* were reported by Biondi et al. (2013), while 55% mortality of 3rd- and 40% of 4th-instar *T. absoluta* larvae were reported by Idriss et al. (2018). The high parasitism rates by *D. gelechiidivoris* will consequently affect the population size of *B. nigricans.* Predation by *N. tenuis* of 1st-instar *T. absoluta* larvae was not found to affect the progeny production and adult emergence of *D. gelechiidivoris* (Aigbedion-Atalor et al. 2021), although *N. tenuis* prefers the egg stage of *T. absoluta* (Sylla et al. 2016).

The number of *T. absoluta* mines drastically decreased from the second week after the parasitoids were introduced into the cages where *T. absoluta* larvae were present, both as standalone treatments as well as in combination, to represent the co-occurrence of the two species. Several studies reported a supplemental effect of more than one natural enemy on control of *T. absoluta*. For example, *T. absoluta* larval parasitism by the parasitoids *D. phthorimaeae* and *P. dignus* resulted in high *T. absoluta* mortality in the field (Luna et al. 2015). A complementary effect in control of *T. absoluta* was also reported for the specialist, *S. japonicus* and omnivorous natural enemy *M. pygmaeus* (Chailleux et al. 2017). The combination of *N. tenuis* and *D. gelechiidivoris* provided the highest reduction in *T. absoluta* populations compared to each natural enemy released on its own (Aigbedion-Atalor et al. 2021). Combined used of *N. tenuis* and *T. achaeae* also resulted in better control compared to *N. tenuis* alone (Calvo et al. 2012). The combined use of the parasitoid *T. achaeae*, and the predator *M. caliginosus* also increased *T. absoluta* control (Kortam et al. 2014). However, an antagonistic effect has been reported between *Spathius agrili* Yang (Hymenoptera: Braconidae) and *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) with larvae from the latter being paralyzed after parasitisation. It served as an indicator to *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) to discriminate between *S. agrili* parasitized and non-parasitized larvae (Yang et al. 2013). *Spathius agrili* cannot detect larvae parasitised by *T. planipennisi*, which is disadvantageous to the parasitoid, since its progeny cannot survive on a host previously parasitized by *T. planipennisi* (Ulyshen et al. 2010).

Conclusion

The endoparasitoid *D. gelechiidivoris* and the ectoparasitoid, *B. nigricans* can co-exist by exploiting the same *T. absoluta* larvae differently. However, the presence of *B. nigricans* negatively affected the population growth of *D. gelechiidivoris* while the presence of *D. gelechiidivoris* does not affect *B. nigricans* population growth. Ecological niche difference created by biotic factors such as host plant, host pest preference and abiotic factors, specifically climate, may drive the augmentation of these two parasitoids for control of *T. absoluta*. Additionally, since *B. nigricans* is a generalist parasitoid of several lepidopteran species such as *Spodoptera littoralis* Boisduval (Lepidoptera Noctuidae) (Becchimanzi et al. 2017) and *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera, Tortricidae) (Loni et al. 2016), a minimal effect is expected on the introduced *D. gelechiidivoris* under field condition. However, the host plant species, and host pest species preferences of *B. nigricans* should be studied to determine the optimal conditions for co-existence of the two *T. absoluta* parasitoids.

References

Abbes K, Biondi A, Zappalà L, et al (2013) Fortuitous parasitoids of the invasive tomato leafminer *Tuta absoluta* in Tunisia. Phytoparasitica 42:85–92. https://doi.org/10.1007/s12600-013-0341-x

Agudelo LL, Kaimowitz DD (1997) Tecnología agrícola sostenible: Restos institucionales y metodológicos. In: retos institucionales y metodológicos: dos estudios de caso en Colombia. Agroamerica. 3. p 37 p

Aigbedion-Atalor PO, Abuelgasim S, Hill MP, et al (2020) Host stage preference and performance of *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae), a candidate for classical biological control of *Tuta absoluta* in Africa. Biol Control 144:104215. https://doi.org/10.1016/j.biocontrol.2020.104215

Aigbedion-Atalor PO, Hill MP, Ayelo PM, et al (2021) Can the combined use of the mirid predator *Nesidiocoris tenuis* and a braconid larval endoparasitoid Dolichogenidea gelechiidivoris improve the biological control of *Tuta absoluta*? Insects 12:1–12. https://doi.org/10.3390/insects12111004

Aigbedion-Atalor PO, Hill MP, Zalucki MP, et al (2019) The South America Tomato Leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), Spreads Its Wings in Eastern Africa:

Distribution and Socioeconomic Impacts. J Econ Entomol 112:2797–2807. https://doi.org/10.1093/jee/toz220

Al-Jboory IJ, Katbeh-Bader A, Shakir AZ (2012) First observation and identification of some natural enemies collected from heavily infested tomato by *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Jordan. World Appl Sci J 17:589–592

Bajonero J, Córdoba N, Cantor F, et al (2008) Biology and life cicle of Apanteles gelechiidivoris (Hymenoptera: Braconidae) parasitoid of *Tuta absoluta*. Agron Colomb 26:417–426

Becchimanzi A, Avolio M, Bostan H, et al (2020) Venomics of the ectoparasitoid wasp *Bracon nigricans*. BMC Genomics 21:1–15. https://doi.org/10.1186/s12864-019-6396-4

Becchimanzi A, Avolio M, Lelio I Di, et al (2017) Host regulation by the ectophagous parasitoid wasp Bracon nigricans. J Insect Physiol. https://doi.org/10.1016/j.jinsphys.2017.07.002

Biondi A, Desneux N, Amiens-Desneux E, et al (2013) Biology and developmental strategies of the Palaearctic parasitoid *Bracon nigricans* (Hymenoptera: Braconidae) on the Neotropical moth *Tuta absoluta* (Lepidoptera: Gelechiidae). J Econ Entomol 106:1638–1647. https://doi.org/10.1603/EC12518

Bográn CE., Heinz KM., Ciomperlik MA. (2002) InterspecificcCompetition among insect parasitoids: field experiments with whiteflies as hosts in cotton. Ecol Soc Am 83:653–668

Calvo FJ, Lorente MJ, Stansly PA, Belda JE (2012) Preplant release of *Nesidiocoris tenuis* and supplementary tactics for control of *Tuta absoluta* and *Bemisa tabaci* in greenhouse tomato. Entomol Exp Appl 1:111–119. https://doi.org/10.1111/j.1570-7458.2012.01238.x

Chailleux A, Desneux N, Arnó J, Gabarra R (2014) Biology of two key Palaearctic larval ectoparasitoids when parasitizing the invasive pest *Tuta absoluta*. J Pest Sci (2004) 87:441–448. https://doi.org/10.1007/s10340-014-0557-7

Chailleux A, Droui A, Bearez P, Desneux N (2017) Survival of a specialist natural enemy experiencing resource competition with an omnivorous predator when sharing the invasive prey *Tuta absoluta*. Ecol Evol 7:8329–8337. https://doi.org/10.1002/ece3.3396

Cusumano A, Peri E, Amodeo V, et al (2013) Intraguild interactions between egg parasitoids: window of opportunity and fitness costs for a facultative hyperparasitoid. PLoS One 8:1–7. https://doi.org/10.1371/journal.pone.0064768

Cusumano A, Peri E, Colazza S (2016) Interspecific competition/facilitation among insect parasitoids. Curr Opin Insect Sci 14:12–16. https://doi.org/10.1016/j.cois.2015.11.006

Denis C, Riudavets J, Alomar O, et al (2022) Naturalized *Dolichogenidea gelechiidivoris* complement the resident parasitoid complex of *Tuta absoluta* in North-eastern Spain. J Appl Entomol 146:461–464. https://doi.org/10.1111/jen.12994

Desneux N, Wajnberg E, Kris WAG, et al (2010) Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, geographic expansion and prospects for biological control. J Pest Sci (2004) 83:197–215. https://doi.org/10.1007/s10340-010-0321-6

Feng Y, Wratten S, Sandhu H, Keller M (2015) Interspecific competition between two generalist parasitoids that attack the leafroller *Epiphyas postvittana* (Lepidoptera: Tortricidae). Bull Entomol Res 105:426–433. https://doi.org/10.1017/S0007485314000923

Fernandez-Triana J, Shaw MR, Boudreault C, et al (2020) Annotated and illustrated world checklist of Microgastrinae parasitoid wasps (Hymenoptera, Braconidae). Zookeys 920:1–1089. https://doi.org/10.3897/zookeys.920.39128

Ferracini C, Bueno VHP, Dindo ML, et al (2019) Natural enemies of *Tuta absoluta* in the Mediterranean basin , Europe and South America. Biocontrol Sci Technol 29:1–32. https://doi.org/10.1080/09583157.2019.1572711

Gabarra R, Arnó J, Lara L, et al (2013) Native parasitoids associated with *Tuta absoluta* in the tomato production areas of the Spanish Mediterranean Coast. BioControl 59:45–54. https://doi.org/10.1007/s10526-013-9545-8

Harvey JA, Poelman EH, Tanaka T (2013) Intrinsic inter- and intraspecific competition in parasitoid wasps. Annu Rev Entomol 58:333–351. https://doi.org/10.1146/annurev-ento-120811-153622

Hassell MP, Godfray HCJ (1994) Natural enemies: The population biology of predators, parasites and diseases. Michael J. Crawley. Q Rev Biol 69:256–257. https://doi.org/10.1086/418554

Haye T, Mason P, Kuhlmann U (2008) Predictive value of biological control agent´s attributes for introduction: *Peristenus digoneutis* as a case study. In: Third International Symposium on Biological Control of Arthropods, Christchurch, New Zealand. pp 403–415

Holt RD, Bonsall MB (2017) Apparent competition. Annu Rev Ecol Evol Syst 48:447–471. https://doi.org/10.1146/annurev-ecolsys-110316-022628

Idriss GEA, Mohamed SA, Khamis F, et al (2018) Biology and performance of two indigenous larval parasitoids on *Tuta absoluta* (Lepidoptera: Gelechiidae) in Sudan. Biocontrol Sci Technol 28:614–628. https://doi.org/10.1080/09583157.2018.1477117

Kortam MN, El Arnaouty SA, Afifi AI, Heikal IH (2014) Efficacy of different biological methods for controlling the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on tomato in greenhouse in egypt. Egypt J Biol Pest Control 24:523–528

Krache F, Boualem M, Fernandez-Triana J, et al (2021) First record in Africa of the parasitoid *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae) on tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae) from tomato fields in Algeria. J Hymenopt Res 88:115–131. https://doi.org/10.3897/jhr.88.75279

Loni A, Samartsev KG, Scaramozzino PL, et al (2016) Braconinae parasitoids (Hymenoptera, Braconidae) emerged from larvae of *Lobesia Botrana* (Denis & Schiffermüller) (Lepidoptera, Tortricidae) feeding on *Daphne gnidium* L. Zookeys 587:125–150. https://doi.org/10.3897/zookeys.587.8478

Luna MG, Pereyra PC, Coviella CE, et al (2015) Potential of biological control agents against *Tuta absoluta* (Lepidoptera: Gelechiidae): current knowledge in Argentina. Florida Entomol 98:489–494. https://doi.org/10.1653/024.098.0215

Mama Sambo S, Akutse KS, du Plessis H, et al (2022a) Interactions between the Entomopathogenic fungus *Metarhizium anisopliae* ICIPE 20 and the endoparasitoid *Dolichogenidea gelechiidivoris*, and implications for combined biocontrol of *Tuta absoluta*. Biology (Basel) 11:1323. https://doi.org/https://doi.org/10.3390/biology11091323

Mama Sambo S, Ndlela S, du Plessis H, et al (2022b) Identification, microhabitat, and ecological niche prediction of two promising native parasitoids of *Tuta absoluta* in Kenya. Insect 16:496. https://doi.org/https://doi.org/10.3390/ insects13060496

Mama Sambo S, Ndlela S, du Plessis H, et al (2022c) Ratio dependence effects of the parasitoid *Dolichogenidea gelechiidivoris* on its associated host *Tuta absoluta*. Biocontrol Sci Technol 32:497–510. https://doi.org/10.1080/09583157.2022.2033702

Mansour R, Brévault T, Chailleux A, et al (2018) Occurrence, biology, natural enemies and management of *Tuta absoluta* in Africa. Entomol Gen 38:83–112. https://doi.org/10.1127/entomologia/2018/0749

Mills NJ (1992) Parasitoid guilds: depning the structure of the parasitoid communities ofendopterigote insect hosts. Environ Entomol 23:1066-1083.

Mirhosseini MA, Fathipour Y, Soufbaf M, Reddy GVP (2019) Implications of using two natural enemies of *Tuta absoluta* (Lepidoptera: Gelechiidae) toward tomato yield enhancement. Bull Entomol Res 109:617–625. https://doi.org/10.1017/S0007485318000998

Mujica N, Kroschel J (2017) Host preference of *Dolichogenidea gelechiidivoris* (Marsh) (Hymenoptera: Braconidae) for *Tuta absoluta* (Meyrick) or *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). In: 15 Siconbiol, Resumo: 385-44. p 423 p

Naselli M, Biondi A, Tropea-Garzia G, et al (2017) Insights into food webs associated with the South American tomato pinworm. Pest Manag Sci 73:1352–1357. https://doi.org/10.1002/ps.4562

Palacios, M. and Cisneros F (1995) Management of the Potato Tuber Moth. Program 4, Integrated pest management. International Potato Center. Program Report

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. URL https//www.R-project.org/.

Reitz SR, Trumble JT (2002) Competitive displacement among insects and arachnids. Annu Rev Entomol 47:435– 465

Rosenheim JA, Kaya HK, Ehler LE, et al (1995) Intraguild predation among biological-control agents: Theory and evidence. Biol Control 5:303–335. https://doi.org/10.1006/bcon.1995.1038

Salas Gervassio NG, Aquino D, Vallina C, et al (2019) A re-examination of *Tuta absoluta* parasitoids in South America for optimized biological control. J Pest Sci (2004) 92:1343–1357. https://doi.org/10.1007/s10340-018-01078-1

Savino V, Luna MG, Gervassio NGS, Coviella CE (2016) Interspecific interactions between two *Tuta absoluta* (Lepidoptera: Gelechiidae) larval parasitoids with contrasting life histories. Bull Entomol Res 107:32–38. https://doi.org/10.1017/S0007485316000547

Shiraku H (2020) ICIPE Makes the first release of parasitoids into the farm in fight against *Tuta absoluta*. Infonet-Biovision. Available online: https://infonet-biovision.org/News/ICIPE-Makes-first-release-parasitoids-farm-fight-against-Tuta-Absoluta (accessed on 14 November 2020).

Sylla S, Brévault T, Streito J, Diarra K (2016) First record of *Nesidiocoris tenuis* (Reuter) (Heteroptera: Miridae), as a predator of the Tomato Leaf Miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), in Senegal. Egypt J Biol Pest Control 26:851–853

Tan X, Hu N, Zhang F, et al (2016) Mixed release of two parasitoids and a polyphagous ladybird as a potential strategy to control the tobacco whitefly *Bemisia tabaci*. Sci Rep 6:1–9. https://doi.org/10.1038/srep28245

Tropea Garzia G, Siscaro G, Biondi A, et al (2012) *Tuta absoluta*, a South American pest of tomato now in the EPPO region: Biology, distribution and damage. EPPO Bull 42:205–210. https://doi.org/10.1111/epp.2556

Ulyshen MD, Duan JJ, Bauer LS (2010) Interactions between *Spathius agrili* (Hymenoptera: Braconidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), larval parasitoids of *Agrilus planipennis* (Coleoptera: Buprestidae). Biol Control 52:188–193. https://doi.org/10.1016/j.biocontrol.2009.10.017

Valencia, L. and Penaloza J (1990) Control Biológico de las palomillas de la papa. Periódico rural. “El Boyacense”, ICA 4 p.

Vallejo FA (1999) Mejoramiento genético y producción de tomate en Colombia. Universidad Nacional de Colombia. pp. 216

van Alphen JJM, Visser ME (1990) Superparasitism as an adaptive strategy for insect parasitoids. Annu Rev Entomol 35:59–79. https://doi.org/https://doi.org/10.1146/annurev.en.35.010190.000423

Wang XG, Bokonon-Ganta AH, Messing RH (2008) Intrinsic inter-specific competition in a guild of tephritid fruit fly parasitoids: Effect of co-evolutionary history on competitive superiority. Biol Control 44:312–320. https://doi.org/10.1016/j.biocontrol.2007.10.012

Xu H, Yang N, Wan F (2013) Competitive interactions between parasitoids provide new insight into host suppression. PLoS One 8:16–21. https://doi.org/10.1371/journal.pone.0082003

Yang S, Duan JJ, Lelito J, Driesche R Van (2013) Multiparasitism by *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) and *Spathius agrili* (Hymenoptera : Braconidae): implication for biological control of the emerald ash borer (Coleoptera : Buprestidae). Biol Control 65:118–123. https://doi.org/10.1016/j.biocontrol.2012.09.010

Yu DSK, Actherberg C (2010) Taxapad Ichneumonoidea. In F. A. Bisby, Y. R. Roskov, T. M. Orrell, D. Nicolson, L. E. Paglinawan, N. Bailly, P. M. Kirk, T. Bourgoin, and G. Baillargeon (eds.), Species 2000 & IT IS. Cat life 2010 Annu checklist DVD, Species 2000, Reading, United Kingdom

Zappalà L, Bernardo U, Biondi A, et al (2012) Recruitment of native parasitoids by the exotic pest *Tuta absoluta* in southern Italy. Bull Insectology 65:51–61

Zappalà L, Biondi A, Alma A, et al (2013) Natural enemies of the South American moth, *Tuta absoluta*, in Europe, North Africa and Middle East, and their potential use in pest control strategies. J Pest Sci (2004) 86:635–647. https://doi.org/10.1007/s10340-013-0531-9

CHAPTER 7

Interactions between the entomopathogenic fungus *Metarhizium anisopliae* ICIPE 20 and the endoparasitoid *Dolichogenidea gelechiidivoris*, and implications for combined biocontrol of *Tuta absoluta*.

Citation:

Mama Sambo, S. Akutse, K.S. du Plessis, H. Aigbedion-Atalor, P.O., Mohamed, S.A. and Ndlela, S. 2022. Interactions between the entomopathogenic fungus *Metarhizium anisopliae* ICIPE 20 and the endoparasitoid *Dolichogenidea gelechiidivoris*, and implications for combined biocontrol of *Tuta absoluta*. *Biology*, 11, 1323. https:// doi.org/10.3390/biology1109132

biology

MDPI

Article

Interactions between the Entomopathogenic Fungus *Metarhizium anisopliae* ICIPE 20 and the Endoparasitoid *Dolichogenidea gelechiidivoris*, and Implications for Combined Biocontrol of *Tuta absoluta*

Sahadatou Mama Sambo [1,2], Komivi Senyo Akutse [1], Hannalene du Plessis [2], Pascal Osa Aigbedion-Atalor [3], Samira Abuelgasim Mohamed [1,*,†] and Shepard Ndlela [1,†]

1 International Centre of Insect Physiology and Ecology (*icipe*), Nairobi P.O. Box 30772-00100, Kenya
2 Unit for Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa
3 Centre for Biological Control, Department of Zoology and Entomology, Rhodes University, Makhanda 6140, South Africa
* Correspondence: sfaris@icipe.org
† These authors contributed equally to this work.

Simple Summary: The theory of beneficial species association in a cropping system can sustain ecosystem services and reduce pest pression under economic injury levels. For the control of the invasive pest, *Tuta absoluta* we assessed the susceptibility of *Dolichogenidea gelechiidivoris* to *Metarhizium anisopliae* ICIPE 20 through adult parasitoid and parasitised larval infection; furthermore, we evaluated the preference and performance of sprayed and non-sprayed host plants. We concluded an additive effect for *Tuta absoluta* control by the two biocontrol technologies even though the entomopathogenic fungus reduces the fitness of the parasitoid, such as adult longevity and its performance, and parasitised larval emergence.

Abstract: The Integrated Pest Management (IPM) approach have been widely promoted and used for the management of native and invasive pests, while the use of various components of the IPM can have a synergetic, additive, or antagonistic effect on each other; this study evaluated the susceptibility of *Dolichogenidea gelechiidivoris* (Marsh) (Hymenoptera: Braconidae), to the *Metarhizium anisopliae* (Metschnikoff) ICIPE 20 through direct and indirect infection approaches. The effect of fungus on parasitoid longevity, survival of parasitized-larvae, preference of the parasitoid to fungal treated and untreated larvae, and percent parasitism of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under different infection scenarios were assessed. The direct application of dry conidia to the parasitoid prior to exposure to the host, reduced *D. gelechiidivoris* longevity, though the infected female wasps still yielded high parasitism (over 70%). Infecting the parasitized larvae at different ages led to a respective reduction of parasitoid emergence by 35% and 23% for infection at 1 and 5 days post-parasitisation. Exposure of healthy-*D. gelechiidivoris* adults to a plant-sprayed with fungus did not affect their longevity, and no discriminatory host selection was observed. The highest mortality (~80%) of *T. absoluta* was achieved when *D. gelechiidivoris* and *M. anisopliae* ICIPE 20 were used in combination, indicating an additive impact on the target pest; however, field validation can shed more light on this outcome.

Keywords: entomopathogenic fungus; parasitoid; intraguild interaction; integrated pest management; *Tuta absoluta*

check for updates

Citation: Mama Sambo, S.; Akutse, K.S.; du Plessis, H.; Aigbedion-Atalor, P.O.; Mohamed, S.A.; Ndlela, S. Interactions between the Entomopathogenic Fungus *Metarhizium anisopliae* ICIPE 20 and the Endoparasitoid *Dolichogenidea gelechiidivoris*, and Implications for Combined Biocontrol of *Tuta absoluta*. *Biology* **2022**, *11*, 1323. https://doi.org/10.3390/biology11091323

Academic Editors: Ramaraj Boopathy, Eldon R. Rene and Mark Zappi

Received: 14 August 2022
Accepted: 31 August 2022
Published: 6 September 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

The South American tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) invasion in Afro-Eurasia is a serious threat to tomato (*Solanum lycopersicum* L.) production and livelihoods [1–4]. Most farmers in sub-Saharan Africa responded to its invasion by using synthetic chemical insecticides as a rapid control method in an attempt to save their tomato crops from this devasting pest [5,6]; however, insecticides, as standalone control tools, are not effective in preventing the pest's damage to below economic thresholds. More importantly, the overuse of synthetic chemical insecticides results in the evolution of resistance in field populations of the pest [7–9]; furthermore, an unrelenting increase in insecticide use is detrimental to human and environmental health because of chemical residues in tomato fruit and disadvantageous effects on non-target organisms, respectively [10–12].

Among the safer alternatives to synthetic chemical insecticides, biological control is an ideal option; this control strategy has no risk to human health and other nontargets, as well as being environmentally friendly and can be incorporated with ecofriendly integrated pest management (IPM) tactics [1,13–16]; this approach is considered an inoffensive method for sustainable agriculture [17,18] with no negative effect on biodiversity, farmers, and consumers [19].

To promote the sustainable management of *T. absoluta* in Africa, the International Centre of Insect Physiology and Ecology (*icipe*) imported the solitary endoparasitoid *Dolichogenidea gelechiidivoris* Marsh (Hymenoptera: Braconidae) from South America, the pest's native region [20], for classical biological control of this pest. *Dolichogenidea gelechiidivoris* parasitised all larval stages of *T. absoluta* with a preference for the early instar larvae [20]. The parasitoid has the potential to achieve a parasitism rate of up to 86% on *T. absoluta* larvae [21]. *Dolichogenidea gelechiidivoris* was lately released in East African countries (Kenya, Uganda, and Ethiopia) to control *T. absoluta* [22], and significant impact is expected from this parasitoid [23,24].

Within the entomopathogenic arena, several microbial organism were documented to be highly pathogenic to the different life stages of *T. absoluta* [25–29]. For example, Akutse et al. [15] reported *M. anisopliae* isolates ICIPE 18, ICIPE 20, and ICIPE 665 with respective mortalities of 95.0, 87.5, and 86.25% in *T. absoluta* adults. Even higher virulence (100% mortality) of these isolates was reported on the fourth instar [15]. In Africa, several isolates of *M. anisopliae* have been registered and commercialized against various insect pests in several African countries; these include *M. anisopliae* ICIPE 69, targeting fruit flies, mealybugs, and thrips and currently also used for the management of *T. absoluta* [10,30].

The combined application of parasitoids and fungal-based biopesticides may enhance the control of *T. absoluta* and the overall success of IPM programs against this pest. Several findings have demonstrated that entomopathogenic fungi and parasitoids/predators can coexist and manage different insect pest species [31–35]; however, detrimental effects of entomopathogenic fungi on adult or larval survival and other fitness parameters have been reported on some parasitoids [31,36–39]. The findings by these authors call for a proper understanding of the nature of the interactions between entomopathogenic fungi and other natural enemies before their potential combined use for effective and sustainable control of insect pests. As highlighted above both *D. gelechiidivoris* and *M. anisopliae* ICIPE 20 isolate have been proved to be very promising candidates for biological control of *T. absoluta*; however, the nature of interactions between these two biocontrol agents has not been elucidated; this research aims to evaluate the effect of direct infection of *D. gelechiidivoris* with *M. anisopliae* ICIPE 20 and the influence of *M. anisopliae* ICIPE 20 infected host larvae on the behavior and performance of *D. gelechiidivoris*.

2. Materials and Methods

2.1. *Metarhizium anisopliae* ICIPE 20 Culture and Viability Assessment

Metarhizium anisopliae ICIPE 20 used in this study was acquired from the Germplasm of the Arthropod Pathology Unit, at *icipe*. The fungus was sub-cultured on Sabouraud dextrose agar (SDA) (OXOID CM0041, Oxoid Ltd., Basingstoke, UK), and kept in an incubator at 25 ± 2 °C in full obscurity. From a mother plate, conidiospores were collected by scratching the surface of two-old sporulated cultures using a sterile spatula. The collected conidia were added to 10 mL sterilized distilled water having 0.05% (*w*/*v*) Triton X-100 (MERCK KGaA, Darmstadt, Germany) and vortexed for five min at 700 rpm to guarantee the homogeneity of the suspension. Conidia concentrations were quantified utilizing an improved Neubauer hemacytometer under a light microscope (LEICA DM 2000, Leica Microsystems, Morrisville, NC, USA) as described by Goettel and Inglis [40]. The conidia suspension concentration of 1×10^8 conidia mL^{-1} was obtained through serial dilution. Before performing any bioassay, the viability of the spores was assessed by spread plating 100 µL of the suspension on a SDA plate under a sterile laminar air flow hood. The inoculated plates were hermetically sealed with a Parafilm membrane and kept at 25 ± 2 °C in total darkness. At 18 h post-incubation, lactophenol aniline cotton blue (Millipore Corporation, Billerica, MA, USA) was added into the plates to end the germination procedure and stain the spores to improve their visibility for counting. The germination rate (%) of conidiospores was evaluated from 100 conidia randomly selected using a light microscope (LEICA DM 2000, Leica Microsystems, Morrisville, NA, USA) following the process explained by Goettel and Inglis [40]. Five plates were assessed, and the average percentage germination of the spores was more than 99% viability for all the bioassays.

2.2. Insect Rearing

Colonies of *T. absoluta* and *D. gelechiidivoris* were reared and maintained in maintained at the Animal Rearing and Containment Unit (ARCU) at *icipe*.

2.2.1. *Tuta absoluta* Colony

The *T. absoluta* colony was established from tomato leaves infested with larvae collected from a tomato farm in Kirinyaga County, Kenya. The leaves were incubated in a ventilated Perspex cage (50 × 50 × 60 cm). The incubated larvae were supplied with clean tomato leaves, sourced from an insecticide-free screenhouse at *icipe*, until larval pupation and moth emergence. The emerged moths represented the 1st generation of the colony. For colony maintenance, four weeks old potted tomato plants (cv. Money maker), grown in the screenhouse, were placed in a Perspex cage of the same size as that used for incubation. After 48 h, the plants were removed and kept until the eggs hatched. Subsequently, leaves, having early instar larvae, were excised from the plants, and placed in a clean Perspex cage lined with a paper towel to absorb excess moisture, caused by the leaves. The larvae were provided with fresh tomato leaves *ad libitum* as a diet until pupation, and 80% honey-drops were applied on the top of the cage as food for the moths that would emerge. Infested leaves were collected from tomato fields in Kirinyaga every three, or four months and adult moths infused into the colony to rejuvenate genetic vigor and avoid deterioration of the colony due to inbreeding. The colony was maintained at 25 ± 2 °C, 70 ± 5% RH, and a 12L:12D photoperiod.

2.2.2. *Dolichogenidea gelechiidivoris* Colony

The initial cohort of the D. gelechiidivoris colony was obtained from the International Potato Center (CIP) and maintained at *icipe* since 2017. The parasitoid was reared according to the protocol described by Mama Sambo et al. [41]. The colony was maintained at 22 ± 1 °C, 70 ± 5% RH, and 12L:12D photoperiod. Four potted tomato plants with early instar larvae of *T. absoluta* were exposed to a cohort of *D. gelechiidivoris* in a Perspex cage (40 × 20 × 50 cm) for parasitisation. After the exposure period of two days, the plants were removed from the cage and the leaves with parasitized larvae were excised, and then kept in another cage without parasitoids, but lined with paper towel. Fresh tomato leaves (i.e., food source) were added as needed until cocoon formation. *Dolichogenidea gelechiidivoris* adults that emerged were aspirated into a clean cage and fed on 80% honey solution, streaked on the top-interior of the cage.

2.3. *Effect of M. anisopliae ICIPE 20 on the Performance and the Longevity of D. gelechiidivoris Adults*

Three newly emerged *D. gelechiidivoris* couples (3 males:3 females) were infected with 0.5 g dry *M. anisopliae* ICIPE 20 conidia in an infection chamber. The infection chamber consisted of a cylindrical plastic tube (11 × 6 cm), covered on the inside with velvet cloth as described by [42]. Three minutes after exposure to the fungus, the infected parasitoids were removed from the infection chamber, and then released into a clean ventilated Perspex cage (20 × 15 × 14 cm). Sixty first-instar larvae of *T. absoluta* were placed on a fresh tomato stem to mine. The infested stem was then introduced to the infected parasitoids for parasitisation for 24 h. For the control, 60 *T. absoluta* first-instar larvae were exposed to three couples of untreated (without fungus) *D. gelechiidivoris*, which were previously introduced into a fungus-free infection chamber. The larvae were removed after 24 h, incubated and maintained under ambient conditions (25 ± 2 °C and 70 ± 5% RH). Mortality of the exposed *D. gelechiidivoris* was monitored daily and the dead wasps were recorded until the death of all individuals. Pupation of the larvae that were exposed to the fungus-infected parasitoids was recorded, as well as the emergence of either *T. absoluta* adults or parasitoids. One week after the last observed emergence of moths or parasitoids, the remaining cocoons from which nothing emerged, were dissected to reveal pharate adults of either *T. absoluta* or parasitoids. The treatments were set up in a randomised complete block design (RCBD), and the trial was replicated 10 times. Parasitism rate was evaluated as the number of emerged *D. gelechiidivoris* plus the number of dissected cocoons, divided by the sum of the number of *T. absoluta*, the number of pupae that did not emerge, the number of *D. gelechiidivoris* emerged, and cocoons dissected. The sex ratio was expressed as the percentage of females out of the total eclosed *D. gelechiidivoris*.

Cadavers of *D. gelechiidivoris* (parents) and their offspring (F1) were surface disinfected by dipping them in 70% ethanol for 1 min, and then by washing twice in sterilised distilled water. The insects were then put into Petri dishes covered with moist filter papers to assess fungal outgrowth on the cadaver. Petri dishes were tightly sealed with Parafilm and kept at 25 ± 2 °C for 5 days. Death as a result of *M. anisopliae* ICIPE 20 infection was confirmed by the existence of hyphae and conidiospores on the cuticula of the cadaver. A sterile pin was used to collect the fungus from the identified insects and to place it on a glass slide with a droplet of distilled water. The glass slide was observed under an oil immersion microscope and compared with a mother solution of *M. anisopliae* ICIPE 20 to record the mycosis (presence or absence of *M. anisopliae* ICIPE 20 on the incubated insect cadaver). All 30 *D. gelechiidivoris* couples directly infected were incubated and observed for mycosis as well as 30 randomly selected dead individuals from their offspring.

2.4. *Effect of Metarhizium anisopliae ICIPE 20 on D. gelechiidivoris Larvae*

To measure the impact of *M. anisopliae* ICIPE 20 on two immature stages of *D. gelechiidivoris* (egg and larvae), infested tomato leaves with 60, first-instar *T. absoluta* larvae were exposed to three, one-day-old *D. gelechiidivoris* couples for 24 h to ensure parasitisation. Thereafter, the leaves with the exposed larvae were removed from the cage and placed into a plastic container (21 × 15 × 15 cm). Then 10 larvae were randomly selected and removed from the tomato leaves using a camel hair brush and put on a paper towel. Ten (10 mL) of *M. anisopliae* ICIPE 20 suspension at a concentration of 10^8 conidia/mL was then prepared with sterile distilled water containing 0.05% (*w*/*v*) Triton X-100. Three (3 mL) of the fungal suspension were applied to the 10 larvae on a paper towel. After three minutes on the sprayed paper towel, larvae were incubated and provided with healthy tomato leaves for feeding and development until parasitoid or *T. absoluta* emergence; this setup was considered as the treatment. A similar number of *T. absoluta* larvae parasitized as above were treated with 3 mL sterile distilled water containing 0.05% (*w*/*v*) Triton X-100 and provided with tomato leaves, and this served as the control. The trial was organized in a randomized complete block design (RCBD) and replicated 10 times. The above setup of treatment and control was repeated at five days post parasitisation of *T. absoluta* larvae.

The number of eclosed *T. absoluta* and *D. gelechiidivoris* as well as the time taken from parasitisation to emergence of the wasps and their sex were recorded.

2.5. *Dolichogenidea gelechiidivoris* Preference for and Performance on *M. anisopliae* ICIPE 20-Sprayed and *T. absoluta* Infested Host Plants

Behavioral activities (landing, walking, resting, probing, and oviposition) of the parasitoid were investigated in no-choice and choice tests to assess the preference of *D. gelechiidivoris* to *T. absoluta* infested host plants treated with *M. anisopliae* ICIPE 20. For the choice test, tomato plants were infested with 30 first-instar *T. absoluta* larvae per plant and sprayed with 10 mL of *M. anisopliae* ICIPE 20 suspension at the concentration of 1×10^8 conidia/mL. Another plant infested with 30 first-instar *T. absoluta* larvae was sprayed with 10 mL sterile distilled water containing 0.05% (*w*/*v*) Triton X-100 solution. The two groups of tomato plants were kept for an hour for the suspension to dry and then exposed simultaneously to three mated, one-day-old *D. gelechiidivoris* females in a cage ($20 \times 15 \times 14$ cm) for 24 h. Behavioral activities of the parasitoid females, such as landing on the plant, walking, resting, probing, and ovipositing were recorded at five-minute intervals for one hour during their most active time, which is in the morning to midday (from 9:00–12:00 h) (Mama Sambo, personal observation). For the no-choice test, tomato plants with 60 larvae were sprayed with either *M. anisopliae* ICIPE 20 or sterile distilled water containing 0.05% (*w*/*v*) Triton X-100 (control treatment) as described in the choice test. The larvae in both the treatment and control plants were exposed in separate cages to three mated parasitoid females for 24 h. Data were recorded as described for the choice test. In addition, mortality of parasitoid females was also recorded daily. To assess the performance of *D. gelechiidivoris* on *M. anisopliae* ICIPE 20-sprayed and non-sprayed *T. absoluta* infested plants, the plants were incubated separately to record the emergence of *T. absoluta* and *D. gelechiidivoris*, and the sex ratio of F1 progeny of the parasitoid. The cocoons from which no parasitoids emerged were dissected to verify the sex of non-eclosed wasps. The female cadavers from each test (choice test, and the respective no-choice tests) were incubated, and a mycosis test was performed as described above in Section 2.1. Thirty couples were also selected from the offspring of each of the tests to perform a mycosis test.

2.6. Efficiency of *D. gelechiidivoris*, *M. anisopliae* ICIPE 20 and Their Combination on *T. absoluta*

Data from the above bioassay in a no-choice test was considered to evaluate the percentage emergence of *T. absoluta*. Additionally, a positive control with only *M. anisopliae* ICIPE 20 applications on an infested host with 60 fist-instar larvae and negative control with only distilled water containing 0.05% (*w*/*v*) Triton X-100 application was settled. *Tuta absoluta* percentage of emergence for the different sets of bioassays was compared.

2.7. Data Analysis

The Kaplan–Meier estimator method was used to estimate the survival function for parasitoid longevity data. Since the behavioral activities were assessed repetitively, normally assumption of parametric tests was violated [43,44], and repeated-measures ANOVA were run to differentiate between the number of activities on *M. anisopliae* ICIPE 20 sprayed and that on non-sprayed host plants in the choice and no-choice tests. Emergence data were analyzed using GLM with a negative binomial distribution, and developmental time data using GLM with a gamma distribution. When significant differences were noticed, multiple means comparisons were done using Tukey's HSD test, at $\alpha = 0.05$. Statistical analyses were performed using R 3.5.1 [45].

3. Results

3.1. Effect of *M. anisopliae* ICIPE 20 on the Performance and the Longevity of *D. gelechiidivoris* Adults

The survival time of *D. gelechiidivoris* was significantly reduced ($p < 0.001$) whereby 50% wasps died by day three post-infection with dry *M. anisopliae* ICIPE 20 conidia (treatment), while uninfected wasps had longer longevity with up to 8 days median survival time

(Figure 1). The reduced survival of infected wasps was confirmed being caused by the fungus and 90% of adult parasitoid cadavers showed mycosis.

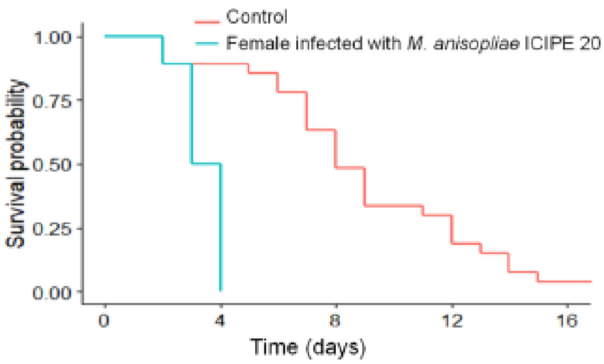


Figure 1. Effect of *M. anisopliae* ICIPE 20 (direct infection with dry conidia) on *D. gelechiidivoris* survival.

The performance of *M. anisopliae* ICIPE 20 infected and uninfected *D. gelechiidivoris* females was measured as percent parasitism of *T. absoluta*, It varied between treatments ($F_{1,18}$ = 4.88, p = 0.040), and was significantly higher (87.79 ± 6.32%) for uninfected wasps. Nonetheless, no transmission of ICIPE 20 conidia by infected *D. gelechiidivoris* parents to offspring was observed. There was also no significant difference between the sex ratio of offspring of infected and uninfected wasps ($F_{1,18}$ = 0.207, p = 0.65), in both cases the sex ratio was male bias (Figure 2).

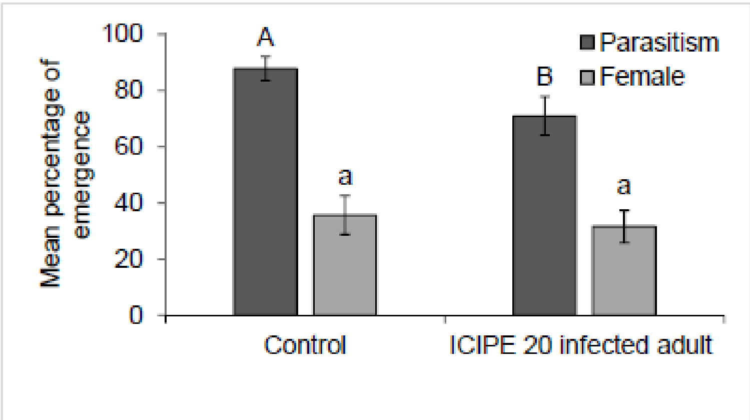


Figure 2. Performance of *M. anisopliae* ICIPE 20 infected and uninfected *D. gelechiidivoris* on *T. absoluta*. Bars capped with the same upper/lower case letters are not significantly different.

3.2. Effect of M. anisopliae ICIPE 20 on the Development of Parasitised T. absoluta Larvae

The developmental time of the immature stages of *D. gelechiidivoris* was not affected by the fungal application to *T. absoluta* larvae one day post-exposure to the parasitoid, for both male ($F_{1,10}$ = 0.97, p = 0.35) and female ($F_{1,13}$ = 0.34, p = 0.60). The developmental time of males was different for the untreated and treated larvae ($F_{1,15}$ = 7.38, p = 0.01). Conversely, female developmental time was comparable between fungal treated and untreated larvae ($F_{1,19}$ = 0.65, p = 0.43), being shorter for treated larvae (Table 1).

Table 1. Developmental time (Mean ± SE) of *D. gelechiidivoris* on *M. anisopliae* ICIPE 20 treated and untreated *T. absoluta* larvae.

Age Before Exposure	Treatments	No. Days ± SE (♂)	No. Days ± SE (♀)
One day	Control	18.8 ± 0.99 a	19.58 ± 1.02 a
	Treatment	16 ± 1.5 a	18.33 ± 0.88 a
Five days	Control	21.33 ± 0.85 a	25.19 ± 4.21 a
	Treatment	17.40 ± 0.75 b	19.0 ± 1.14 a

Means with the same letter within a column are not significantly different (Tukey's HSD, α = 0.05).

The percent eclosed *D. gelechiidivoris* wasps varied between *M. anisopliae* ICIPE 20 infected and non-infected larvae for both one day old post-exposure ($F_{1,18} = 18.15$, $p < 0.001$) and five days old post-exposure ($F_{1,18} = 26.3$, $p < 0.001$), being lowest (5 ± 1.67%) for one day old post-exposure to the fungus (Figure 3).

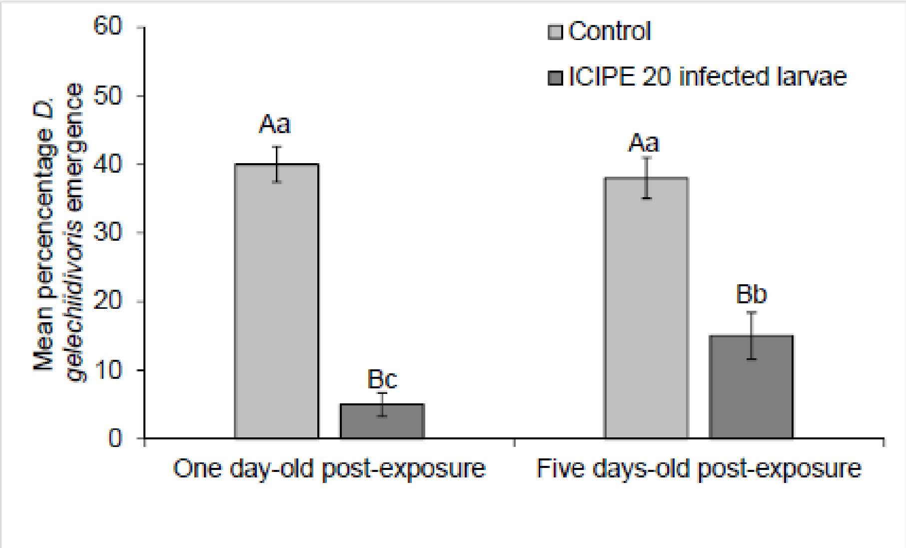


Figure 3. Percentage of *D. gelechiidivoris* that emerged from larvae exposed to the parasitoids infected with *M. anisopliae* ICIPE 20 at one day and five days post-exposure. Bars capped with the same uppercase letter indicate no difference between control and treatment within the same age exposed-larvae, bars capped with the same lowercase letter indicate no difference between one day and five days post-exposure.

3.3. *Dolichogenidea gelechiidivoris* Preference for and Performance on *M. anisopliae* ICIPE 20-Sprayed and *T. absoluta* Infested Host Plants

The preference of *Dolichogenidea gelechiidivoris* to fungal sprayed and non-sprayed plants as measured by the different behavioral response of foraging females was not affect by the host plant status (Table 2).

Table 2. Number of behavioral activities (means ± SE) performed by three females of *D. gelechiidivoris*/5 min on sprayed and non-sprayed host plant in choice and no-choice tests.

Behavioral Activities	Choice Test			No-Choice Test		
	Means ± SE		Statistics	Means ± SE		Statistics
	Non-Sprayed	Sprayed		Non-Sprayed	Sprayed	
Landing	1.10 ± 0.25	0.63 ± 0.15	$F_{1,58}$ = 2.57, p = 0.11	0.50 ± 0.14	0.63 ± 0.16	$F_{1,58}$ = 1.59, p = 0.21
walking	1.50 ± 0.30	1.00 ± 0.31	$F_{1,58}$ = 1.30, p = 0.26	1.43 ± 0.49	0.77 ± 0.42	$F_{1,58}$ = 0.32, p = 0.57
Resting	0.9 ± 0.2	0.67 ± 0.16	$F_{1,58}$ = 0.49, p = 0.49	0.57 ± 0.23	0.83 ± 0.25	$F_{1,58}$ = 1.07, p = 0.30
Probing	5.33 ± 1.10	3.27 ± 1.10	$F_{1,58}$ = 2.08, p = 0.15	4.77 ± 0.88	2.80 ± 1.20	$F_{1,58}$ = 1.72, p = 0.19
Oviposition	0.36 ± 0.13	0.33 ± 0.17	$F_{1,58}$ = 0.02, p = 0.88	0.67 ± 0.31	1.03 ± 0.30	$F_{1,58}$ = 0.72, p = 0.40

The longevity of *D. gelechiidivoris* females foraging on fungal-infected and uninfected host plants did not differ significantly (p = 0.14). The median survival time of females exposed to infected and uninfected hosts was nine and eight days, respectively (Figure 4). Furthermore, only 43% of *D. gelechiidivoris* cadavers from larvae in plants that received an *M. anisopliae* ICIPE 20 application were found to have mycosis Percent parasitism of *T. absoluta* by *D. gelechiidivoris* differed between *M. anisopliae* ICIPE 20 sprayed and non-sprayed plants in choice test ($F_{1,18}$ = 4.68, p = 0.044), being higher (62%) on the latter. While in the no-choice test, there was no significant difference ($F_{1,18}$ = 0.10, p = 0.75); however, the proportion of females that emerged from parasitised larvae did not differ significantly between *M. anisopliae* ICIPE 20 sprayed and non-sprayed plants in the choice ($F_{1,18}$ = 0.12, p = 0.73), as well as in no-choice scenario ($F_{1,18}$ = 3.16, p = 0.09) (Figure 5). From the F1 of the offspring of *D. gelechiidivoris* that foraged on fungal sprayed host plants, 77% were infected with *M. anisopliae* ICIPE 20.

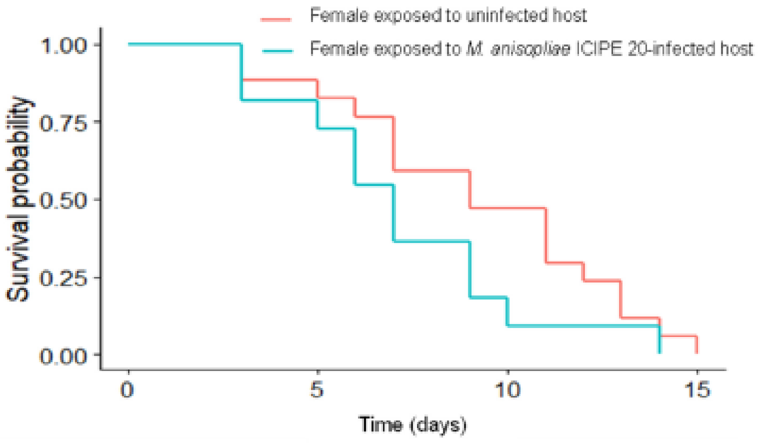


Figure 4. Survival of *D. gelechiidivoris* female foraging on *Metarhizium anisopliae* ICIPE 20 sprayed and unsprayed host plants.

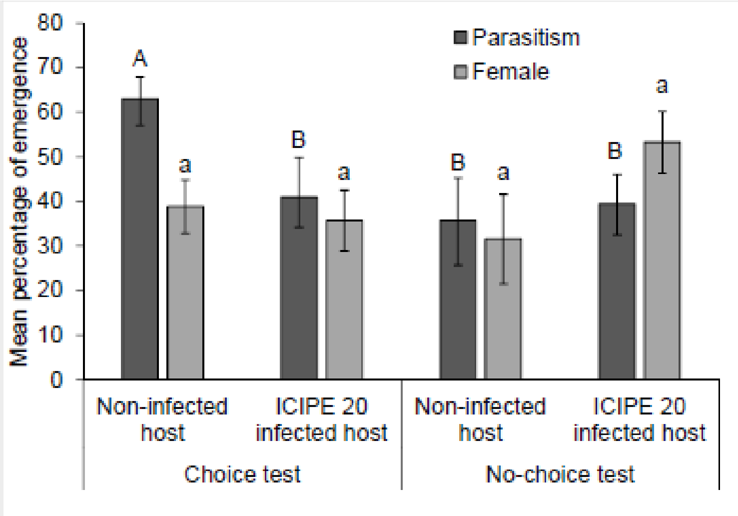


Figure 5. Performance of *D. gelechiidivoris* on *M. anisopliae* ICIPE 20 sprayed and unsprayed plants Bars capped with same upper/lower case letters are not significantly different.

3.4. Efficiency of D. gelechiidivoris, M. anisopliae ICIPE 20 and Their Combination on T. absoluta

The percent emerged moths of *T. absoluta* varied considerably ($F_{3,36}$ = 14.56, $p < 0.001$) among the treatments (when exposed to *D. gelechiidivoris* alone, subjected to infection by *M. anisopliae* ICIPE 20, and subjected to the fungal infection followed by exposure to the parasitoid and untreated control plants). More *T. absoluta* adults emerged from the untreated control plants, while the lowest emergence was observed from plants exposed to *D. gelechiidivoris* that were previously sprayed with *M. anisopliae* ICIPE 20 (Figure 6).

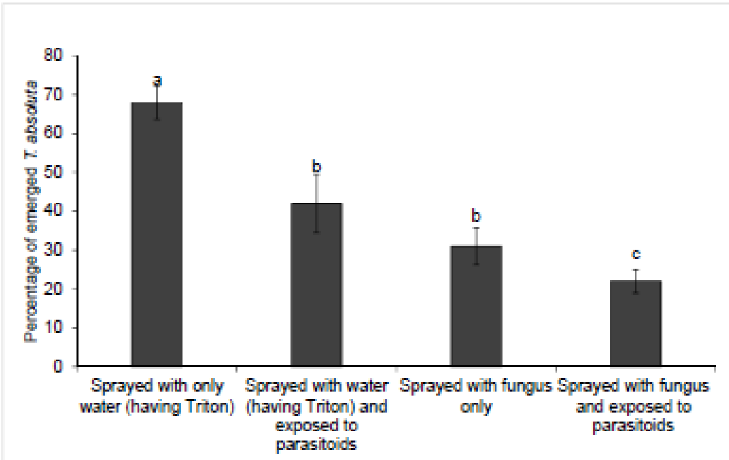


Figure 6. Effect of sole and combined use of two biocontrol agents on *Tuta absoluta* emergence. Bar capped with the same letters are not significantly different, (Tukey's HSD, α = 0.05).

4. Discussion

Several *Metarhizium* species and strains have been reported to be pathogenic to different life stages of *T. absoluta* [15,25,46–48]. Different strategies of using these fungal isolates including their integration with pheromone traps using dry conidia through an autodissemination method [15], and inundative application of the fungus as direct sprays [28,49] have been demonstrated for the control of *T. absoluta*. When several management strategies such as the use of parasitoids, entomopathogens and mass trapping are implemented in the context of IPM, parasitoids could be attracted to or accidentally enter the *T. absoluta* trap impregnated with *M. anisopliae* ICIPE 20, thus resulting in detrimental effects to the functioning of the parasitoids. Furthermore, although parasitoids may avoid infected hosts, they can be in contact with the inoculum and their survival inside or on the infected host may be hampered.

In the current study, infecting *D. gelechiidivoris* directly with dry conidia of *M. anisopliae* ICIPE 20 reduced percent parasitism as well as the longevity of the parasitoid. Similar results were reported for other parasitoid species. For example Nozad-Bonab et al. [50] working on the same host used in this study, found that infecting *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) with *M. anisopliae*, reduced the longevity of the parasitoid. Similarly, in a study by Presa-Parra et al. [51] the longevity of *M. anisopliae* infected Diachasmimorpha longicaudata Ashmead (Hymenoptera: Braconidae) was much shorter compared to their untreated counterparts. *Dolichogenidea gelechiidivoris* lays the highest number of eggs at one to three days after female emergence [20], when the parasitoid was *infected* with *M. anisopliae* ICIPE 20 due to the attraction to *T. absoluta* pheromone traps as found by Ayelo et al. [52]. Considering the findings by these authors and in the light of the result of this study that the median survival time of fungus-infected females were 3 days coupled with the fact that they cause a parasitism level of more than 70%, the parasitoid population growth might not be significantly affected by the fungal application. Our result of percent mycosed wasps (90%) following infection with dry *M. anisopliae* ICIPE 20, was different from that (50%) reported by Nielsen et al. [31] for the same fungus when tested on *Spalangia cameroni* Perkins (Hymenoptera: Pteromalidae). The discrepancy between our results and that by Nielsen et al. [31], could be due to the fact that these authors used fungal conidial suspensions while we used dry conidia. Another possible explanation could be due to difference in the fungal isolate used in the two studies.

We have demonstrated that infecting the *T. absoluta* parasitized larvae *with M. anisopliae* ICIPE 20 reduced the survival of immature stages of *D. gelechiidivoris*, with the egg stage more affected than the larval stage. Using the same fungus different levels of pathogenicity against *T. absoluta* have been documented. For example, Contreras et al. [28] reported more than 80% pathogenicity to pupae of *T. absoluta* from different populations, while Rodríguez et al. [53] reported more than 90% mortality of third instar *T. absoluta larvae*, and Akutse et al. [15] found 100% mortality of fourth instar *T. absoluta* larvae. The differential survival of the immature stages of *D. gelechiidivoris* reared on fungal infected host larvae could be due to the fact that parasitoid larvae release fungicidal substances inside the host to stop fungus growth, thus facilitating the development of their offspring as argued by Fransen and van Lenteren, [54]. Although the survival of the immature stages of *D. gelechiidivoris* was reduced by fungal treatment of their host, interestingly in general the developmental time of the eclosed wasps was not affected by the fungal treatment. Similarly, Ramos Aguila et al. [55] found that the developmental time of *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae) reared on *Beauveria bassiana* infected Diaphorina citri Kuwayama (Hemiptera: Liviidae) was similar to the parasitoids being reared on healthy hosts.

With regard to *D. gelechiidivoris* preference, the female wasps could not distinguish between *M. anisopliae* ICIPE 20-infected and uninfected hosts, nonetheless high percent parasitism was recorded on an uninfected host in choice experimental conditions. Similarly, non-discriminatory behavior was also reported for the parasitoid, *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae) exposed to *Diuraphis noxia* Kurdjumov (Hemiptera: Aphididae) populations infected with the fungus *Isaria fumosorosea* (Paecilomyces) compared to uninfected host [56]. The non-discrimination behavior of *D. gelechiidivoris* between infected and uninfected hosts could be due to the absence of odor of *M. anisopliae*, as reported in the case of mosquitoes species [57]. Additionally the response of a species involves a co-evolutionary phenomenon [58] and could explained the non-discriminatory behavior of *D. gelechiidivoris* towards the fungal-infected and uninfected host. Contrary to our findings, *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) discriminated against the *M. anisopliae* infected eggs of *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae) when given a choice [34]. Similarly, Miranda-fuentes et al. [38] found that the cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) infected with *Metarhizium brunneum* Petch (Hypocreales: Clavicipitaceae) was less preferred by the endoparasitoid, *Hyposoter didymator* (Thunberg) (Hymenoptera: Ichneumonidae) with parasitism being almost three times higher in the uninfected compared to the infected larvae.

In terms of parasitoid emergence, a lower parasitism rate was recorded on the sprayed host plant in a choice test, suggesting that the fungus might have affected the growth and development of the parasitoid. In a previous study by Potrich et al. [34], the eggs of *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae) infected with *Metarhizium anisopliae* Unioeste 43 and *M. anisopliae* ESALQ 1641 were 44% and 41%, respectively less parasitised compared to the healthy host when offered to *T. pretiosum* in a choice test. On the other hand, spraying eggs of *Duponchelia fovealis* (Zeller) (Lepidoptera: Crambidae) with commercial *M. anisopliae* IBCB348 at 1.5 × 105 conidia mL^{-1} did not affect the choice of *Trichogramma atopovirilia* Oatman and Platner and *T. pretiosum* (Hymenoptera: Trichogrammatidae) to parasitise and no effect on the parasitoid emergence, or sex ratio of the progeny was reported [59]. Furthermore, Domingues et al. [39] reported comparable parasitism rates by the parasitoid *Cleruchoides noackae* Lin and Huber (Hymenoptera: Mymaridae) between infected and uninfected eggs of *Thaumastocoris peregrinus* Carpintero and Dellapé (Hemiptera: Thaumastocoridae) treated with *M. anisopliae*. The simultaneous release of the parasitoid *D. gelechiidivoris* and the spraying of *M. anisopliae* will therefore have limited effects on the parasitoid since a choice scenario might be observed in real conditions.

The highest level of *T. absoluta* mortality occurred with the combined use of the two biocontrol agents, whereby *M. anisopliae* ICIPE 20 was applied to an infested plant followed by the release of *D. gelechiidivoris*. In all scenarios, when the fungus was applied first, and the parasitoid encountered an already infected host, longevity and parasitism were not affected. Against this background, we envisage that under field conditions, with application of *M. anisopliae* ICIPE 20 prior to the release of the parasitoid, the likelihood of the fungus negatively impacting on the parasitoid performance will be negligible. Similarly Nozad-Bonab et al. [50] reported 94–95% mortality of *T. absoluta* when *T. brassicae* was used in combination with *M. anisopliae*. In contrast, Presa-Parra et al. [51] using the same fungus found that there was no difference in mortality of parasitised and non-parasitised *Anastrepha ludens* Loew (Diptera: Tephritidae) larvae sprayed with *M. anisopliae*.

5. Conclusions

Although direct infection of *D. gelechiidivoris* adults with *M. anisopliae* ICIPE 20 reduced the longevity of the parasitoid, the infected females were able to achieve a considerable level of parasitism (more than 70%). The direct infection of *D. gelechiidivoris* adults did not result in any infection of the offspring. The study also demonstrated that mortality of the parasitoid following the infection of host larvae was a function of the time at which the host was subjected to *M. anisopliae* ICIPE 20 infection, with more time between parasitism and fungal infection resulting in lower mortality of the parasitoid. With regard the potential use of the two biocontrol agents evaluated in this study, sequential use *D. gelechiidivoris and M. anisopliae* ICIPE 20 yielded the highest mortality of the host, suggesting and additive effect on the target pest. Therefore, the combination of *M. anisopliae* ICIPE 20 application and *D. gelechiidivoris* for management of *T. absoluta* can offer a promising alternative to chemical control applications. However, field or semi-field trials on the combined use of these two important biocontrol agents (*D. gelechiidivoris* and *M. anisopliae* ICIPE 20) will shed more light on their performance in suppression of *T. absoluta*, a study which we are currently undertaking.

Author Contributions: Conceptualization, S.M.S., S.N. and S.A.M.; methodology and validation, S.M.S., K.S.A., S.N., P.O.A.-A., H.d.P. and S.A.M.; data collection and curation, S.M.S.; formal statistical analysis, S.M.S.; writing—original draft preparation, S.M.S.; writing—review and editing, S.M.S., K.S.A., H.d.P., P.O.A.-A., S.N. and S.A.M.; supervision, S.A.M., K.S.A., H.d.P. and S.N.; project administration and funding acquisition, S.A.M. All authors have read and agreed to the published version of the manuscript.

Funding: The authors gratefully acknowledge the financial support for this research by the following organizations and agencies: Biovision Foundation grant number BV DPP-012/2019-2022, Norwegian Agency for Development Cooperation-(NORAD), the section for research, innovation and higher education grant number RAF-3058 KEN-18/0005, Scaling-up Climate-Smart Pest Management Approaches for Enhanced Maize and Tomato Systems Productivity in Eastern Africa (SCLAMP) grant number 81250394, the Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); the Federal Democratic Republic of Ethiopia; and the Government of the Republic of Kenya. The first author was supported by the German Academic Exchange Service (DAAD) through the African Regional Postgraduate Programme in Insect Science (ARPPIS) tenable at ICIPE. The views expressed herein do not necessarily reflect the official opinion of the donors.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data of these results is available on the International Centre of Insect Physiology and Ecology (*icipe*) data repository at the link: https://dmmg.icipe.org/dataportal/dataset/interactions-between-the-entomopathogenic-fungus-metarhizium-anisopliae-icipe-20.

Acknowledgments: The authors acknowledge the technical support of Esther Awuor Owino, Sospeter Wafula, Patrick Kipkorir Koech, Alex Muthembwa, Francis Obala, Omar Jimali Ibrahim, and all the African Fruit Fly Program (AFFP) and Arthropod Pathology Unit (APU) colleagues.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Desneux, N.; Wajnberg, E.; Kris, W.A.G.; Burgio, G.; Arpaia, S.; Narváez-Vasquez, C.A.; González-Cabrera, J.; Ruescas, D.C.; Tabone, E.; Frandon, J.; et al. Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, geographic expansion and prospects for biological control. *J. Pest. Sci.* **2010**, *83*, 197–215. [CrossRef]
2. Chidege, M.; A-zaildi, S.; Hassan, N.; Julie, A.; Kaaya, E.; Mrogoro, S. First record of tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Tanzania. *Agric. Food Secur.* **2016**, *5*, 17. [CrossRef]
3. Karlsson, F.M.; Rachidatou, S.; Sahadatou, M.S.; Joseph, Z.A.; Georg, G. First report of *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) in the Republic of Benin. *BioInvasions Rec.* **2018**, *7*, 463–468. [CrossRef]
4. Han, P.; Bayram, Y.; Shaltiel-Harpaz, L.; Sohrabi, F.; Saji, A.; Esenali, U.T.; Jalilov, A.; Ali, A.; Shashank, P.R.; Ismoilov, K.; et al. *Tuta absoluta* continues to disperse in Asia: Damage, ongoing management and future challenges. *J. Pest. Sci.* **2019**, *92*, 1317–1327. [CrossRef]
5. Mansour, R.; Brévault, T.; Chailleux, A.; Cherif, A.; Grissa-Lebdi, K.; Haddi, K.; Mohamed, S.A.; Nofemela, R.S.; Oke, A.; Sylla, S.; et al. Occurrence, biology, natural enemies and management of *Tuta absoluta* in Africa. *Entomol. Gen.* **2018**, *38*, 83–112. [CrossRef]
6. Aigbedion-Atalor, P.O.; Hill, M.P.; Zalucki, M.P.; Obala, F.; Idriss, G.E.; Midingoyi, S.K.; Chidege, M.; Ekesi, S.; Mohamed, S.A. The South America Tomato Leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), Spreads Its Wings in Eastern Africa: Distribution and Socioeconomic Impacts. *J. Econ. Entomol.* **2019**, *112*, 2797–2807. [CrossRef]
7. Biondi, A.; Guedes, R.N.C.R.; Wan, F.-H.F.; Desneux, N. Ecology, Worldwide Spread, and Management of the Invasive South American Tomato Pinworm, *Tuta absoluta*: Past, Present, and Future. *Annu. Rev. Entomol.* **2018**, *63*, 239–258. [CrossRef]
8. Grant, C.; Jacobson, R.; Bass, C. Parthenogenesis in UK field populations of the tomato leaf miner, *Tuta absoluta*, exposed to the mating disruptor Isonet T. *Pest. Manag. Sci.* **2021**, *77*, 3445–3449. [CrossRef]
9. Guedes, R.N.C.; Roditakis, E.; Campos, M.R.; Haddi, K.; Bielza, P.; Siqueira, H.A.A.; Tsagkarakou, A.; Vontas, J.; Nauen, R. Insecticide resistance in the tomato pinworm *Tuta absoluta*: Patterns, spread, mechanisms, management and outlook. *J. Pest. Sci.* **2019**, *92*, 1329–1342. [CrossRef]
10. Rwomushana, I.; Day, R.; Gonzalez-moreno, P. *Evidence Note Tomato Leafminer Impacts and Coping Strategies for Africa*; CABI: Wallingford, UK, 2019.
11. Marete, G.M.; Lalah, J.O.; Mputhia, J.; Wekesa, V.W. Pesticide usage practices as sources of occupational exposure and health impacts on horticultural farmers in Meru County, Kenya. *Heliyon* **2021**, *7*, e06118. [CrossRef]

12. Mama Sambo, S.; Ndlela, S.; Plessis, H.; Obala, F.; Mohamed, S.A. Identification, Microhabitat, and Ecological Niche Prediction of Two Promising Native Parasitoids of *Tuta absoluta* in Kenya. *Insect* **2022**, *16*, 496. [CrossRef] [PubMed]
13. Salas Gervassio, N.G.; Aquino, D.; Vallina, C.; Biondi, A.; Luna, M.G. A re-examination of *Tuta absoluta* parasitoids in South America for optimized biological control. *J. Pest. Sci.* **2019**, *92*, 1343–1357. [CrossRef]
14. Agbessenou, A.; Akutse, K.S.; Yusuf, A.A.; Ekesi, S.; Subramanian, S.; Khamis, F.M. Endophytic fungi protect tomato and nightshade plants against *Tuta absoluta* (Lepidoptera: Gelechiidae) through a hidden friendship and cryptic battle. *Sci. Rep.* **2020**, *10*, 22195. [CrossRef]
15. Akutse, K.S.; Subramanian, S.; Khamis, F.M.; Ekesi, S.; Mohamed, S.A. Entomopathogenic fungus isolates for adult *Tuta absoluta* (Lepidoptera: Gelechiidae) management and their compatibility with Tuta pheromone. *J. Appl. Entomol.* **2020**, *144*, 777–787. [CrossRef]
16. Aigbedion-Atalor, P.O.; Hill, M.P.; Ayelo, P.M.; Ndlela, S.; Zalucki, M.P.; Mohamed, S.A. Can the combined use of the mirid predator *Nesidiocoris tenuis* and a braconid larval endoparasitoid *Dolichogenidea gelechiidivoris* improve the biological control of *Tuta absoluta*? *Insects* **2021**, *12*, 1004. [CrossRef] [PubMed]
17. Benjamin, E.O.; Wesseler, J.H.H. A socioeconomic analysis of biocontrol in integrated pest management: A review of the effects of uncertainty, irreversibility and flexibility. *NJAS-Wageningen J. Life Sci.* **2016**, *77*, 53–60. [CrossRef]
18. Farrar, J.J.; Baur, M.E.; Elliott, S.F. Measuring IPM impacts in California and Arizona. *J. Integr. Pest. Manag.* **2016**, *7*, 1–6. [CrossRef]
19. van Lenteren, J.C. The state of commercial augmentative biological control: Plenty of natural. *BioControl* **2018**, *57*, 1–20. [CrossRef]
20. Aigbedion-Atalor, P.O.; Abuelgasim, S.; Hill, M.P.; Zalucki, M.P.; Azrag, A.G.A.; Srinivasan, R.; Ekesi, S. Host stage preference and performance of *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae), a candidate for classical biological control of *Tuta absoluta* in Africa. *Biol. Control.* **2020**, *144*, 104215. [CrossRef]
21. Morales, J.A.; Muñóz, L.Y.; Rodriguez Caicedo, D.; Cantor, F. Combined Action of Sex Pheromone and Wasp *Apanteles gelechiidivoris* in Greenhouse Tomato Crops. *Acta Biol Colomb* **2014**, *19*, 175–184. [CrossRef]
22. Mohamed, S.A.; Dubois, T.; Azrag, A.G.A.; Ndlela, S.; Neuenschwander, P. Classical biological of key horticultural pests in Africa: Successes, challenges, and opportunities. *Curr. Opin. Insect Sci.* **2022**, *53*, 100945. [CrossRef] [PubMed]
23. Aigbedion-Atalor, P.O.; Hill, M.P.; Azrag, A.G.A.; Zalucki, M.P.; Mohamed, S.A. Disentangling thermal effects using life cycle simulation modelling on the biology and demographic parameters of *Dolichogenidea gelechiidivoris*, a parasitoid of *Tuta absoluta*. *J. Therm. Biol.* **2022**, *107*, 103260. [CrossRef] [PubMed]
24. Agboka, K.M.; Tonnang, H.E.Z.; Abdel-rahman, E.M.; Odindi, J.; Mutanga, O.; Mohamed, S.A. A Fuzzy-Based Model to Predict the Spatio-Temporal Performance of the *Dolichogenidea gelechiidivoris* Natural Enemy against *Tuta absoluta* under Climate Change. *Biology* **2022**, *11*, 1280. [CrossRef]
25. Abdel-baky, N.F.; Alhewairini, S.S.; Al-azzazy, M.M.; Qureshi, Z.; Al-deghairi, M.A.; Hajjar, J. Efficacy of *Metarhizium anisopliae* and *Beauveria bassiana* against *Tuta absoluta* (Lepidoptera: Gelechiidae) eggs under laboratory conditions. *Pakistan J. Agric. Sci.* **2021**, *58*, 743–750. [CrossRef]
26. Ndereyimana, A.; Nyalala, S.; Murerwa, P.; Gaidashova, S. Pathogenicity of some commercial formulations of entomopathogenic fungi on the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Egypt. J. Biol. Pest. Control.* **2019**, *29*, 70. [CrossRef]
27. Mantzoukas, S.; Denaxa, N.K. Efficacy of *Metarhizium anisopliae* and *Bacillus thuringiensis* against Tomato Leafminer *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae). *Curr. Agric. Res. J.* **2019**, *7*, 37–45. [CrossRef]
28. Contreras, A.J.; Mendoza, J.E.; Izquierdo, J.; Bielza, P.; Garci, L. Efficacy of Enthomopathogenic Fungus *Metarhizium anisopliae* Against *Tuta absoluta* (Lepidoptera: Gelechiidae). *Biol. Microb. Control.* **2014**, *107*, 121–124. [CrossRef]
29. Erasmus, R.; Van Den Berg, J.; du Plessis, H. Susceptibility of *Tuta absoluta* (Lepidoptera: Gelechiidae) Pupae to Soil Applied Entomopathogenic Fungal Biopesticides. *Insects* **2021**, *12*, 515. [CrossRef]
30. Zekeya, N.; Dubois, T.; Smith, J.; Ramasamy, S. Field effectiveness of *Metarhizium anisopliae* and pheromone traps against *Phthorimaea absoluta* on tomato in Tanzania. *Crop Prot.* **2022**, *156*, 105942. [CrossRef]
31. Nielsen, C.; Skovgård, H.; Steenberg, T. Effect of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) on survival and reproduction of the filth fly parasitoid, *Spalangia cameroni* (Hymenoptera: Pteromalidae). *Environ. Entomol.* **2005**, *34*, 133–139. [CrossRef]
32. Labbé, R.M.; Gillespie, D.R.; Cloutier, C.; Brodeur, J. Compatibility of an entomopathogenic fungus with a predator and a parasitoid in the biological control of greenhouse whitefly. *Biocontrol. Sci. Technol.* **2009**, *19*, 429–446. [CrossRef]
33. Rännbäck, L.M.; Cotes, B.; Anderson, P.; Rämert, B.; Meyling, N.V. Mortality risk from entomopathogenic fungi affects oviposition behavior in the parasitoid wasp *Trybliographa rapae*. *J. Invertebr. Pathol.* **2015**, *124*, 78–86. [CrossRef] [PubMed]
34. Potrich, M.; Alves, L.F.A.; Lozano, E.R.; Bonini, A.K.; Neves, P.M.O.J. Potential Side Effects of the Entomopathogenic Fungus *Metarhizium anisopliae* on the Egg Parasitoid *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) under Controlled Conditions. *J. Econ. Entomol.* **2017**, *110*, 2318–2324. [CrossRef]
35. Mas, N.G.; Medina, M.C.; Sánchez, F.G.; Moraga, E.Q. Bottom-up effects of endophytic *Beauveria bassiana* on multitrophic interactions between the cotton aphid, *Aphis gossypii*, and its natural enemies in melon. *J. Pest. Sci.* **2019**, *92*, 1271–1281. [CrossRef]
36. Wu, S.; Gao, Y.; Xu, X.; Goettel, M.S.; Lei, Z. Compatibility of *Beauveria bassiana* with *Neoseiulus barkeri* for Control of *Frankliniella occidentalis*. *J. Integr. Agric.* **2015**, *14*, 98–105. [CrossRef]

37. Fazeli-Dinan, M.; Talaei-Hassanloui, R.; Goettel, M. Virulence of the entomopathogenic fungus *Lecanicillium longisporum* against the greenhouse whitefly, *Trialeurodes vaporariorum* and its parasitoid *Encarsia formosa*. *Int. J. Pest. Manag.* **2016**, *62*, 251–260. [CrossRef]
38. Miranda-fuentes, P.; Quesada-moraga, E.; Aldebis, H.K.; Yousef-naef, M. Compatibility between the endoparasitoid *Hyposoter didymator* and the entomopathogenic fungus *Metarhizium brunneum*: A laboratory simulation for the simultaneous use to control *Spodoptera littoralis*. *Pest. Manag. Sci.* **2019**, *76*, 1060–1070. [CrossRef]
39. Domingues, M.M.; Becchi, L.K.; Graziele, S.; Velozo, M.; de Souza, A.R.; Barbosa, L.R.; Soares, M.A.; Serrão, J.E.; Zanuncio, J.C.; Wilcken, C.F. Selectivity of mycoinsecticides and a pyrethroid to the egg parasitoid *Cleruchoides noackae* (Hymenoptera: Mymaridae). *Sci. Rep.* **2020**, *10*, 14617. [CrossRef]
40. Goettel, M.S.; Inglis, D.G. Fungi: Hyphomycetes. In *Manual of Techniques in Insect Pathology*; Lacey, L.A., Ed.; Academic Press: London, UK, 1997; pp. 213–249.
41. Mama Sambo, S.; Ndlela, S.; Plessis, H.; Obala, F.; Mohamed, S.A. Ratio dependence effects of the parasitoid *Dolichogenidea gelechiidivoris* on its associated host *Tuta absoluta*. *Biocontrol. Sci. Technol.* **2022**, *32*, 497–510. [CrossRef]
42. Wamiti, L.G.; Khamis, F.M.; Abd-Alla, A.M.M.; Ombura, F.L.O.; Akutse, K.S.; Subramanian, S.; Odiwuor, S.O.; Ochieng, S.J.; Ekesi, S.; Maniania, N.K. *Metarhizium anisopliae* infection reduces *Trypanosoma congolense* reproduction in *Glossina fuscipes fuscipes* and its ability to acquire or transmit the parasite. *BMC Microbiol.* **2018**, *18*, 272–292. [CrossRef]
43. Park, E.; Cho, M.; Ki, C.S. Correct use of repeated measures analysis of variance. *Korean J. Lab. Med.* **2009**, *29*, 1–9. [CrossRef]
44. Bathke, A.C.; Friedrich, S.; Pauly, M.; Konietschke, F.; Staffen, W.; Strobl, N.; Höller, Y. Testing Mean Differences among Groups: Multivariate and Repeated Measures Analysis with Minimal Assumptions. *Multivariate Behav. Res.* **2018**, *53*, 348–359. [CrossRef] [PubMed]
45. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2018. Available online: https//www.R-project.org/ (accessed on 2 January 2022).
46. Hajek, A.E.; Delalibera, I. Fungal pathogens as classical biological control agents against arthropods. *Ecol. Fungal. Entomopathog.* **2009**, *55*, 147–158. [CrossRef]
47. Magda, S.M.; Soliman, N. Evaluations of Two *Metarhizium* varieties against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Egypt. *Int. J. Sci. Res.* **2014**, *3*, 2067–2073. [CrossRef]
48. Alikhani, M.; Safavi, S.A.; Iranipour, S. Effect of the entomopathogenic fungus, *Metarhizium anisopliae* (Metschnikoff) sorokin, on demographic fitness of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Egypt. J. Biol. Pest. Control.* **2019**, *29*, 23. [CrossRef]
49. Abd El-Ghany, N.M.; Abdel-Razek, A.S.; Ebadah, I.M.A.; Mahmoud, Y.A. Evaluation of some microbial agents, natural and chemical compounds for controlling tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *J. Plant Prot. Res.* **2016**, *56*, 372–379. [CrossRef]
50. Nozad-Bonab, Z.; Hejazi, M.J.; Iranipour, S.; Arzanlou, M.; Biondi, A. Lethal and sublethal effects of synthetic and bio-insecticides on *Trichogramma brassicae* parasitizing *Tuta absoluta*. *PLoS ONE* **2021**, *16*, e0243334. [CrossRef]
51. Presa-Parra, E.; Hernández-Rosas, F.; Bernal, J.S.; Valenzuela-González, J.E.; Martinez-Tlapa, J.; Birke, A. Impact of *Metarhizium robertsii* on adults of the parasitoid *Diachasmimorpha longicaudata* and parasitized *Anastrepha ludens* larvae. *Insects* **2021**, *12*, 125. [CrossRef]
52. Ayelo, P.M.; Mohamed, S.A.; Chailleux, A.; Yusuf, A.A.; Pirk, C.W.W.; Deletre, E. The parasitoid *Dolichogenidea gelechiidivoris* eavesdrops on semiochemicals from its host *Tuta absoluta* and tomato. *J. Pest. Sci.* **2022**, *95*, 633–652. [CrossRef]
53. Rodriguez, M.; Gerding, M.; France, A. Efectividad de Aislamientos de Hongos Entomopatógenos Sobre Larvas de Polilla del Tomate *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae). *Agric. Técnica* **2006**, *66*, 159–165. [CrossRef]
54. Fransen, J.J.; van Lenteren, J.C. Survival of the parasitoid *Encarsia formosa* after treatment of parasitized greenhouse whitefly larvae with fungal spores of *Aschersonia aleyrodis*. *Entomol. Exp. Appl.* **1994**, *71*, 235–243. [CrossRef]
55. Ramos Aguila, L.C.; Akutse, K.S.; Ashraf, H.J.; Bamisile, B.S.; Lin, J.; Dai, J.; Wang, H.; Wang, L. The survival and parasitism rate of *Tamarixia radiata* (Hymenoptera: Eulophidae) on its host exposed to *Beauveria bassiana* (Ascomycota: Hypocreales). *Agronomy* **2021**, *11*, 1496. [CrossRef]
56. Mesquita, A.L.M.; Lacey, L.A. Interactions among the Entomopathogenic Fungus, *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes), the Parasitoid, *Aphelinus asychis* (Hymenoptera: Aphelinidae), and Their Aphid Host. *Biol. Control.* **2001**, *22*, 51–59. [CrossRef]
57. Mnyone, L.L.; Koenraadt, C.J.M.; Lyimo, I.N.; Mpingwa, M.W.; Takken, W.; Russell, T.L. Anopheline and culicine mosquitoes are not repelled by surfaces treated with the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*. *Parasit. Vectors* **2010**, *3*, 2–7. [CrossRef] [PubMed]
58. Mburu, D.M.; Ochola, L.; Maniania, N.K.; Njagi, P.G.N.; Gitonga, L.M.; Ndung'u, M.W.; Wanjoya, A.K.; Hassanali, A. Relationship between virulence and repellency of entomopathogenic isolates of *Metarhizium anisopliae* and *Beauveria bassiana* to the termite *Macrotermes michaelseni*. *J. Insect Physiol.* **2009**, *55*, 774–780. [CrossRef] [PubMed]
59. Araujo, E.S.; Poltronieri, A.S.; Poitevin, C.G.; Mirás-Avalos, J.M.; Zawadneak, M.A.C.; Pimentel, I.C. Compatibility between Entomopathogenic Fungi and Egg Parasitoids (Trichogrammatidae): A Laboratory Study for Their Combined Use to Control *Duponchelia fovealis*. *Insects* **2020**, *11*, 630. [CrossRef]

CHAPTER 8

Dispersion of *Dolichogenidea gelechiidivoris* in open-field tomato in central Kenya and its performance in combination with *Metarhizium anisopliae* under greenhouse conditions

Citation:

Prepared for submission to *Entomologia Generalis (Schweizerbart Science Publishers)*

Dispersion of *Dolichogenidea gelechiidivoris* in open-field tomato in central Kenya and its performance in combination with *Metarhizium anisopliae* under greenhouse conditions

Short title**:** Exotic parasitoid field effectiveness

Sahadatou Mama Sambo[1,2*], Shepard Ndlela[1], Hannalene du Plessis[2], Komivi S. Akutse[1] and Samira A. Mohamed[1*]

[1]International Centre of Insect Physiology and Ecology (*icipe*), Nairobi P.O. Box 30772-00100, Kenya.

[2]Unit for Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa.

Correspondence: sfaris@icipe.org or smama@icipe.org P.O. BOX 30772-00100, Nairobi, Kenya.

Sahadatou Mama Sambo, smama@icipe.org, *icipe*, P.O. BOX 30772-00100, Nairobi, Kenya, +254757420186

Shepard Ndlela, sndlela@icipe.org, *icipe*, P.O. BOX 30772-00100, Nairobi, Kenya, +254741749993

Hannalene du Plessis, Hannalene.DuPlessis@nwu.ac.za, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa +27844910102

Komivi S. Akutse, Kakutse@icipe.org, *icipe*, P.O. BOX 30772-00100, Nairobi, Kenya, +254799716741

Samira A. Mohamed, sfaris@icipe.org, *icipe*, P.O. BOX 30772-00100, Nairobi, Kenya, +254769228569

Abstract

The parasitoid *Dolichogenidea gelechiidivoris* (Marsh) (Hymenoptera: Braconidae) and the entomopathogenic fungus, *Metarhizium anisopliae* (Metschnikoff), have been identified as promising agents for biocontrol of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). This study evaluated the greenhouse performance of *D. gelechiidivoris* as a standalone and used in combination with *M. anisopliae*. The five treatments consisted of two *D. gelechiidivoris* release frequencies, *viz*. weekly and monthly releases, monthly releases of *D. gelechiidivoris* in combination with fungus-contaminated pheromone trap placements, monthly placement of fungus-contaminated pheromone traps alone, and an untreated control treatment. *Dolichogenidea gelechiidivoris* dispersal ability was also evaluated in the open field by releasing 500 couples and monitoring their spread at different distances from the release point after 24h, five months, and one year after release. In the experimental greenhouse, we found no difference in percentage of leaves mined between the different treatments, except for the untreated treatment where plants were 100% infested from 8 weeks after transplanting. The highest parasitism by *D. gelechiidivoris* (86%) occurred under weekly releases of parasitoids for 12 weeks. The highest marketable yield (70%) was achieved with the combined use of *M. anisopliae* and monthly releases of *D. gelechiidivoris* compared to monthly and weekly parasitoid releases, and the *M. anisopliae* application alone. *Dolichogenidea gelechiidivoris* dispersed well, with parasitoids recovered 1.8 and 4.4 km from the release point within five- and 12-months post-release, respectively. Results from this study provided evidence that *D. gelechiidivoris* is established in Kenya and that this parasitoid can be combined with the entomopathogenic fungus, *M. anisopliae* for effective management of *T. absoluta* in tomato production.

Keywords: invasive pest, exotic parasitoid, entomopathogen fungus, Integrated Pest Management, parasitoid establishment

1 Introduction

Tomato production has been threatened by the tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in its invaded areas since 2006 (Biondi et al. 2018). Tomato production is drastically reduced by the larvae consuming the mesophyll of leaves, stems, and fruits, resulting in fruit that are unsuitable for trade (Desneux et al. 2011). Endemic natural enemy species of *T. absoluta* have been investigated but economic thresholds using these species have not been determined for the control of this pest (Mansour et al. 2018; Mama Sambo, et al. 2022a). The performance of *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) under greenhouse tomato production has been evaluated in different invaded areas (Chailleux et al. 2013; Zouba et al. 2013; Cherif et al. 2019). *Trichogramma* spp. are, however, generalist parasitoids of various lepidopteran species (Brotodjojo & Walter, 2006; Laurentis et al. 2019), with varying performance due to the learning capability of these species to new cues (Gonthier et al. 2022).

An alternative solution is to introduce exotic parasitoid species for biological control of *T. absoluta*. It has been initiated in East Africa by importing the natural enemy, *Dolichogenidea gelechiidivoris* (Marsh) (Hymenoptera: Braconidae) from the pests' origin (Aigbedion-Atalor et al. 2020). *Dolichogenidea gelechiidivoris* is indigenous to Peru, South America, and it is a specialist parasitoid of Gelechiidae, mainly *T. absoluta* and *Phthorimaea operculella* (Zeller) (Bajonero et al. 2008; Aigbedion-Atalor et al. 2020). It is considered as the most important parasitoid for natural and augmentative biological control in Colombian tomato crops with parasitism of *T. absoluta* as high as 90% (Morales et al. 2014). Despite the known importance of

D. gelechiidivoris as a biocontrol agent, there are no studies that confirm its effectiveness, as well as information related to the conditions or factors that affect its effectiveness in areas outside its native area.

Approximately 10% of natural enemy introductions succeed in establishing in new areas and in many of these cases, the population progressively reduces and disappears (Cock, 2016). Several reasons affecting parasitoid establishment and performance have been suggested (Stiling, 1990; Tscharntke et al. 2016; Seehausen et al. 2021). According to Stiling (1990), characteristics of both the host insect as well as the parasitoid play a role in parasitoid establishment. For the host, these include the order it belongs to, its feeding location, voltinism, origin (invasive/native), diet (polyphagy/monophagy), and habitat. Parasitoid characteristics such as climate adaptation and feeding location (ectophagy/endophagy) are also important (Stiling, 1990). The success rate in control of the pest increases with an increase in the number of introductions of biological control agents (Seehausen et al. 2021). Another explanation for the failure of establishment is the composition of the landscape surrounding the farm (Tscharntke et al. 2016). Contact with specific semiochemical cues by parasitoids during mass rearing, age of the natural enemy released, transportation delays on the way to release sites, and premature termination of projects could also affect the success of the process (Beirne, 1984; Mohamed, et al. 2022).

Biological control can be strengthened by complementarity among natural enemies, e.g. if multiple enemies attack a pest during different periods of its occurrence in the field, or different stages of the lifecycle (Jonsson et al. 2017). The selection of the most appropriate combination of arthropods as natural enemies for *T. absoluta* control in tomato fields will depend on their pest suppression capacity when used alone, or in an integrated pest management (IPM) scenario. *Tuta* *absoluta* is also susceptible to various entomopathogenic fungi (Agbessenou et al. 2021; Akutse et al. 2020; Ndereyimana et al. 2019). The entomopathogenic fungus, *Metarhizium anisopliae* is pathogenic to both the adult and larval stages of this pest (Akutse et al. 2020). An additive effect in the control of *T. absoluta* was reported by Mama Sambo et al. (2022b) with the combined use of *D. gelechiidivoris* and *M. anisopliae*. The technique to auto-disseminate the *M. anisopliae*-infection in a pest population through a trap containing the fungus and the pest being lured to a trap was described by (Migiro et al. 2010). It can be applied to *T. absoluta* also, but the attraction of *D. gelechiidivoris* females to the *T. absoluta* lure (Ayelo et al. 2021) is unknown. It may have a negative effect on the performance of the parasitoid. Thus, the aim of this study was to develop an efficient method to control *T. absoluta* by releasing *D. gelechiidivoris* at different frequencies, using a lure trap contaminated with fungus, as well as combined use of the parasitoid and the lure trap contaminated with fungus in greenhouses. Additionally, the parasitoid dispersion in an open field area where it was released was assessed.

2 Material and methods

2.1 Tomato planting

Tomato seedlings (cv. Moneymaker) were transplanted in pots (37 cm in diameter) containing soil mixed with goat manure. Three seedlings were planted per pot and four pots were placed in a greenhouse (115 (L) × 115 (W) × 190 cm (H)) covered with a fine mesh for aeration, under environmental conditions. The pots were watered and weeded regularly. The experiment was conducted between December 2021 and May 2022.

2.2 Insect production

Tuta absoluta were obtained by means of collection of infested tomato leaves from from Nakuru and Kirinyaga. Parasitoids were obtained from the colony kept at the International Center of Insect Physiology and Ecology (*icipe*) Nairobi, Kenya, which was initially imported in 2017 from the International Potato Center (CIP), Peru. Both *T. absoluta* and *D. gelechiidivoris* were reared using the method described by Mama Sambo et al. (2022c). *Tuta absoluta* was reared on four-week-old tomato plant. The parasitoid was reared on *T. absoluta* larvae present in plants after they were exposed to the parasitoids while in the 1st larval stage.

2.3 *Metarhizium anisopliae* ICIPE 20 mass production

Metarhizium anisopliae ICIPE 20 was mass-produced on a rice substrate. A liquid media was prepared with 2% dextrose, 1% peptone, 0.25% yeast extract, and 250 ml distilled water in a 1L conical flask. The flasks containing the mixture were then sterilized at 121°C for 15-20 min in an autoclave. After cooling at room temperature, 0.02% antibacterial chloramphenicol followed by 0.05 g of *M. anisopliae* ICIPE 20 dry conidia were added to the broth, and the flasks were continuously shaken for 4 days in a refrigerated orbital shaker (New Brunswick Scientific™ Innova ™ 44, Germany) at 25 ± 2°C and 100 rpm. Pishori rice (2.5 kg) was washed for 30-45 minutes until all the starch was removed. The water was drained from the rice for 10-15 minutes before the rice was placed into Milner bags (60 cm long × 35 cm wide), which were filled with air and sealed with an electric sealer following the technique described by Maniania, (1998). These rice-containing bags were autoclaved and sterilized at 121°C for 1 hour and cooled down at room temperature (Opisa et al. 2019). A small cut was made in each bag and 5 ml of the mycelia solution was added. The bags were re-sealed using an electric sealer before they were vigorously shaken to homogeneously mix the mycelia with rice grains (Jenkins et al. 1998). The inoculated bags were placed in a tray at 20–26°C and 40%–70% relative humidity (RH) and shaked and manipulated 48-hourly until the fungus matured. Conidia from these bags were harvested 30 days after inoculation. Conidial viability was determined before application in the field, by spreading and incubation on Sabouraud Dextrose Agar media plates as described by Goettel and Inglis (1997). The viability of the harvested conidia was more than 98%. The dry conidia were stored at 4 °C for the period of application in the greenhouses.

2.4 Application of the treatment in the greenhouse

The bioassay was performed at the Duduville campus of the *icipe* (1°13'18.3"S 36°53'48.1"E; Altitude 1,604m) in greenhouses as described above for tomato planting. Three weeks after tomato seedling transplanting, 20 *T. absoluta* male-female pairs were released into each of the greenhouses. At four weeks after transplanting, when *T. absoluta* mines were observed, a cohort of 15 pairs of naïve mated parasitoids were released. A week later, one delta trap (Tutrack, Kenya Biologics, Nairobi) to which a velvet fabric was secured (to retain the dry conidia), was placed per greenhouse. Dry *M. anisopliae* conidia were weighed (3 g) and transported in a piece of aluminum foil to the greenhouse. The fungus was spread homogeneously on the velvet fabric using a camel-hair brush. *Tuta absoluta* pheromone lures (3E, 8Z, 11Z-tetradecatrien-1-ylacetate and 3E, 8Z-tetradecadien-1-yl acetate) were placed inside the traps. The traps were hung in the respective greenhouses without making contact with any foliage. Three replicates were done per treatment. The five treatments were as follows:

- T1 = Untreated, with no biocontrol method;
- T2 = Pheromone trap contaminated with dry *M. anisopliae* ICIPE 20 conidia replaced

every month for four months;

- T3 = Weekly release of *D. gelechiidivoris* for 12 weeks;
- T4 = Monthly release of *D. gelechiidivoris* released for four months;
- T5 = Monthly release of *D. gelechiidivoris* in combination with pheromone trap contaminated with dry *M. anisopliae* ICIPE 20 conidia replace every month for 4 months.

2.5 Data collection

Five leaves (i.e. a branch) were randomly sampled per plant from each greenhouse, at two-week intervals. The leaves from the middle part were sampled since they are the most infested (Da Silva Galdino et al. 2015). Percentage damage was calculated as (the number of leaflets with mines/ the total number ofleaflets) × 100. Tomato leaves with approximately 10 *T. absoluta* larvae were additionally sampled. The collected leaves were placed in plastic containers in the laboratory and moths and parasitoids that emerged were recorded. Parasitism by *D. gelechiidivoris* was calculated as number of *D. gelechiidivoris/*(number of *T. absoluta* + total number of *T. absoluta* parasitoid emerged) ×100. The percentage of marketable fruit was calculated as: (number of *T. absoluta*-undamaged fruits/total of fruits harvested) ×100. Monitoring commenced one week after the installation of the traps containing *M. anisopliae* and two weeks after the first release of parasitoids.

2.6 *Dolichogenidea gelechiidivoris* dispersion

In October 2020, 500 *D. gelechiidivoris* couples were transported to a farmer field in Kirinyaga south subcounty (S 00.63222°, E 037.38222°, Alt 1194 m; 1 ha in size) in a net cage with honey droplets provided as food. The parasitoids were released at the center of the farms. The tomato farm was an *icipe* demonstration plot at flowering stage where IPM strategies were used to control *T. absoluta* infestation. Moreover, before the release, the infested material collection was done regularly from that farm and all the tomato farms in that region to supplement laboratory colonies, pest density monitoring, and native parasitoids monitoring. *Dolichogenidea gelechiidivoris* was not recorded at this site prior to this study. Five months after release, the first random sampling of infested tomato leaves was done in the county where the parasitoids were released. For each sampling time, the Global Positioning System (GPS) coordinate of each collection point was recorded. The collected leaves were incubated per collection point, and *D. gelechiidivoris* that emerged from infested *T. absoluta* were recorded. A second sampling was done one year after the parasitoids were released.

2.7 Data analysis

To assess the effect of the treatments on the percentage *T. absoluta* mine density and *D. gelechiidivoris* parasitism over time, the linear mixed-effects models, *lmer* function was used. Data on the percentage marketable fruits i.e., fruit without *T. absoluta* mines was tested for normality using the Shapiro-Wilk test. Since the data were found to be normally distributed and homogenous, it was subjected to analyses of variance (ANOVA), followed by Tukey's HSD post hoc tests to separate the means where significant differences between treatments occurred. The data were analyzed using the software R, version 3.5 (R Core Team, 2018). We used QGIS 3.10.6 (QGIS. ORG, Zürich, Switzerland) (QGIS.org, 2021) software to project sampling points and parasitoid recovery points in Kirinyaga county, Kenya to show *D. gelechiidivoris* dispersion.

3 Results

3.1 Effectiveness of the treatments

The percentage *T. absoluta* infested leaves and *D. gelechiidivoris* parasitism in the treatments with an absolute t-value >2, were significantly different (Table 1). The percentage *T. absoluta* infested leaves in all treatments were significantly lower compared to the untreated control (Table1). In all treatments, the percentage *T. absoluta* infested leaves reduced significantly from week 8 onwards compared to the untreated control (Fig. 1). The percentage *T. absoluta* larval parasitism by *D. gelechiidivoris* in the treatment where this parasitoid was released monthly in combination with a pheromone trap contaminated with dry *M. anisopliae* ICIPE 20 conidia (which was replace monthly for four months), was significantly lower compared to the treatment where the parasitoids were released weekly for a period of 12 weeks (Table 1). The percentage parasitism of *T. absoluta* larvae by *D. gelechiidivoris* in the treatment where *D. gelechiidivoris* was released monthly in combination with the use of a pheromone trap contaminated with dry *M. anisopliae* ICIPE 20 conidia, which was replaced every month for a period of four months, was comparable to the scenario where *D. gelechiidivoris* was released monthly for four months (Table1). Parasitism by *D. gelechiidivoris* remained high (24-86%) when *D. gelechiidivoris* was released weekly for a period of 12 weeks (Fig. 2). .

Table 1. Parameter estimates for treatments effect in relation to percentage of *Tuta absoluta* infested leaves and parasitism by *Dolichogenidea gelechiidivoris* (Linear Mixed Effects Model (*lmer*)).

Attribute	Parameter	Estimate	SE	t value
% *T. absoluta* infested leaves	Intercept	16.83	5.41	3.11
	T1 (untreated)	56.5	13.82	4.09
	T2 (Pheromone trap contaminated with dry *M. anisopliae* ICIPE 20 conidia every month for four months)	17.33	5.92	-2.93
	T4 (Monthly release of *D. gelechiidivoris* for four months)	12.37	6.18	2.00
	T5 (Monthly release of *D. gelechiidivoris* in combination with the use of a pheromone trap contaminated with dry *M. anisopliae* ICIPE 20 conidia, which was replaced every month for 4 months.	-2.83	5.40	-0.52
% Parasitism by *D. gelechiidivoris*	Intercept	20.95	6.41	3.27
	T3 (Weekly release of *D. gelechiidivoris* for 12 weeks)	34.86	9.41	3.70
	T4 (Monthly release of *D. gelechiidivoris* for a period of four months)	-5.84	6.61	-0.88

A negative t-value indicates a negative correlation.

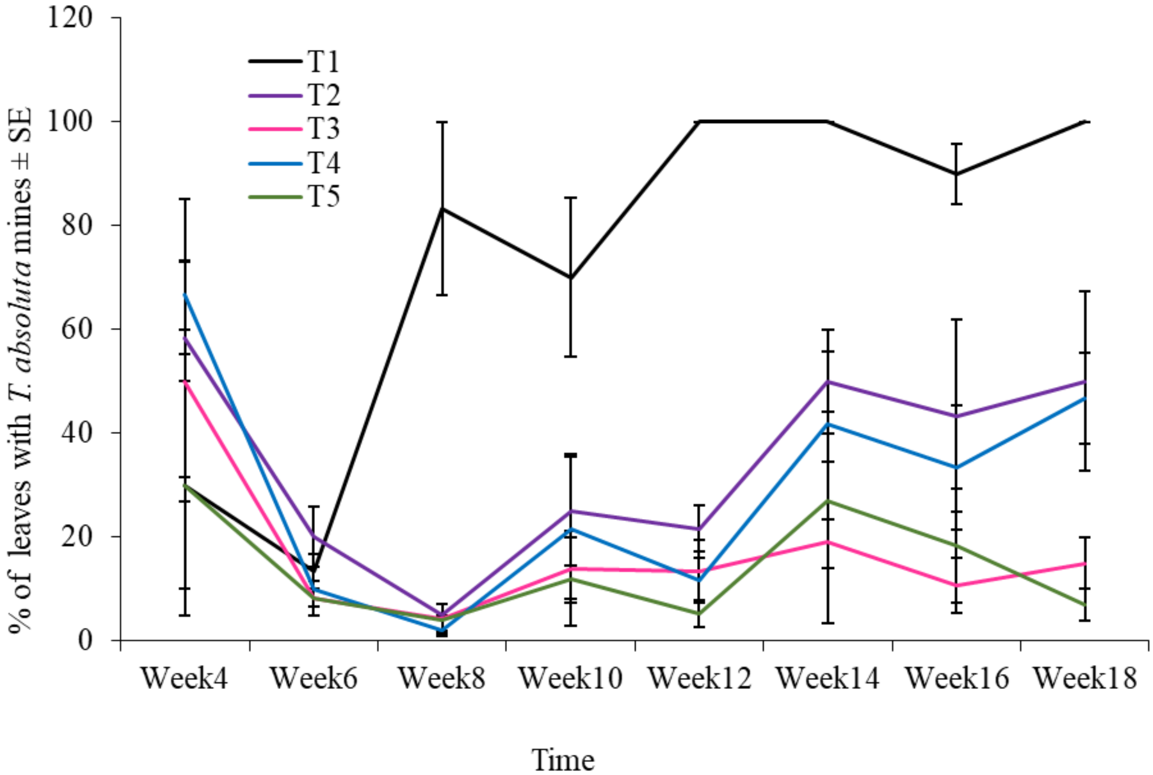


Fig. 1. Percentage *Tuta absoluta* infested leaves ± SE following application of the respective treatments: T1 = untreated, T2 = Pheromone trap contaminated with dry *M. anisopliae* ICIPE 20 conidia, which was replaced every month for a period of four months, T3 = Weekly release of *D. gelechiidivoris* for 12 weeks, T4 = Monthly release of *D. gelechiidivoris* for a period of four months, and T5 = Monthly release of *D. gelechiidivoris* in combination with the use of a pheromone trap contaminated with dry *M. anisopliae* ICIPE 20 conidia, which was replaced every month for a period of four months. Means followed by the same letters did not differ significantly (Tukey's HSD, α = 0.05).

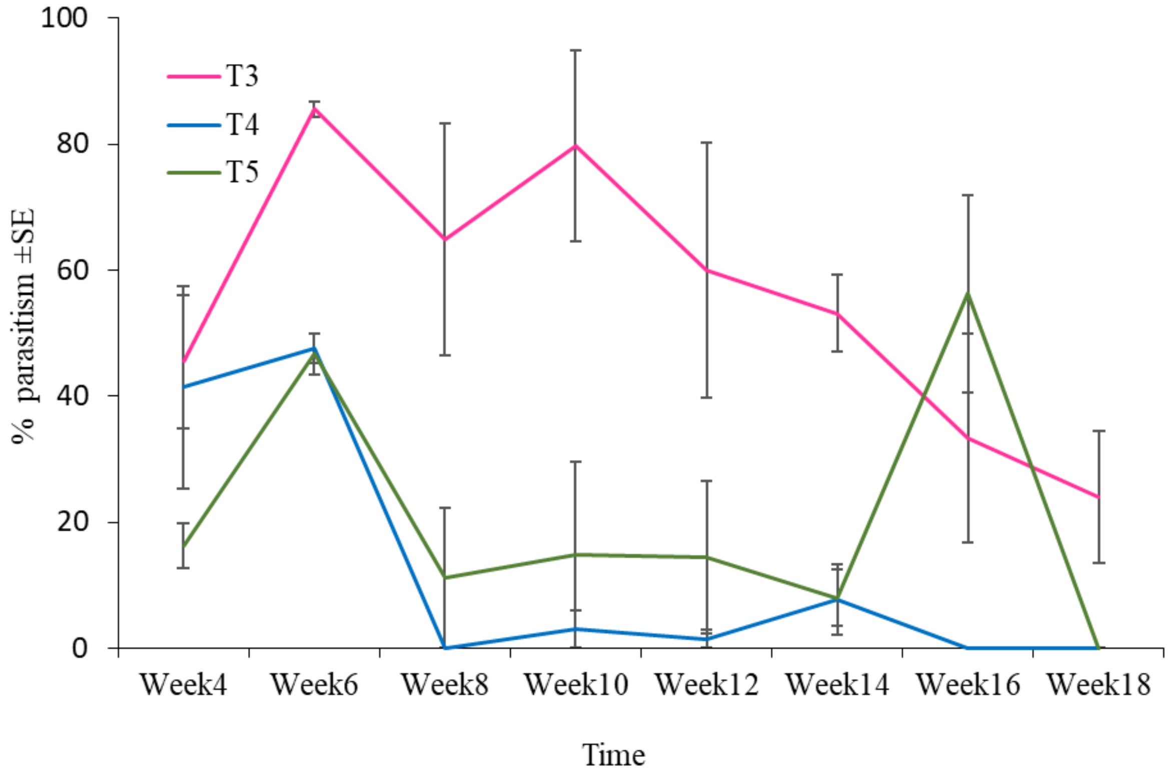


Fig. 2. Percentage *Tuta absoluta* larval parasitism by *Dolichogenidea gelechiidivoris* ± SE following application of the respective treatments: T3 = Weekly release of *D. gelechiidivoris* for 12 weeks, T4 = Monthly release of *D. gelechiidivoris* for a period of four months, and T5 = Monthly release of *D. gelechiidivoris* in combination with the use of a pheromone trap contaminated with dry *M. anisopliae* ICIPE 20 conidia, which was replaced every month for a period of four months. Means followed by the same letters did not differ significantly (Tukey's HSD, $\alpha = 0.05$).

The percentage marketable fruit estimated from the number of fruits harvested and the *T. absoluta* infested fruit varied significantly between the treatments ($F_{4, 10} = 6.07$, $P = 0.001$). The highest percentage yield was achieved with the combination of *D. gelechiidivoris* released and placement of a pheromone trap contaminated with dry *M. anisopliae* ICIPE 20 conidia (Fig. 3).

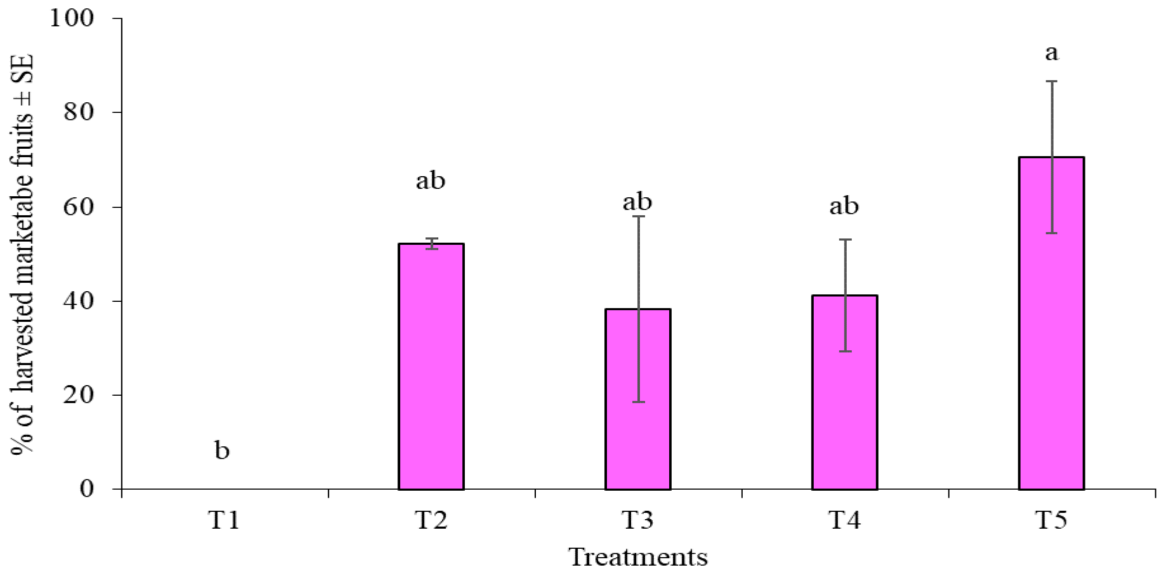
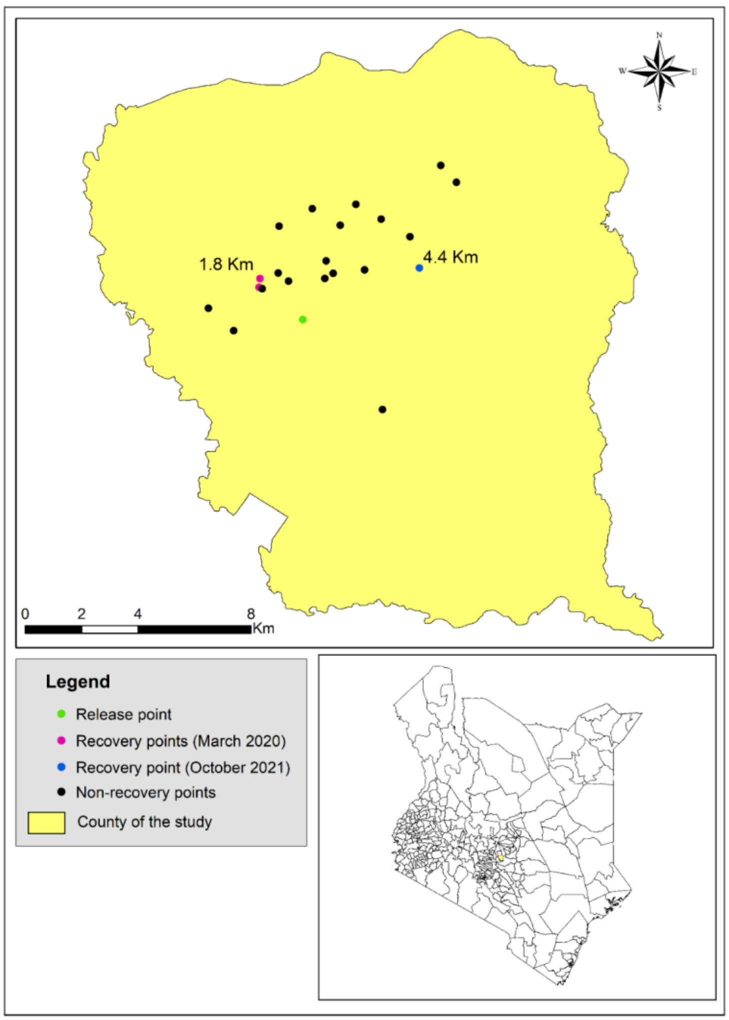


Fig. 3. Percentage of non-infested tomato fruit harvested from greenhouses following application of the respective treatments: T1 = untreated, T2 = Pheromone trap contaminated with dry *M. anisopliae* ICIPE 20 conidia, which was replaced every month for a period of four months, T3 = Weekly release of *D. gelechiidivoris* for 12 weeks, T4 = Monthly release of *D. gelechiidivoris* for a period of four months, and T5 = Monthly release of *D. gelechiidivoris* in combination with the use of a pheromone trap contaminated with dry *M. anisopliae* ICIPE 20 conidia, which was replaced every month for a period of four months. Means followed by the same letters did not differ significantly (Tukey's HSD, α = 0.05).

3.2 *Dolichogenidea gelechiidivoris* dispersion

Five months after *D. gelechiidivoris* parasitoids were released, parasitized *T. absoluta* larvae were found up to 1.8 km from the point of release (Fig. 4). One year after release, the parasitoids were reared from *T. absoluta* larvae collected 4.4 km from the initial point of release (Fig. 4). Dispersion towards the north-western and north-eastern direction was observed in the release field (Fig. 4).



.

Fig. 4. Recovery of *D. gelechiidivoris* five months and one year after release at Kirinyaga south subcounty, Kenya.

4 Discussion

Our findings confirmed the success of *D. gelechiidivoris* as a biological control agent of *T. absoluta* as well as its compatibility with, and use in combination with the entomopathogenic fungus, *M. anisopliae* ICIPE 20 contaminated to a pheromone trap. The successful establishment of *D. gelechiidivoris* in Kenya was also confirmed. Several cases of successful establishment of other introduced natural enemies and their success as biocontrol agents in the areas invaded by co-evolved pests have been reported (Cock, 2016; Mohamed et al. 2022; Ndiaye et al. 2015). However, a single natural enemy of a pest often fails to reduce the pest to levels below the economic injury level (Chailleux et al. 2013; Farrar et al. 2016; Labbé et al. 2009; Mas et al. 2019). A variety of control measures, combined in an IPM strategy for control of *T. absoluta* should be promoted.

In this study, *D. gelechiidivoris* release frequencies and the combination of parasitoid releases with the use of a *T. absoluta* pheromone trap contaminated with *M. anisopliae* ICIPE 20, did not affect the percentage of *T. absoluta* infested leaves in general. Morales et al. (2014) reported similar results, with the number of mined leaves, which were similar with the release of *D. gelechiidivoris* only, and where *D. gelechiidivoris* parasitoids were released in combination with *T. absoluta* pheromone traps. Weekly releases of 20 and 30 *Trichogramma cacoeciae* (Marchal) (Hymenoptera: Trichogrammatidae)/plant was equally effective and significantly decreased *T. absoluta* densities in greenhouses (Cherif et al., 2019). Successful control of *T. absoluta* with *Trichogramma* spp. was also reported by Zouba et al. (2013), with a reduction in leaf damage of 78.89% and 87.62% by *T. cacoeciae* and *Trichogramma bourarachae* Pintureau & Babault, (Hymenoptera: Trichogrammatidae) respectively after inundative releases of 25000 parasitoids/week in a greenhouse. A positive correlation was also reported between the number of *Trichogramma euproctidis* Girault (Hymenoptera: Trichogrammatidae) or *T. achaeae* Nagaraja & Nagarkatti parasitoids released at 25, 50, and 75 parasitoids/m^2 and the level of *T. absoluta* control (El-Arnaouty et al., 2014).

In the present study, the highest percentage parasitism rate by *D. gelechiidivoris* (24-86%) was recorded when the parasitoids were released every week for a period of 12 weeks. Monthly parasitoid releases for a period of four months, were similar for parasitoids released in association with or without the *M. anisopliae* ICIPE 20-contaminated trap. Several authors reported on the performance of *D. gelechiidivoris* in controlling Gelechiidae species (Bajonero et al. 2008; Morales et al. 2014, Aigbedion-Atalor et al. 2020; Mama Sambo et al. 2022c). Morales et al. (2014) recorded up to 86.38 % *T. absoluta* larval parasitism with *Apanteles gelechiidivoris* (Hymenoptera: Braconidae) when this parasitoid was used in combination with a pheromone trap. Similarly, Mama Sambo et al. (2022b) reported 86% of 1st-instar larvae parasitism by *D. gelechiidivoris* under laboratory conditions. Various factors affect parasitism performance of parasitoids, which was evident from the lower percentage parasitism of *T. absoluta* larvae by *D. gelechiidivoris* in laboratory studies, reported by Bajonero et al. (2008) (75%), Aigbedion-Atalor et al. (2020) (50%), and Mama Sambo et al. (2022c) (59%) under scenarios that included differences in temperature, larval stages, host densities, and parasitoid density. However, Morales et al. (2013) reported more than 90% parasitism with 4, 8, and 12 *D. gelechiidivoris* females exposed to a *T. absoluta*-infested plant under laboratory conditions.

The release of *D. gelechiidivoris* in a greenhouse, in combination with a *T. absoluta* pheromone trap contaminated with *M. anisopliae* ICIPE 20, resulted in higher marketable tomato yield. A similar trend was reported by Mama Sambo et al. (2022b) where the combined use of the two biocontrol agents, *M. anisopliae* ICIPE 20 applied as a spray onto a *T. absoluta* infested host plant, followed by the release of *D. gelechiidivoris,* resulted in higher mortality of this pest, compared to use of the two agents individually. In contrast, the combination of *T. bactrae* released and mass trapping of *T. absoluta* resulted in higher tomato yields compared to a scenario where biopesticides (Biotrine and Fytomax) were used in combination with mass trapping, and with an insecticide application (Goda et al. 2015). Other combinations of biocontrol agents of *T. absoluta* under protected cropping that influenced tomato yield, included a combination of parasitoids and predators. For example, better *T. absoluta* control was achieved with a combination of *Macrolophus pygmaeus* Rambur (Hemiptera: Meridae) and the release of *T. achaeae* compared to the individual use of the respective agents (Chailleux et al. 2013). More uninfested fruit were harvested in a greenhouse where *T. evanescens* and *Nesidiocoris tenuis* (Reuter) (Hemiptera: Meridae) were released, compared to *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) alone and *N. tenuis* alone (Öztemiz, 2013). However, Mirhosseini et al. (2019), found the highest percentage of undamaged fruits, with *N. tenuis* regardless of the application of *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae).

Dolichogenidea gelechiidivoris was recovered a year after its first release in Kenya, at a distance of 4.4 km from the release area. Dispersion might have been achieved through wind or transportation of infested material. *Dolichogenidea gelechiidivoris* has also recently been recovered in the palearctic region (Denis et al. 2022; Krache et al. 2021). A steady increase in endoparasitism levels of *T. absoluta* larvae occurred during the tomato production season in 2020, in samples collected from tomato fields in northeastern Spain. By October, 21.8% of *T. absoluta* larvae were parasitized by Braconidae, all identified as *D. gelechiidivoris* (Denis et al. 2022). Although the speed of dispersal and spread of several introduced parasitoid species are available (Baoua et al. 2018; Bokonon-Ganta et al. 2013; Salazar-mendoza et al. 2020; Sallam et al. 2001), it has not been published for *D. gelechiidivoris.* The establishment of *Diachasmimorpha kraussii* (Fullaway) (Hymenoptera: Braconidae) in Hawaii for the control of *Bactrocera latifrons* (Hendel) (Diptera: Tephritidae) infesting turkey berry, *Solanum torvum* Swartz, (Solanaceae) was confirmed after three years, 5 km away from the release point (Bokonon-Ganta et al. 2013). The exotic *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), a larval endoparasitoid of stemborers, was recorded 64 m away from the point of its release after four days, with the number of parasitised larvae that decreased with distance (Sallam et al. 2001). In contrast, parasitism by both *Tetrastichus julis* (Walker) of *Oulema melanopus* (Linnaeus) (Coleoptera: Chrysomelidae) (Hymenoptera: Eulophidae) and *Bathyplectes curculionis* (Thomson) (Hymenoptera: Ichneumonidae) of *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) did not change with increasing distance (Evans, 2018). Parasitism by *Telenomus remus* Nixon (Hymenoptera: Platygastridae), a native parasitoid of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) eggs in Brazil, also declined linearly with increasing distance from the parasitoid release point, 48 hours post-release (Salazar-Mendoza et al. 2020). Parasitism levels of *Heliocheilus albipunctella* de Joannis (Lepidoptera: Noctuidae) larvae by *Habrobracon hebetor* Say (Hymenoptera: Braconidae) after augmentative releases in Burkina Faso and Niger, were initially higher at the site of dissemination compared to sites at 3 km and 5 km away. However, 5 weeks after release, parasitism of *H. albipunctella* 3 km away, was similar to those at the release point (Baoua et al. 2018). These findings indicate that for optimization of *D. gelechiidivoris* distribution in control of *T. absoluta*, the distance between release points needs to be taken into account. Since the parasitoid has now spread in Kenya. Decision-makers in the inhabited area could consider this parasitoid for classical biological control programs as an eco-friendly and economically sustainable management tool for the control of *T. absoluta*.

Control of *T. absoluta* in a greenhouse, expressed as an increase in marketable yield was similar between weekly and monthly *D. gelechiidivoris* releases. A once-off release of a substantial number of *D. gelechiidivoris,* will therefore be sufficient to provide adequate control for the duration of a crop cycle. If tomato or an alternative *T. absoluta* host plant such as potato or nightshade is planted in succession, the parasitoids will be able to survive and reproduce and no further releases will be necessary. However, cropping these crops in rotation, is not recommended since it will serve as host plants for the pest to persist and multiply between seasons. In this study, the parasitoid, *D. gelechiidivoris*, the *T. absoluta* pheromone lure, and entomopathogenic fungus, *M. anisopliae* ICIPE 20 were found to be compatible and can therefore be used in combination as biocontrol agents of *T. absoluta*. The combined use also resulted in the highest marketable tomato yield where it was applied.

The concomitant use of *D. gelechiidivoris* and insecticide application for control of *T. absoluta* should, however, be evaluated in the laboratory as well as in field trials. The cost-effectiveness of this technology should also be determined. The implementation of an IPM program taking into consideration more than one insect pest as well as diseases should be considered to achieve a higher marketable tomato yield.

Funding: This research was supported by the following organizations: Biovision Foundation grant number BV DPP-012/2019-2022, Norwegian Agency for Development Cooperation-(NORAD), the section for research, innovation and higher education grant number RAF-3058 KEN-18/0005, Scaling-up Climate-Smart Pest Management Approaches for Enhanced Maize and Tomato Systems Productivity in Eastern Africa (SCLAMP) grant number 81250394, the

Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); the Federal Democratic Republic of Ethiopia; and the Government of the Republic of Kenya. The first author was supported by the German Academic Exchange Service (DAAD) through the African Regional Postgraduate Programme in Insect Science (ARPPIS) tenable at *icipe*. The views expressed herein do not necessarily reflect the official opinion of the donors.

Acknowledgments: The authors recognize the technical assistance of Esther Awuor Owino, Omar Jimali Ibrahim, Alex Muthembwa, Francis Obala, Patrick Kipkorir Koech, all the African Fruit Fly Program (AFFP). The authors also appreciated the support of Sospeter Wafula at Arthropod Pathology Unit (APU).

References

Agbessenou, A., Akutse, K. S., Yusuf, A. A., Wekesa, S. W., Khamis, F. M. (2021). Temperature-dependent modelling and spatial prediction reveal suitable geographical areas for deployment of two Metarhizium anisopliae isolates for *Tuta absoluta* management. *Scientific Report*, *11*, 23346, doi:10.1038/s41598-021-02718-w.

Aigbedion-Atalor, P. O., Abuelgasim, S., Hill, M. P., Zalucki, M. P., Azrag, A. G. A., Srinivasan, R., & Ekesi, S. (2020). Host stage preference and performance of *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae), a candidate for classical biological control of *Tuta absoluta* in Africa. *Biological Control*, *144*, 104215. https://doi.org/10.1016/j.biocontrol.2020.104215

Akutse, K. S., Subramanian, S., Khamis, F. M., Ekesi, S., & Mohamed, S. A. (2020). Entomopathogenic fungus isolates for adult *Tuta absoluta* (Lepidoptera: Gelechiidae) management and their compatibility with Tuta pheromone. *Journal of Applied Entomology*, *144*(9), 777–787. https://doi.org/10.1111/jen.12812

Ayelo, P. M., Mohamed, S. A., Chailleux, A., Yusuf, A. A., Pirk, C. W. W., & Deletre, E. (2021). The parasitoid *Dolichogenidea gelechiidivoris* eavesdrops on semiochemicals from its host *Tuta absoluta* and tomato. *Journal of Pest Science*, *95,* 633–652. https://doi.org/https://doi.org/10.1007/s10340-021-01424-w

Bajonero, J., Córdoba, N., Cantor, F., Rodríguez, D., & Cure, R. (2008). Biology and life cicle of *Apanteles gelechiidivoris* (Hymenoptera: Braconidae) parasitoid of *Tuta absoluta*. *Agronomía Colombiana*, *26*(3), 417–426.

Baoua, I. B., Ba, M. N., Amadou, L., Kabore, A., & Dabire-binso, C. L. (2018). Field dispersal of the parasitoid wasp *Habrobracon hebetor* (Hymenoptera: Braconidae) following augmentative release against the millet head miner *Heliocheilus albipunctella* (Lepidoptera: Noctuidae) in the Sahel Field dispersal of the parasitoid wasp. *Biocontrol Science and Technology*, *28*(4), 404–415. https://doi.org/10.1080/09583157.2018.1450842

Beirne, B. P. (1984). Avoidable obstacles to colonization in classical biological control of insects. *Canadian Journal of Zoology*, *63*(4), 743–747. https://doi.org/10.1139/z85-108

Biondi, A., Guedes, R., Wan, F., & Desneux, N. (2018). Ecology, worldwide spread, and management of the invasive South American Tomato Pinworm, *Tuta absoluta*: Past, present, and future. *Annual Review of Entomology*, *63*, 239–258. https://doi.org/10.1146/annurev-ento-031616

Bokonon-Ganta, A. H., McQuate, G. T., Messing, R. H., & Jang, E. B. (2013). Release and establishment of the parasitoid *Diachasmimorpha kraussii* against the tephritid fruit fly

Bactrocera latifrons in Hawaii. *Journal of Insect Science*, *13*(7), 1–15. https://doi.org/10.1673/031.013.0701

Brotodjojo, R. R. R., & Walter, H. G. (2006). Oviposition and reproductive performance of a generalist parasitoid (*Trichogramma pretiosum*) exposed to host species that differ in their physical characteristics. *Biological Control*, *39*(3), 300–312. https://doi.org/10.1016/J.BIOCONTROL.2006.08.011

Chailleux, Anaïs, Biondi, A., Han, P., Tabone, E., & Desneux, N. (2013). Suitability of the Pest – Plant System *Tuta absoluta* (Lepidoptera: Gelechiidae)– Tomato for *Trichogramma* (Hymenoptera: Trichogrammatidae) Parasitoids and Insights for Biological Control. *Biological and Microbial Control*, *106*(6), 2310–2321. https://doi.org/https://doi.org/10.1603/EC13092

Cherif, A., Mansour, R., Attia-Barhoumi, S., Zappalà, L., & Grissa-Lebdi, K. (2019). Effectiveness of different release rates of *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae) against *Tuta absoluta* (Lepidoptera: Gelechiidae) in protected and open field tomato crops in Tunisia. *Biocontrol Science and Technology*, *29*(2), 149–161. https://doi.org/10.1080/09583157.2018.1542485

Cock, M. (2016). The impacts of some classical biological control successes. *CAB Reviews*, *10*(042), 1–58. https://doi.org/10.1079/pavsnnr201510042

Da Silva Galdino, T. V., Picanço, M. C., Ferreira, D. O., Silva, G. A. R., De Souza, T. C., & Silva, G. A. (2015). Is the performance of a specialist herbivore affected by female choices and the adaptability of the offspring? *PLoS ONE*, *10*(11): e0143389. https://doi.org/10.1371/journal.pone.0143389

Denis, C., Riudavets, J., Alomar, O., Agustí, N., Gonzalez-Valero, H., Cubí, M., … Arnó, J. (2022). Naturalized *Dolichogenidea gelechiidivoris* complement the resident parasitoid complex of Tuta absoluta in North-eastern Spain. *Journal of Applied Entomology*, *146*(4), 461–464. https://doi.org/10.1111/jen.12994

Desneux, N., Luna, M. G., Guillemaud, T., & Urbaneja, A. (2011). The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond: the new threat to tomato world production. *J Pest Sci*, *84*, 403. https://doi.org/doi.org/10.1007/s10340-011-0398-6

El-Arnaouty, S. A., Pizzol, J., Galal, H. H., Kortam, M. N., Afifi, A. I., Beyssat, et al. (2014). Assessment of two *Trichogramma* species for the control of *Tuta absoluta* in North African tomato greenhouses. *African Entomology*, *22*(4), 801–809. https://doi.org/10.4001/003.022.0410

Evans, E. W. (2018). Dispersal in Host – Parasitoid Interactions : Crop Colonization by Pests and Specialist Enemies. *Insects*, *9*(4). https://doi.org/10.3390/insects9040134

Farrar, J. J., Baur, M. E., & Elliott, S. F. (2016). Measuring IPM impacts in California and Arizona. *Journal of Integrated Pest Management*, *7*(1), 0–5. https://doi.org/10.1093/jipm/pmw012

Goda, N. F., El-Heneidy, A. H., Djelouah, K., & Hassan, N. (2015). Integrated pest management of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in tomato fields in Egypt. *International Medical Journal*, *25*(3), 655–661.

Goettel, M. S., & Inglis, D. G. (1997). *Fungi: Hyphomycetes*. *Manual of Techniques in Insect Pathology*. https://doi.org/10.1016/B978-012432555-5/50013-0

Gonthier, J., Bo, Y., Gui, Z., Zhang, F., Romeis, J., & Collatz, J. (2022). Odor learning improves efficacy of egg parasitoids as biocontrol agents against *Tuta absoluta*. *Journal of Pest Science*. https://doi.org/10.1007/s10340-022-01484-6

Jenkins, N. E., Heviefo, G., Langewald, J., Cherry, A. J., & Lomer, C. J. (1998). Development of mass production technology for aerial conidia for use as mycopesticides. *Biocontrol News and Information*, *19*, 21–32.

Jonsson, M., Kaartinen, R., & Straub, C. S. (2017). Relationships between natural enemy diversity and biological control. *Current Opinion in Insect Science*, *20*, 1–6. https://doi.org/10.1016/j.cois.2017.01.001

Krache, F., Boualem, M., Fernandez-Triana, J., Bass, A., Arnó, J., & Benourad, F. (2021). First record in Africa of the parasitoid *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae) on tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae) from tomato fields in Algeria. *Journal of Hymenoptera Research*, *88*, 115–131. https://doi.org/10.3897/jhr.88.75279

Labbé, R. M., Gillespie, D. R., Cloutier, C., & Brodeur, J. (2009). Compatibility of an entomopathogenic fungus with a predator and a parasitoid in the biological control of greenhouse whitefly. *Biocontrol Science and Technology*, *19*(4), 429–446. https://doi.org/10.1080/09583150902803229

Laurentis, V. L., Ramalho, D. G., Santos, N. A., Fabíola, V., Carvalho, P., Vacari, A. M., … Dami, B. G. (2019). Performance of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) on eggs of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Scientific Reports*, *9*, 1156. https://doi.org/10.1038/s41598-018-37797-9

Mama Sambo, S., Ndlela, S., du Plessis, H., Obala, F., & Mohamed, S. A. (2022a). Identification, Microhabitat, and Ecological Niche Prediction of Two Promising Native Parasitoids of *Tuta absoluta* in Kenya. *Insect*, *16*(6), 496. https://doi.org/https://doi.org/10.3390/ insects13060496

Mama Sambo, S., Akutse, K. S., du Plessis, H., Osa, P., Atalor, A., Mohamed, S. A., & Ndlela, S. (2022b). Interactions between the Entomopathogenic Fungus *Metarhizium anisopliae* ICIPE 20 and the Endoparasitoid *Dolichogenidea gelechiidivoris*, and Implications for Combined Biocontrol of *Tuta absoluta*. *Biology*, *11*(9), 1323. https://doi.org/https://doi.org/10.3390/biology11091323

Mama Sambo, S., Ndlela, S., du Plessis, H., Obala, F., & Mohamed, S. A. (2022c). Ratio dependence effects of the parasitoid *Dolichogenidea gelechiidivoris* on its associated host *Tuta absoluta*. *Biocontrol Science and Technology*, *32*(4), 497–510. https://doi.org/10.1080/09583157.2022.2033702

Maniania, N. K. (1998). A Device for Infecting Adult Tsetse Flies, *Glossina* spp., with an Entomopathogenic Fungus in the Field. *Biological Control*, *11*, 248–254. https://doi.org/https://doi.org/10.1006/bcon.1997.0580

Mansour, R., Brévault, T., Chailleux, A., Cherif, A., Grissa-Lebdi, K., Haddi, K., et al. (2018). Occurrence, biology, natural enemies and management of *Tuta absoluta* in Africa. *Entomologia Generalis*, *38*(2), 83–112. https://doi.org/10.1127/entomologia/2018/0749

Mas, N. G., Medina, M. C., Sánchez, F. G., & Moraga, E. Q. (2019). Bottom-up effects of endophytic *Beauveria bassiana* on multitrophic interactions between the cotton aphid, *Aphis gossypii*, and its natural enemies in melon. *Journal of Pest Science*, *92*(3), 1271–

1281. https://doi.org/10.1007/s10340-019-01098-5

Migiro, L. N., Maniania, N. K., Chabi-Olaye, A., & Vandenberg, J. (2010). Pathogenicity of Entomopathogenic Fungi *Metarhizium anisopliae* and *Beauveria bassiana* (Hypocreales: Clavicipitaceae) Isolates to the Adult Pea Leafminer (Diptera: Agromyzidae) and Prospects of an Autoinoculation Device for Infection in the Field. *Environmental Entomology*, *39*(2), 468–475. https://doi.org/10.1603/EN09359

Mirhosseini, M. A., Fathipour, Y., Soufbaf, M., & Reddy, G. V. P. (2019). Implications of using two natural enemies of *Tuta absoluta* (Lepidoptera: Gelechiidae) toward tomato yield enhancement. *Bulletin of Entomological Research*, *109*(5), 617–625. https://doi.org/10.1017/S0007485318000998

Mohamed, S. A., Dubois, T., Azrag, A. G. A., Ndlela, S., & Neuenschwander, P. (2022). Classical biological of key horticultural pests in Africa: successes, challenges, and opportunities. *Current Opinion in Insect Science*, *53*(100945), 1–6. https://doi.org/10.1016/j.cois.2022.100945

Morales, J., Rodríguez, D., & Cantor, F. (2013). Standardization of a massive rearing system of *Apanteles gelechiidivoris* Marsh (Hymenoptera: Braconidae), biological control for *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae). *Revista Facultad de Ciencias Básicas*, *9*(1), 20. https://doi.org/10.18359/rfcb.353

Morales, J., Sc, M., Muñoz, L., Sc, M., Rodríguez, D., & Cantor, F. (2014). Combined Action of Sex Pheromone and Wasp *Apanteles gelechiidivoris* in Greenhouse Tomato Crops. *Acta Biologica Colombiana*, *19*(2), 175–184. https://doi.org/https://doi.org/10.15446/abc.v19n2.38202

Ndereyimana, A., Nyalala, S., Murerwa, P., & Gaidashova, S. (2019). Pathogenicity of some commercial formulations of entomopathogenic fungi on the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Egyptian Journal of Biological Pest Control*, *29*(70), 1–5. https://doi.org/https://doi.org/10.1186/s41938-019-0184-y

Ndiaye, O., Ndiaye, S., Djiba, S., Ba, C. T., Vaughan, L., Rey, J., & Vayssières, J. (2015). Preliminary surveys after release of the fruit fly parasitoid *Fopius arisanus* Sonan (Hymenoptera Braconidae) in mango production systems in Casamance (Senegal). *Fruits*, *70*(2), 91–99. https://doi.org/DOI: 10.1051/fruits/2015001

Opisa, S., du Plessis, H., Akutse, K. S., Fiaboe, K. K. M., & Ekesi, S. (2019). Horizontal transmission of *Metarhizium anisopliae* between *Spoladea recurvali*s (Lepidoptera: Crambidae) adults and compatibility of the fungus with the attractant phenylacetaldehyde. *Microbial Pathogenesis*, *131*, 197–204. https://doi.org/10.1016/j.micpath.2019.04.010

Oztemiz, S. (2013). Population of *Tuta absoluta* and natural enemies after releasing on tomato grown greenhouse in Turkey. *African Journal of Biotechnology*, *12*(15), 1882–1887. https://doi.org/10.5897/ajb12.726

QGIS.org. (2021). QGIS Geographic Information System. QGIS Association. http://www.qgis.org.

R Core Team. (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing. *Vienna, Austria. URL Https://Www.R-Project.Org/.*

Salazar-mendoza, P., Rodriguez-saona, C., & Aparecido, O. (2020). Release density, dispersal capacity, and optimal rearing conditions for *Telenomus remus*, an egg parasitoid of *Spodoptera frugiperda*, in maize. *Biocontrol Science and Technology*, *30*(10), 1040–1050.

https://doi.org/10.1080/09583157.2020.1776841

Sallam, M. N., Overholt, W. A., & Kairu, E. (2001). Dispersal of the exotic parasitoid *Cotesia flavipes* in a new ecosystem. *Entomologia Experimentalis et Applicata*, *98*(2), 211–217. https://doi.org/https://doi.org/10.1046/j.1570-7458.2001.00776.x

Seehausen, M. L., Afonso, C., Jactel, H., & Kenis, M. (2021). Classical biological control against insect pests in Europe, North Africa, and the Middle East: What influences its success? *NeoBiota*, *191*, 169–191. https://doi.org/10.3897/neobiota.65.66276

Stiling, P. (1990). Calculating the Establishment Rates of Parasitoids in Classical Biological Control. *American Entomologist*, *36*(3), 225–230. https://doi.org/10.1093/ae/36.3.225

Tscharntke, T., Karp, D. S., Chaplin-Kramer, R., Batáry, P., DeClerck, F., Gratton, C., … Zhang, W. (2016). When natural habitat fails to enhance biological pest control – Five hypotheses. *Biological Conservation*, *204*, 449–458. https://doi.org/10.1016/j.biocon.2016.10.001

Zouba, A., Chermiti, B., Chraiet, R., & Mahjoubi, K. (2013). Effect of Two Indigenous *Trichogramma* Species on the Infestation Level by Tomato Miner *Tuta absoluta* in Tomato Greenhouses in the South-West of Tunisia. *Tunisian Journal of Plant Protection*, *87*(2).

CHAPTER 9

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Classical biological control (CBC) has been defined as ‘‘the intentional introduction of an exotic biological control agent for permanent establishment and long-term pest control’’ (Eilenberg *et al*., 2001). Success of CBC can be defined ecologically as the establishment of the introduced natural enemy, and economically as the reduction of the pest population to such an extent that it is economical valuable (Hokkanen and Sailer, 1985). Known successes in the field of CBC in Africa are those of cassava mealybug, mango mealybug, fruit flies, and *Liriomyza* leafminer (Cock, 2016; Gnanvossou *et al*., 2016; Mohamed *et al*., 2022). Ekesi *et al*. (2011) did, however, warn that although biocontrol-based integrated pest management (IPM) is successful in some cases, long-term sustainability might be adversely affected by emerging issues such as climate change, occurrence of more invasive species, changing consumer demands related to quality and standards, sanitary and phytosanitary requirements, and trade issues. Integrated pest management are therefore widely recommended for control of insect pests (Ekesi *et al*., 2011; Niassy *et al*., 2022), including *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Desneux *et al*., 2022). *Trichrogramma* spp. have been tested in Chile for classical biocontrol of *T. absoluta* (Desneux et al., 2010), while *D. gelechiidivoris* have been released as classical biological agent in Colombia (Rojas, 1997), from where establishment and spread to areas such as Chile was expected. This introduction resulted in establishment, and it is now considered as one of the most important parasitoids of *T. absoluta* in Chile (Desneux *et al*., 2010). The aim of this project was to investigate the management of *T. absoluta* with introduced and native biocontrol agents in Kenya. The aim was divided into objectives that were addressed and provided as chapters in this thesis. The outcome of this study and recommendations for future research will be discussed per each of the five hypotheses set out in the first chapter of this thesis (Heading 1.2.3).

9.1 Study outcomes and recommendations for future research

9.1.1 Hypothesis 1: The host: parasitoid density ratio will optimise mass production of *Dolichogenidea gelechiidivoris*.

The International Center for Insect Physiology and Ecology (icipe) imported the micrograstrinae koinobiont endoparasitoid, *Dolichogenidea gelechiidivoris* Masch (Syn.: *Apanteles* *gelechiidivoris* Marsh) (Hymenoptera: Braconidae) from Peru and introduced it into Kenya (Aigbedion-Atalor *et al.*, 2020). It is important to be able to mass produce the parasitoid and release it in numbers to achieve a significant reduction in *T. absoluta* populations. The results in chapter 3 of this thesis, indicated that host and parasitoid density affected superparsitism. Intraspecific competition enhanced the parasitism rate of *D. gelechiidivoris* females, but their performance was reduced with intraspecific competition. The efficiency in control of *T. absoluta* by *D. gelechiidivoris* was evident from the type II functional response and the high percentage of wasps that emerged from larvae exposed to the parasitoid. Based on these results a ratio of one *D. gelechiidivoris* female to 100 first instar *T. absoluta* larvae are recommended to optimize mass rearing of the parasitoid. The first hypothesis is therefore accepted.

Recommendations for future research

The main problem in successful mass production of *T. absoluta* parasitoids is the lack of a suitable diet for mass rearing of this pest. During this study, attempts to rear first instar *T. absoluta* larvae on an artificial diet reported by Bajonero and Parra (2017) and Genç (2017) were unsuccessful, similar to results reported by Cherif (2018). Future research should therefore focus on formulating a suitable artificial diet for this pest to facilitate in rearing *T. absoluta* parasitoids in mass. Other mass rearing aspects such as alternative hosts, age of the parasitoid, and temperature at which cocoons can be stored should also be investigated.

9.1.2 Hypothesis 2: Two native parasitoids, *B. nigricans* and *Stenomesius* sp. near *japonicus* present in Kenya, could establish in sub-Saharan Africa and can be implemented as biocontrol agents in IPM programs for control of *T. absoluta* in Africa.

An IPM strategy is widely promoted for management of native and invasive pest species. Biocontrol as one of the pillars of IPM, can be enhanced by combining different biocontrol agents. The compatibility of these agents is, however, not always known. Interactions between biocontrol agents could be synergistic – i.e., the cumulative efficacy of both species being significantly higher than that of the two natural enemies alone, or additive – when both natural enemies are more effective than the most effective species alone, but less effective than the added efficacy by each species alone (Turner *et al*., 2010). Inhibitory interactions may also result when both natural enemies are significantly less effective than the most effective species alone (Bilu and Coll, 2007; Turner *et al*., 2010).

Results of a survey on the native parasitoids of *T. absoluta* from three regions in Kenya, are reported in chapter 4. Two native endogenous parasitoid species of *T. absoluta* were recorded, *viz*. *Bracon nigricans*, and *Stenomesius* sp. near *japonicus.* The ecological niche prediction reported in chapter 4 showed many parts in Africa to be highly to very highly suitable for persistence of *B. nigricans,* including the areas where *D. gelechiidivoris* had been released. Hypothesis 2 is, however, only partly accepted, since limited areas in sub-Saharan Africa are ecological suited for establishment of *Stenomesius* sp. near *japonicus.* Areas indicated by the predictions in chapter 4 can be used as a guide for future recovery surveys and for the implementation of different biological control strategies against *T. absoluta*.

9.1.3 Hypothesis 3: The exotic *Dolichogenidea gelechiidivoris* can be used together with the native *Stenomesius* sp. nr. *japonicus* and *Bracon nigricans* for control of *Tuta absoluta.*

Results from chapter 5 indicated that the exotic *D. gelechiidivoris* performed much better than the native *Stenomesius* sp. near *japonicus*, and that their simultaneous use had an additive effect on control of *T. absoluta*, with no negative effects on either of the parasitoid species.

The same level of pest control was achieved where *D. gelechiidivoris* and *B. nigricans* co-occurred as well as where each species of parasitoid was individually present. The native parasitoid *B. nigricans* did, however, reduce the population growth of the exotic *D. gelechiidivoris*. Major parts of sub-Saharan Africa are suited for establishment of *B. nigricans.* Hypothesis 3 is partly accepted since *D. gelechiidivoris* and *Stenomesius* sp. nr. *japonicus* can be released together to achieve better control of *T. absoluta*. A reduction in population growth of *D. gelechiidivoris* as a result of its co-existence with *B. nigricans,* was, however, reported in chapter 6 of this study, resulting in a partly rejection of hypothesis 3 pending future research.

Recommendations for future research

Results from this study reported on the performance and population growth of parasitoid species that co-existed at one specific ratio. Future research should be elaborated to also include co-existence of parasitoids at different ratios. Parasitism performance and population growth of *B. nigricans* and *D. gelechiidivoris* in co-existence might also be influenced when multiple lepidopteran pests from different families are present on the same crop or, alternatively, where tomato is intercropped with another crop hosting an additional host of *B. nigricans*. For example, *B. nigricans* acting as an ectoparasitic idiobiont species of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctudae) (Becchimanzi *et al*., 2017), and *D. gelechiidivoris*, as a koinobiont solitary larval endoparasitoid of *T. absoluta*. Tomato planted together with a host plant of the pest, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), may provide for an alternative host of *B. nigricans* (Loni e*t al*., 2016). These aspects warrant future research. The possibility of using the venom of an ectoparasitoid such as *B. nigricans* to develop new biopesticides lines could also be explored and host finding by parasitoids may be enhanced by pheromones and plant kairomones that attract females. Future research on these aspects may improve parasitism by parasitoids.

9.1.4 Hypothesis 4: The combined use of *Dolichogenidea gelechiidivoris* and *Metarhizium anisopliae* ICIPE 20 has an additive effect in biocontrol of *T. absoluta*.

To develop an eco-friendly, sustainable control strategy for *T. absoluta*, many control options should be available to be integrated in an IPM program. The combined application of parasitoids and fungal-based biopesticides may enhance the control of *T. absoluta* and the overall success of IPM programs against this pest. This could potentially be achieved with an entomopathogenic fungus (EPF)-contaminated trap containing a *T. absoluta* pheromone lure, an auto-dissemination technique of fungus conidia, originally described by Migiro *et al*. (2010). For this strategy to be successful, a few requirements must be met. For example, the pest should be attracted to the trap, acquires the fungus and disseminates it to its conspecifics, and the fungus should not be pathogenetic to the parasitoid. *Dolichogenidea gelechiidivoris* is also attracted to the *T. absoluta* pheromone (Ayelo *et al*., 2021). Results reported in chapter 7 of this thesis showed that direct application of dry *Metarhizium anisopliae* (Metschnikoff) ICIPE 20 conidia to the parasitoid, prior to exposure to the host, reduced the longevity of the parasitoid. Emergence of infected *D. gelechiidivoris* larvae was also reduced by the fungus, but a plant sprayed with this fungus did not affect its longevity and host selection. *Metarhizium anisopliae* ICIPE 20 sprays can be recommended to reduce the pest infestation, before release of *D. gelechiidivoris*, a strategy which will have an additive effect on control of *T. absoluta* control.

Recommendations for future research

Results from this study (laboratory results) should be confirmed under field conditions. Future research should also be aimed to determining the most appropriate time of application of the respective biocontrol agents used in combination. For example, to minimise the pathogenic effect of *M. anisopliae* 20 on the parasitoids attracted to the *T. absoluta* lure in traps, the time that should lapse between release of parasitoids and installing the EPF contaminated traps, should be investigated.

9.1.5 Hypothesis 5: The imported endoparasitoid, *Dolichogenidea gelechiidivoris*, will establish in Kenya and could be used with the entomopathogenic fungus, *M. anisopliae* for biological control of *T. absoluta.*

Biological control can be strengthened by complementarity among natural enemies, e.g. if multiple enemies attack a pest during different periods of its occurrence in the field, or in different stages of the lifecycle (Jonsson et al., 2017). *Dolichogenidea* g*elechiidivoris* is considered as the most important parasitoid for natural and augmentative biological control in Colombian tomato crops (Bajonero, 2017) with parasitism of *T. absoluta* as high as 90% (Morales et al., 2014). Despite the known importance of *D. gelechiidivoris* as a biocontrol agent, knowledge on its effectiveness, as well as information related to the conditions or factors that affect its effectiveness in areas outside its native area, is lacking.

The combined use of biopesticides, parasitoids and predators are effective in controlling *T. absoluta* under greenhouse (Kortam *et al*., 2014) and field conditions (Khidr *et al*., 2013). A greenhouse trial from this study confirmed the efficacy of *D. gelechiidivoris* and *M. anisopliae* ICIPE 20 in controlling *T. absoluta* applied as stand-alone as well as combined treatments (Chapter 8). The establishment of *D. gelechiidivoris* one year after its release in Kenya, was confirmed with wasps sampled at approximately 4.4 km from the point of release, co-existing with *Stenomesius* sp. near *japonicus* and *Bracon nigricans* (Chapter 4). Their speed of distribution was much lower compared to that of *Habrobracon hebetor* Say (Hymenoptera: Braconidae) a parasitoid of *Heliocheilus albipunctella* de Joannis (Lepidoptera: Noctuidae), which spread up to 3 km, five weeks after release in pearl millet fields (Baoua *et al*., 2018). It is, however, faster than that of *Diachasmimorpha kraussii* (Fullaway) (Hymenoptera: Braconidae in Hawaii, which was found 5 km away from their point of release, three years later (Bokonon-

Ganta *et al*., 2013). However, landscape structure affects parasitoid dispersion and should also be taken into account (Mama Sambo *et al*., 2019; Rohrig *et al*., 2008; Romeis *et al*., 2005).

Recommendations for future research

The four biological control agents, *viz*. the imported exotic parasitoid *D. gelechiidivoris*, the fungus *M. anisopliae* ICIPE 20, and two native parasitoids, *S.* sp. nr. *japonicus* and *B. nigricans* were all found to effectively control *T. absoluta*. The co-existence and performance of these four biological control agents in combination should, however, be tested under greenhouse conditions. The effects of abiotic and biotic factors such as insecticide applications, tomato varieties, crop association and rotation and plant composition to optimise control with these biocontrol agents, individually or in combination. Cost benefit analysis of different combinations of these technologies for control of *T. absoluta* in tomato production should also be investigated to facilitate farmers and policies makers with decisions.

9.2 References

Aigbedion-Atalor, P.O., Mohamed, S.A., Hill, M.P., Zalucki, M.P., Azrag, A.G.A., Srinivasan, R. and Ekesi, S. 2020. Host stage preference and performance of *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae), a candidate for classical biological control of *Tuta absoluta* in Africa. *Biol. Control* 144:104215. https://doi.org/10.1016/j.biocontrol.2020.104215

Ayelo, P.M., Mohamed, S.A., Chailleux, A., Yusuf, A.A., Pirk, C.W.W., Deletre, E. 2021. The parasitoid *Dolichogenidea gelechiidivoris* eavesdrops on semiochemicals from its host *Tuta absoluta* and tomato. *J. Pest Sci.* (2004). 95:633–652. https://doi.org/https://doi.org/10.1007/s10340-021-01424-w

Bajonero, J.G. and Parra, J.R.P. 2017. Selection and suitability of an artificial diet for *Tuta absoluta* (Lepidoptera: Gelechiidae) based on physical and chemical characteristics. *J. Insect Sci*. 17:1–16. https://doi.org/10.1093/jisesa/iew105

Bajonero J. G. 2017. Release of *Apanteles gelechiidivoris* for biological control of *Tuta absoluta* in tomato greenhouse in Colombia. n15° Simp. Control. Biológico, Ribeirao Preto.

Baoua, I.B., Ba, M.N., Amadou, L., Kabore, A. and Dabire-binso, C.L. 2018. Field dispersal of the parasitoid wasp *Habrobracon hebetor* (Hymenoptera: Braconidae) following augmentative release against the millet head miner *Heliocheilus albipunctella* (Lepidoptera: Noctuidae) in the Sahel field dispersal of the parasitoid wasp. *Biocontrol Sci. Technol*. 28:404–415. https://doi.org/10.1080/09583157.2018.1450842

Becchimanzi, A., Avolio, M., Lelio, I. Di, Marinelli, A., Varricchio, P., Grimaldi, A., Eguileor, M. and De Caccia, S. 2017. Host regulation by the ectophagous parasitoid wasp *Bracon nigricans*. *J. Insect Physiol.* https://doi.org/10.1016/j.jinsphys.2017.07.002

Bilu, E. and Coll, M. 2007. The importance of intraguild interactions to the combined effect of a parasitoid and a predator on aphid population suppression. *BioControl* 52:753-763.

Bokonon-Ganta, A.H., McQuate, G.T., Messing, R.H. and Jang, E.B. 2013. Release and establishment of the parasitoid *Diachasmimorpha kraussii* against the tephritid fruit fly *Bactrocera latifrons* in Hawaii. *J. Insect Sci*. 13:1–15. https://doi.org/10.1673/031.013.0701

Cherif, A. 2018. La mineuse de la tomate *Tuta absoluta* (Lepidoptera : Gelechiidae) : Paramètres biologiques, écologiques et alternatives de lutte. Thèse de doctorat, Ecole Doctorale : Sciences et Techniques de L'Agronomie et de l'Environnement, Tunisie, 11 Janvier 2018.

Cock, M. 2016. The impacts of some classical biological control successes. CAB Rev. 10, 1–58. https://doi.org/10.1079/pavsnnr201510042

Desneux, N., Wajnberg, E., Kris, W.A.G., Burgio, G., Arpaia, S., Narváez-Vasquez, C.A., González-Cabrera, J., Ruescas, D.C., Tabone, E., Frandon, J., Pizzol, J., Poncet, C., Cabello, T. and Urbaneja, A. 2010. Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, geographic expansion and prospects for biological control. *J. Pest Sci.* 83:197–215. https://doi.org/10.1007/s10340-010-0321-6

Desneux, N., Han, P., Mansour, R., Arnó, J., Brévault, T., Campos, M.R., Chailleux, A., Guedes, R.N.C., Karimi, J., Konan, K.A.J., Lavoir, A. violette, Luna, M.G., Perez-Hedo, M., Urbaneja, A., Verheggen, F.J., Zappalà, L., Abbes, K., Ali, A., Bayram, Y., Cantor, F., Cuthbertson, A.G.S., De Vis, R., Erler, F., Firake, D.M., Haddi, K., Hajjar, M.J., Ismoilov, K., Jaworski, C.C., Kenis, M., Liu, H. tian, Madadi, H., Martin, T., Mazih, A., J. Messelink, G., Mohamed, S.A., Nofemela, R.S., Oke, A., Ramos, C., Ricupero, M., Roditakis, E., Shashank, P.R., Wan, F.H., Wang, M. hui, Wang, S., Zhang, Y.B. and Biondi, A. 2022.

Integrated pest management of *Tuta absoluta*: practical implementations across different world regions. *J. Pest Sci. (2004).* 95:17–39. https://doi.org/10.1007/s10340-021-01442-8

Eilenberg, J., Hajek, A. and Lomer, C. 2001. Suggestions for unifying the terminology in biological control. *BioControl* 46:387–400. https://doi.org/10.1023/A:1014193329979

Ekesi, S., Chabi-Olaye, A., Subramanian, S. and Borgemeister, C. 2011. Horticultural pest management and the African economy: Successes, challenges and opportunities in a changing global environment. *Acta Hortic.* 911:165–183. https://doi.org/10.17660/ActaHortic.2011.911.17

Genç, H. 2017. Laboratory Rearing of Tomato Leaf Miner, Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) on Artificial Diet. Türk Tarım ve Doğa Bilim. Dergisi-Trkish *J. Agric. Nat. Sci*. 4:528–536.

Gnanvossou, D., Hanna, R., Bokonon-Ganta, A.H., Ekesi, S. and Mohamed, S. 2016. Release, establishment and spread of the natural enemy *Fopius arisanus* (Hymenoptera: Braconidae) for control of the invasive oriental fruit fly *Bactrocera dorsalis* (Diptera: Tephritidae) in Benin, West Africa. In M. De Ekesi, S., Mohamed, S.A., Meyer (Ed.) 575–600.

Hokkanen, H.M.T. and Sailer, R.I. 1985. Success in classical biological control. *CRe Crit. Rev. Plant Sci*. 3:35–72. https://doi.org/10.1080/07352688509382203

Jonsson, M., Kaartinen, R. and Straub, C.S. 2017. Relationships between natural enemy diversity and biological control. *Curr. Opin. Insect Sci*. 20:1–6. https://doi.org/10.1016/j.cois.2017.01.001

Khidr, A.A., Gaffar, S.A., Nada, M.S., Taman, A.A. and Salem, F.A. 2013. New approaches for controlling Tomato leafminer, *Tuta absoluta* (Meyrick) in tomato fields in Egypt. *J. Agric. Res* 91: 335–348.

Kortam, M.N., El Arnaouty, S.A., Afifi, A.I. and Heikal, I.H. 2014. Efficacy of different biological methods for controlling the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on tomato in greenhouse in Egypt. *Egypt. J. Biol. Pest Control* 24:523–528.

Loni, A., Samartsev, K.G., Scaramozzino, P.L., Belokobylskij, S.A. and Lucchi, A. 2016. Braconinae parasitoids (Hymenoptera, Braconidae) emerged from larvae of *Lobesia* *botrana* (Denis & Schiffermüller) (Lepidoptera, Tortricidae) feeding on *Daphne gnidium* L. *Zookeys* 587:125–150. https://doi.org/10.3897/zookeys.587.8478

Mama Sambo, S., Togbe, D.R., Sinzogan, A.A.C., Bokonon-Ganta, H.A., Adomou, A., Karlsson, M.F. 2019. Habitat factors associated with *Fopius caudatus* parasitism and population level of its host, *Ceratitis cosyra*. *Entomol. Exp. Appl.* 168:1–13. https://doi.org/10.1111/eea.12858

Migiro, L.N., Maniania, N.K., Chabi-Olaye, A. and Vandenberg, J. 2010. Pathogenicity of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* (Hypocreales: Clavicipitaceae) isolates to the adult pea leafminer (Diptera: Agromyzidae) and prospects of an autoinoculation device for infection in the field. *Environ. Entomol.* 39:468–475. https://doi.org/10.1603/EN09359

Mohamed, S.A., Dubois, T., Azrag, A.G.A., Ndlela, S. and Neuenschwander, P. 2022. Classical biological of key horticultural pests in Africa: successes, challenges, and opportunities. *Curr. Opin. Insect Sci.* 53:1–6. https://doi.org/10.1016/j.cois.2022.100945

Morales, J., Muñoz, L., Rodríguez, D. and Cantor, F. 2014. Combined action of sex pheromone and wasp *Apanteles gelechiidivoris* in greenhouse tomato crops. Acta Biol Colomb. 19:175–184.

Niassy, S., Murithii, B., Omuse, E.R., Kimathi, E., Tonnang, H., Ndlela, S., Mohamed, S. and Ekesi, S. 2022. Insight on fruit Fly IPM technology uptake and barriers to scaling in Africa. *Sustain*. 14:2954. https://doi.org/10.3390/su14052954

Rohrig, E., Sivinski, J., Teal, P., Stuhl, C., and Aluja, M. 2008. A floral-derived compound attractive to the Tephritid fruit fly parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). *J. Chem. Ecol.* 34:549–557. https://doi.org/10.1007/s10886-008-9438-y

Rojas, S. 1997. Establecimiento de enemigos naturales. *Agric Técnica*, 57:297–298.

Romeis, J., Babendreier, D., Wäckers, F.L. and Shanower, T.G. 2005. Habitat and plant specificity of *Trichogramma* egg parasitoids — underlying mechanisms and implications. *Basic Appl. Ecol.* 6:215–236. https://doi.org/10.1016/j.baae.2004.10.004

Turner, P.J., Morin, L., Williams, D.G. and Kriticos, D.J. 2010. Interactions between a leafhopper and rust fungus on the invasive plant *Asparagus asparagoides* in Australia: a case of two agents being better than one for biological control. *Biol. Control*, 54: 322-330.

APPENDIX A

Instructions to authors: Biocontrol Science and Technology (Taylor and Francis)

Preparing Your Paper

Research Article

Should be written with the following elements in the following order: title page; abstract; keywords; acknowledgments; declaration of interest statement; references; appendices (as appropriate); table(s) with caption(s) (on individual pages); figures; figure captions (as a list)

Should contain an unstructured abstract of 250 words.

Should contain between 3 and 6 keywords. Read making your article more discoverable, including information on choosing a title and search engine optimization.

Please include a word count for your paper and ensure line numbers are present in the file.

Review Articles

Should be written with the following elements in the following order: title page; abstract; keywords; main text introduction, materials and methods, results, discussion; acknowledgments; declaration of interest statement; references; appendices (as appropriate); table(s) with caption(s) (on individual pages); figures; figure captions (as a list)

Should contain an unstructured abstract of 250 words.

Should contain between 3 and 6 keywords. Read making your article more discoverable, including information on choosing a title and search engine optimization.

Please include a word count for your paper and ensure line numbers are present in the file.

Short Communcation

Should be written with the following elements in the following order: abstract; main body; references

Should contain an unstructured abstract of 100 words.

Read making your article more discoverable, including information on choosing a title and search engine optimization.

Short communications should report results of importance that justify presentation in advance of a more detailed investigation or which do not justify an extensive presentation. Short communications have a limit of 2500 words, which are not subdivided, other than acknowledgements and references. No more than 10 references and there should be no more than 2 figures or tables.

Please ensure line numbers are present in the file.

Book Review

Should be written with the following elements in the following order:

Should contain an unstructured abstract of 250 words.

Read making your article more discoverable, including information on choosing a title and search engine optimization.

Please ensure line numbers are present in the file.

Letter to the Editor

No tables or figures are allowed and there is a limit of 4 references.

Style Guidelines

Please refer to these quick style guidelines when preparing your paper, rather than any published articles or a sample copy.

Please use British (-ise) spelling style consistently throughout your manuscript.

Please use single quotation marks, except where ‘a quotation is “within” a quotation’. Please note that long quotations should be indented without quotation marks.

Formatting and Templates

Papers may be submitted in Word format. Figures should be saved separately from the text. To assist you in preparing your paper, we provide formatting template(s).

Word templates are available for this journal. Please save the template to your hard drive, ready for use.

If you are not able to use the template via the links (or if you have any other template queries) please contact us here.

References

Please use this reference guide when preparing your paper.

An EndNote output style is also available to assist you.

Taylor & Francis Editing Services

To help you improve your manuscript and prepare it for submission, Taylor & Francis provides a range of editing services. Choose from options such as English Language Editing, which will ensure that your article is free of spelling and grammar errors, Translation, and Artwork Preparation. For more information, including pricing, visit this website.

Checklist: What to Include

Author details.Please ensure all listed authors meet the Taylor & Francis authorship criteria. All authors of a manuscript should include their full name and affiliation on the cover page of the manuscript. Where available, please also include ORCiDs and social media handles (Facebook, Twitter or LinkedIn). One author will need to be identified as the corresponding author, with their email address normally displayed in the article PDF (depending on the journal) and the online article. Authors' affiliations are the affiliations where the research was conducted. If any of the named co-authors moves affiliation during the peer-review process, the new affiliation can be given as a footnote. Please note that no changes to affiliation can be made after your paper is accepted. Read more on authorship.

Graphical abstract (optional). This is an image to give readers a clear idea of the content of your article. It should be a maximum width of 525 pixels. If your image is narrower than 525 pixels, please place it on a white background 525 pixels wide to ensure the dimensions are maintained. Save the graphical abstract as a .jpg, .png, or .tiff. Please do not embed it in the manuscript file but save it as a separate file, labelled GraphicalAbstract1.

You can opt to include a video abstract with your article. Find out how these can help your work reach a wider audience, and what to think about when filming.

Funding details. Please supply all details required by your funding and grant-awarding bodies as follows:

For single agency grants

This work was supported by the [Funding Agency] under Grant [number xxxx].

For multiple agency grants

This work was supported by the [Funding Agency #1] under Grant [number xxxx]; [Funding Agency #2] under Grant [number xxxx]; and [Funding Agency #3] under Grant [number xxxx].

Disclosure statement. This is to acknowledge any financial or non-financial interest that has arisen from the direct applications of your research. If there are no relevant competing interests to declare please state this within the article, for example: *The authors report there are no competing interests to declare.* Further guidance on what is a conflict of interest and how to disclose it.

Data availability statement. If there is a data set associated with the paper, please provide information about where the data supporting the results or analyses presented in the paper can be found. Where applicable, this should include the hyperlink, DOI or other persistent identifier associated with the data set(s). Templates are also available to support authors.

Data deposition. If you choose to share or make the data underlying the study open, please deposit your data in a recognized data repository prior to or at the time of submission. You will be asked to provide the DOI, pre-reserved DOI, or other persistent identifier for the data set.

Geolocation information. Submitting a geolocation information section, as a separate paragraph before your acknowledgements, means we can index your paper’s study area accurately in JournalMap’s geographic literature database and make your article more discoverable to others. More information.

Supplemental online material. Supplemental material can be a video, dataset, fileset, sound file or anything which supports (and is pertinent to) your paper. We publish supplemental material online via Figshare. Find out more about supplemental material and how to submit it with your article.

Figures. Figures should be high quality (1200 dpi for line art, 600 dpi for grayscale and 300 dpi for colour, at the correct size). Figures should be supplied in one of our preferred file formats: EPS, PS, JPEG, TIFF, or Microsoft Word (DOC or DOCX) files are acceptable for figures that have been drawn in Word. For information relating to other file types, please consult our Submission of electronic artwork document.

Tables. Tables should present new information rather than duplicating what is in the text. Readers should be able to interpret the table without reference to the text. Please supply editable files.

Equations. If you are submitting your manuscript as a Word document, please ensure that equations are editable. More information about mathematical symbols and equations.

Units. Please use SI units (non-italicized).

Using Third-Party Material in your Paper

You must obtain the necessary permission to reuse third-party material in your article. The use of short extracts of text and some other types of material is usually permitted, on a limited basis, for the purposes of criticism and review without securing formal permission. If you wish to include any material in your paper for which you do not hold copyright, and which is not covered by this informal agreement, you will need to obtain written permission from the copyright owner prior to submission. More information on requesting permission to reproduce work(s) under copyright.

Submitting Your Paper

This journal uses Taylor & Francis' Submission Portal to manage the submission process. The Submission Portal allows you to see your submissions across Taylor & Francis' journal portfolio in one place. To submit your manuscript please click here.

Please note that *Biocontrol Science and Technology* uses Crossref™ to screen papers for unoriginal material. By submitting your paper to *Biocontrol Science and*

Technology you are agreeing to originality checks during the peer-review and production processes.

On acceptance, we recommend that you keep a copy of your Accepted Manuscript. Find out more about sharing your work.

Data Sharing Policy

This journal applies the Taylor & Francis Basic Data Sharing Policy. Authors are encouraged to share or make open the data supporting the results or analyses presented in their paper where this does not violate the protection of human subjects or other valid privacy or security concerns.

Authors are encouraged to deposit the dataset(s) in a recognized data repository that can mint a persistent digital identifier, preferably a digital object identifier (DOI) and recognizes a long-term preservation plan. If you are uncertain about where to deposit your data, please see this information regarding repositories.

Authors are further encouraged to cite any data sets referenced in the article and provide a Data Availability Statement.

At the point of submission, you will be asked if there is a data set associated with the paper. If you reply yes, you will be asked to provide the DOI, pre-registered DOI, hyperlink, or other persistent identifier associated with the data set(s). If you have selected to provide a pre-registered DOI, please be prepared to share the reviewer URL associated with your data deposit, upon request by reviewers.

Where one or multiple data sets are associated with a manuscript, these are not formally peer reviewed as a part of the journal submission process. It is the author's responsibility to ensure the soundness of data. Any errors in the data rest solely with the producers of the data set(s).

Publication Charges

There are no submission fees, publication fees or page charges for this journal.

Colour figures will be reproduced in colour in your online article free of charge. If it is necessary for the figures to be reproduced in colour in the print version, a charge will apply.

Charges for colour figures in print are £300 per figure ($400 US Dollars; $500 Australian Dollars; €350). For more than 4 colour figures, figures 5 and above will be charged at £50 per figure ($75 US Dollars; $100 Australian Dollars; €65). Depending on your location, these charges may be subject to local taxes.

Copyright Options

Copyright allows you to protect your original material, and stop others from using your work without your permission. Taylor & Francis offers a number of different license and reuse options, including Creative Commons licenses when publishing open access. Read more on publishing agreements.

Complying with Funding Agencies

We will deposit all National Institutes of Health or Wellcome Trust-funded papers into PubMedCentral on behalf of authors, meeting the requirements of their respective open access policies. If this applies to you, please tell our production team when you receive your article proofs, so we can do this for you. Check funders' open access policy mandates. Find out more abouts haring your work.

My Authored Works

On publication, you will be able to view, download and check your article's metrics (downloads, citations and Altmetric data) via My Authored Works on Taylor & Francis Online. This is where you can access every article you have published with us, as well as your free eprints link, so you can quickly and easily share your work with friends and colleagues.

We are committed to promoting and increasing the visibility of your article. Here are some tips and ideas on how you can work with us to promote your research.

APPENDIX B

Instructions to authors: Insects and Biology (MDPI)

General Considerations

Research manuscripts should comprise:

Front matter: Title, Author list, Affiliations, Abstract, Keywords

Research manuscript sections: Introduction, Materials and Methods, Results, Discussion, Conclusions (optional).

Back matter: Supplementary Materials, Acknowledgments, Author Contributions, Conflicts of Interest, References.

Review manuscripts should comprise the front matter, literature review sections and the back matter. The template file can also be used to prepare the front and back matter of your review manuscript. It is not necessary to follow the remaining structure. Structured reviews and meta-analyses should use the same structure as research articles and ensure they conform to the PRISMA guidelines.

Graphical Abstract:

A graphical abstract (GA) is an image that appears alongside the text abstract in the Table of Contents. In addition to summarizing the content, it should represent the topic of the article in an attention-grabbing way. Moreover, it should not be exactly the same as the Figure in the paper or just a simple superposition of several subfigures. Note that the GA must be original and unpublished artwork. Any postage stamps, currency from any country, or trademarked items should not be included in it.

The GA should be a high-quality illustration or diagram in any of the following formats: PNG, JPEG, TIFF, or SVG. Written text in a GA should be clear and easy to read, using one of the following fonts: Times, Arial, Courier, Helvetica, Ubuntu or Calibri.

The minimum required size for the GA is 560 × 1100 pixels (height × width). The size should be of high quality in order to reproduce well.

Acronyms/Abbreviations/Initialisms should be defined the first time they appear in each of three sections: the abstract; the main text; the first figure or table. When defined for the first time, the acronym/abbreviation/initialism should be added in parentheses after the written-out form.

SI Units (International System of Units) should be used. Imperial, US customary and other units should be converted to SI units whenever possible.

Accession numbers of RNA, DNA and protein sequences used in the manuscript should be provided in the Materials and Methods section. Also see the section on Deposition of Sequences and Expression Data.

Equations: If you are using Word, please use either the Microsoft Equation Editor or the MathType add-on. Equations should be editable by the editorial office and not appear in a picture format.

Research Data and supplementary materials: Note that publication of your manuscript implies that you must make all materials, data, and protocols associated with the publication available to readers. Disclose at the submission stage any restrictions on the availability of materials or information. Read the information about Supplementary Materials and Data Deposit for additional guidelines.

Preregistration: Where authors have preregistered studies or analysis plans, links to the preregistration must be provided in the manuscript.

Guidelines and standards: MDPI follows standards and guidelines for certain types of research. See https://www.mdpi.com/editorial_process for further information.

New Species Description: Manuscripts that describe new or revised taxon names must be registered in ZooBank, as required by the International Code of Zoological Nomenclature, after article acceptance following peer review. This ensures that your article is officially recorded as the first paper to describe the new species. The ZooBank unique identification code (LSID—Life Science Identifier) should be provided at the final proofreading stage, on the first page of your manuscript, following the affiliations, so that it is included in your published article. An LSID is represented as a uniform resource name (URN) with the following format: urn:lsid:<Authority>:<Namespace>:<ObjectID>[:<Version>]. Authors will be asked to alert ZooBank with the final citation following publication. For further help registering with ZooBank, please go to Help.

Front Matter

These sections should appear in all manuscript types

Title: The title of your manuscript should be concise, specific and relevant. It should identify if the study reports (human or animal) trial data, or is a systematic review, meta-analysis or replication study. When gene or protein names are included, the abbreviated name rather than full name should be used. Please do not include abbreviated or short forms of the title, such as a running title or head. These will be removed by our Editorial Office.

Author List and Affiliations: Authors' full first and last names must be provided. The initials of any middle names can be added. The PubMed/MEDLINE standard format is used for affiliations: complete address information including city, zip code, state/province, and country. At least one author should be designated as corresponding author, and his or her email address and other details should be included at the end of the affiliation section. Please read the criteria to qualify for authorship.

Simple Summary: It is vitally important that scientists are able to describe their work simply and concisely to the public, especially in an open-access on-line journal. The simple summary consists of no more than 200 words in one paragraph and contains a clear statement of the problem addressed, the aims and objectives, pertinent results, conclusions from the study and how they will be valuable to society. This should be written for a lay audience, i.e., no technical terms without explanations. No references are cited and no abbreviations. Submissions without a simple summary will be returned directly. Example could be found at https://www.mdpi.com/2075-4450/11/8/508.

Abstract: The abstract should be a total of about 200 words maximum. The abstract should be a single paragraph and should follow the style of structured abstracts, but without headings: 1) Background: Place the question addressed in a broad context and highlight the purpose of the study; 2) Methods: Describe briefly the main methods or treatments applied. Include any relevant preregistration numbers, and species and strains of any animals used. 3) Results: Summarize the article's main findings; and 4) Conclusion: Indicate the main conclusions or interpretations. The abstract should be an objective representation of the article: it must not contain results which are not presented and substantiated in the main text and should not exaggerate the main conclusions.

Keywords: Three to ten pertinent keywords need to be added after the abstract. We recommend that the keywords are specific to the article, yet reasonably common within the subject discipline.

Research Manuscript Sections

Introduction: The introduction should briefly place the study in a broad context and highlight why it is important. It should define the purpose of the work and its significance, including specific hypotheses being tested. The current state of the research field should be reviewed carefully and key publications cited. Please highlight controversial and diverging hypotheses when necessary. Finally, briefly mention the main aim of the work and highlight the main conclusions. Keep the introduction comprehensible to scientists working outside the topic of the paper.

Materials and Methods: They should be described with sufficient detail to allow others to replicate and build on published results. New methods and protocols should be described in detail while well-established methods can be briefly described and appropriately cited. Give the name and version of any software used and make clear whether computer code used is available. Include any pre-registration codes.

Results: Provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.

Discussion: Authors should discuss the results and how they can be interpreted in perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible and limitations of the work highlighted. Future research directions may also be mentioned. This section may be combined with Results.

Conclusions: This section is not mandatory but can be added to the manuscript if the discussion is unusually long or complex.

Patents: This section is not mandatory but may be added if there are patents resulting from the work reported in this manuscript.

Back Matter

Supplementary Materials: Describe any supplementary material published online alongside the manuscript (figure, tables, video, spreadsheets, etc.). Please indicate the name and title of each element as follows Figure S1: title, Table S1: title, etc.

Funding: All sources of funding of the study should be disclosed. Clearly indicate grants that you have received in support of your research work and if you received funds to cover publication costs. Note that some funders will not refund article processing charges (APC) if the funder and grant number are not clearly and correctly identified in the paper. Funding information can be entered separately into the submission system by the authors during submission of their manuscript. Such funding information, if available, will be deposited to FundRef if the manuscript is finally published.

Please add: “This research received no external funding” or “This research was funded by [name of funder] grant number [xxx]” and “The APC was funded by [XXX]” in this section. Check carefully that the details given are accurate and use the standard spelling of funding agency names at https://search.crossref.org/funding, any errors may affect your future funding.

Acknowledgments: In this section you can acknowledge any support given which is not covered by the author contribution or funding sections. This may include administrative and technical support, or donations in kind (e.g., materials used for experiments).

Author Contributions: Each author is expected to have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; or have drafted the work or substantively revised it; AND has approved the submitted version (and version substantially edited by journal staff that involves the author’s contribution to the study); AND agrees to be personally accountable for the author’s own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature.

For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, X.X. and Y.Y.; Methodology, X.X.; Software, X.X.; Validation, X.X., Y.Y. and Z.Z.; Formal Analysis, X.X.; Investigation, X.X.; Resources, X.X.; Data Curation, X.X.; Writing – Original Draft Preparation, X.X.; Writing – Review & Editing, X.X.; Visualization, X.X.; Supervision, X.X.; Project Administration, X.X.; Funding Acquisition, Y.Y.", please turn to the CRediT taxonomy for the term explanation. For more background on CRediT, see here. "Authorship must include and be limited to those who have contributed substantially to the work. Please read the section concerning the criteria to qualify for authorship carefully".

Data Availability Statement: In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Please refer to suggested Data Availability Statements in section "MDPI Research Data Policies". You might choose to exclude this statement if the study did not report any data.

Conflicts of Interest: Authors must identify and declare any personal circumstances or interest that may be perceived as influencing the representation or interpretation of reported research results. If there is no conflict of interest, please state "The authors declare no conflict of interest." Any role of the funding sponsors in the choice of research project; design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results must be declared in this section. *Insects* does not publish studies funded partially or fully by the tobacco industry. Any projects funded by industry must pay special attention to the full declaration of funder involvement. If there is no role, please state "The sponsors had no role in the design, execution, interpretation, or writing of the study". For more details please see Conflict of Interest.

References: References must be numbered in order of appearance in the text (including table captions and figure legends) and listed individually at the end of the manuscript. We recommend preparing the references with a bibliography software package, such as EndNote, ReferenceManager or Zotero to avoid typing mistakes and duplicated references. We encourage citations to data, computer code and other citable research material. If available online, you may use reference style 9. below.

Citations and References in Supplementary files are permitted provided that they also appear in the main text and in the reference list.

In the text, reference numbers should be placed in square brackets [ ], and placed before the punctuation; for example [1], [1–3] or [1,3]. For embedded citations in the text with pagination, use both parentheses and brackets to indicate the reference number and page numbers; for example [5] (p. 10). or [6] (pp. 101–105).

The reference list should include the full title, as recommended by the ACS style guide. Style files for Endnote and Zotero are available.

References should be described as follows, depending on the type of work:

- Journal Articles:

1. Author 1, A.B.; Author 2, C.D. Title of the article. *Abbreviated Journal Name* Year, *Volume*, page range.

- Books and Book Chapters:

2. Author 1, A.; Author 2, B. *Book Title*, 3rd ed.; Publisher: Publisher Location, Country, Year; pp. 154–196.

3. Author 1, A.; Author 2, B. Title of the chapter. In *Book Title*, 2nd ed.; Editor 1, A., Editor 2, B., Eds.; Publisher: Publisher Location, Country, Year; Volume 3, pp. 154–196.

- Unpublished materials intended for publication:

4. Author 1, A.B.; Author 2, C. Title of Unpublished Work (optional). Correspondence Affiliation, City, State, Country. year, *status* (*manuscript in preparation*; *to be submitted*).

5. Author 1, A.B.; Author 2, C. Title of Unpublished Work. *Abbreviated Journal Name* year, *phrase indicating stage of publication* (*submitted*; *accepted*; *in press*).

- Unpublished materials not intended for publication:

6. Author 1, A.B. (Affiliation, City, State, Country); Author 2, C. (Affiliation, City, State, Country). Phase describing the material, year. (phase: Personal communication; Private communication; Unpublished work; etc.)

- Conference Proceedings:

7. Author 1, A.B.; Author 2, C.D.; Author 3, E.F. Title of Presentation. In *Title of the Collected Work* (if available), Proceedings of the Name of the Conference, Location of Conference, Country, Date of Conference; Editor 1, Editor 2, Eds. (if available); Publisher: City, Country, Year (if available); Abstract Number (optional), Pagination (optional).

- Thesis:

8. Author 1, A.B. Title of Thesis. Level of Thesis, Degree-Granting University, Location of University, Date of Completion.

- Websites:

9. Title of Site. Available online: URL (accessed on Day Month Year). Unlike published works, websites may change over time or disappear, so we encourage you create an archive of the cited website using a service such as WebCite. Archived websites should be cited using the link provided as follows:

10. Title of Site. URL (archived on Day Month Year).

See the Reference List and Citations Guide for more detailed information.

Preparing Figures, Schemes and Tables

File for Figures and Schemes must be provided during submission in a single zip archive and at a sufficiently high resolution (minimum 1000 pixels width/height, or a resolution of 300 dpi or higher). Common formats are accepted, however, TIFF, JPEG, EPS and PDF are preferred.

Insects can publish multimedia files in articles or as supplementary materials. Please contact the editorial office for further information.

All Figures, Schemes and Tables should be inserted into the main text close to their first citation and must be numbered following their number of appearance (Figure 1, Scheme I, Figure 2, Scheme II, Table 1, *etc.*).

All Figures, Schemes and Tables should have a short explanatory title and caption.

All table columns should have an explanatory heading. To facilitate the copy-editing of larger tables, smaller fonts may be used, but no less than 8 pt. in size. Authors should use the Table option of Microsoft Word to create tables.

Authors are encouraged to prepare figures and schemes in color (RGB at 8-bit per channel). There is no additional cost for publishing full color graphics.

Original Images for Blots and Gels Requirements

For the main text, please ensure that:

All experimental samples and controls used for one comparative analysis are run on the same blot/gel.

Image processing methods, such as adjusting the brightness or contrast, do not alter or distort the information in the figure and are applied to every pixel. High-contrast blots/gels are discouraged.

Cropped blots/gels present in the main text retain all important information and bands.

You have checked figures for duplications and ensured the figure legends are clear and accurate. Please include all relevant information in the figure legends and clearly indicate any re-arrangement of lanes.

In order to ensure the integrity and scientific validity of blots (including, but not limited to, Western blots) and the reporting of gel data, original, uncropped and unadjusted images should be uploaded as Supporting Information files at the time of initial submission.

A single PDF file or a zip folder including all the original images reported in the main figure and supplemental figures should be prepared. Authors should annotate each original image, corresponding to the figure in the main article or supplementary materials, and label each lane or loading order. All experimental samples and controls used for one comparative analysis should be run on the same blot/gel image. For quantitative analyses, please provide the blots/gels for each independent biological replicate used in the analysis.

Supplementary Materials, Data Deposit and Software Source Code

MDPI Research Data Policies

MDPI is committed to supporting open scientific exchange and enabling our authors to achieve best practices in sharing and archiving research data. We encourage all authors of articles published in MDPI journals to share their research data. Individual journal guidelines can be found at the journal 'Instructions for Authors' page. Data sharing policies concern the minimal dataset that supports the central findings of a published study. Generated data should be publicly available and cited in accordance with journal guidelines.

MDPI data policies are informed by TOP Guidelines and FAIR Principles.

Where ethical, legal or privacy issues are present, data should not be shared. The authors should make any limitations clear in the Data Availability Statement upon submission. Authors should ensure that data shared are in accordance with consent provided by participants on the use of confidential data.

Data Availability Statements provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study.

Below are suggested Data Availability Statements:

Data available in a publicly accessible repository

The data presented in this study are openly available in [repository name e.g., FigShare] at [doi], reference number [reference number].

Data available in a publicly accessible repository that does not issue DOIs

Publicly available datasets were analyzed in this study. This data can be found here: [link/accession number]

Data available on request due to restrictions eg privacy or ethical

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to [insert reason here]

3rd Party Data

Restrictions apply to the availability of these data. Data was obtained from [third party] and are available [from the authors / at URL] with the permission of [third party].

Data sharing not applicable

No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Data is contained within the article or supplementary material

The data presented in this study are available in [insert article or supplementary material here]

Data citation:

[dataset] Authors. Year. Dataset title; Data repository or archive; Version (if any); Persistent identifier (e.g., DOI).

Computer Code and Software

For work where novel computer code was developed, authors should release the code either by depositing in a recognized, public repository such as GitHub or uploading as supplementary information to the publication. The name, version, corporation and location information for all software used should be clearly indicated. Please include all the parameters used to run software/programs analyses.

Supplementary Material

Additional data and files can be uploaded as "Supplementary Files" during the manuscript submission process. The supplementary files will also be available to the referees as part of the peer-review process. Any file format is acceptable; however, we recommend that common, non-proprietary formats are used where possible. For more information on supplementary materials, please refer to https://www.mdpi.com/authors/layout#_bookmark83.

References in Supplementary Files

Citations and References in Supplementary files are permitted provided that they also appear in the reference list of the main text.

Unpublished Data

Restrictions on data availability should be noted during submission and in the manuscript. "Data not shown" should be avoided: authors are encouraged to publish all observations related to the submitted manuscript as Supplementary Material. "Unpublished data" intended for publication in a manuscript that is either planned, "in preparation" or "submitted" but not yet accepted, should be cited in the text and a reference should be added in the References section. "Personal Communication" should also be cited in the text and reference added in the References section. (see also the MDPI reference list and citations style guide).

Remote Hosting and Large Data Sets

Data may be deposited with specialized service providers or institutional/subject repositories, preferably those that use the DataCite mechanism. Large data sets and files greater than 60 MB must be deposited in this way. For a list of other repositories specialized in scientific and experimental data, please consult databib.org or re3data.org. The data repository name, link to the data set (URL) and accession number, doi or handle number of the data set must be provided in the paper. The journal Data also accepts submissions of data set papers.

Deposition of Sequences and Expression Data

New sequence information must be deposited to the appropriate database prior to submission of the manuscript. Accession numbers provided by the database should be included in the submitted manuscript. Manuscripts will not be published until the accession number is provided.

New nucleic acid sequences must be deposited in one of the following databases: GenBank, EMBL, or DDBJ. Sequences should be submitted to only one database.

New high throughput sequencing (HTS) datasets (RNA-seq, ChIP-Seq, degradome analysis, …) must be deposited either in the GEO database or in the NCBI's Sequence Read Archive (SRA).

New microarray data must be deposited either in the GEO or the ArrayExpress databases.The "Minimal Information About a Microarray Experiment" (MIAME) guidelines published by the Microarray Gene Expression Data Society must be followed.

New protein sequences obtained by protein sequencing must be submitted to UniProt (submission tool SPIN). Annotated protein structure and its reference sequence must be submitted to RCSB of Protein Data Bank.

All sequence names and the accession numbers provided by the databases must be provided in the Materials and Methods section of the article.

Deposition of Proteomics Data

Methods used to generate the proteomics data should be described in detail and we encourage authors to adhere to the "Minimum Information About a Proteomics Experiment". All generated mass spectrometry raw data must be deposited in the appropriate public database such as ProteomeXchange, PRIDE or jPOST. At the time of submission, please include all relevant information in the materials and methods section, such as repository where the data was submitted and link, data set identifier, username and password needed to access the data.

Research and Publication Ethics

Research Ethics

Research Involving Human Subjects

When reporting on research that involves human subjects, human material, human tissues, or human data, authors must declare that the investigations were carried out following the rules of the Declaration of Helsinki of 1975 (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/), revised in 2013. According to point 23 of this declaration, an approval from the local institutional review board (IRB) or other appropriate ethics committee must be obtained before undertaking the research to confirm the study meets national and international guidelines. As a minimum, a statement including the project identification code, date of approval, and name of the ethics committee or institutional review board must be stated in Section 'Institutional Review Board Statement' of the article.

Example of an ethical statement: "All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of XXX (Project identification code)."

For non-interventional studies (e.g. surveys, questionnaires, social media research), all participants must be fully informed if the anonymity is assured, why the research is being conducted, how their data will be used and if there are any risks associated. As with all research involving humans, ethical approval from an appropriate ethics committee must be obtained prior to conducting the study. If ethical approval is not required, authors must either provide an exemption from the ethics committee or are encouraged to cite the local or national legislation that indicates ethics approval is not required for this type of study. Where a study has been granted exemption, the name of the ethics committee which provided this should be stated in Section 'Institutional Review Board Statement' with a full explanation regarding why ethical approval was not required.

A written informed consent for publication must be obtained from participating patients. Data relating to individual participants must be described in detail, but private information identifying participants need not be included unless the identifiable materials are of relevance to the research (for example, photographs of participants' faces that show a particular symptom). Patients' initials or other personal identifiers must not appear in any images. For manuscripts that include any case details, personal information, and/or images of patients, authors must obtain signed informed consent for publication from patients (or their relatives/guardians) before submitting to an MDPI journal. Patient details must be anonymized as far as possible, e.g., do not mention specific age, ethnicity, or occupation where they are not relevant to the conclusions. A template permission form is available to download. A blank version of the form used to obtain permission (without the patient names or signature) must be uploaded with your submission. Editors reserve the right to reject any submission that does not meet these requirements.

You may refer to our sample form and provide an appropriate form after consulting with your affiliated institution. For the purposes of publishing in MDPI journals, a consent, permission, or release form should include unlimited permission for publication in all formats (including print, electronic, and online), in sublicensed and reprinted versions (including translations and derived works), and in other works and products under open access license. To respect patients' and any other individual's privacy, please do not send signed forms. The journal reserves the right to ask authors to provide signed forms if necessary.

If the study reports research involving vulnerable groups, an additional check may be performed. The submitted manuscript will be scrutinized by the editorial office and upon request, documentary evidence (blank consent forms and any related discussion documents from the ethics board) must be supplied. Additionally, when studies describe groups by race, ethnicity, gender, disability, disease, etc., explanation regarding why such categorization was needed must be clearly stated in the article.

Ethical Guidelines for the Use of Animals in Research

The editors will require that the benefits potentially derived from any research causing harm to animals are significant in relation to any cost endured by animals, and that procedures followed are unlikely to cause offense to the majority of readers. Authors should particularly ensure that their research complies with the commonly-accepted '3Rs [1]':

Replacement of animals by alternatives wherever possible,

Reduction in number of animals used, and

Refinement of experimental conditions and procedures to minimize the harm to animals.

Authors must include details on housing, husbandry and pain management in their manuscript.

For further guidance authors should refer to the Code of Practice for the Housing and Care of Animals Used in Scientific Procedures [2], American Association for Laboratory Animal Science [3] or European Animal Research Association [4].

If national legislation requires it, studies involving vertebrates or higher invertebrates must only be carried out after obtaining approval from the appropriate ethics committee. As a minimum, the project identification code, date of approval and name of the ethics committee or institutional review board should be stated in Section 'Institutional Review Board Statement'. Research procedures must be carried out in accordance with national and institutional regulations. Statements on animal welfare should confirm that the study complied with all relevant legislation. Clinical studies involving animals and interventions outside of routine care require ethics committee oversight as per the American Veterinary Medical Association. If the study involved client-owned animals, informed client consent must be obtained and certified in the manuscript report of the research. Owners must be fully informed if there are any risks associated with the procedures and that the research will be published. If available, a high standard of veterinary care must be provided. Authors are responsible for correctness of the statements provided in the manuscript.

If ethical approval is not required by national laws, authors must provide an exemption from the ethics committee, if one is available. Where a study has been granted exemption, the name of the ethics committee that provided this should be stated in Section 'Institutional Review Board Statement' with a full explanation on why the ethical approval was not required.

If no animal ethics committee is available to review applications, authors should be aware that the ethics of their research will be evaluated by reviewers and editors. Authors should provide a statement justifying the work from an ethical perspective, using the same utilitarian framework that is used by ethics committees. Authors may be asked to provide this even if they have received ethical approval.

MDPI endorses the ARRIVE guidelines (arriveguidelines.org/) for reporting experiments using live animals. Authors and reviewers must use the ARRIVE guidelines as a checklist, which can be found at https://arriveguidelines.org/sites/arrive/files/documents/ARRIVE%20Compliance%20Questionnaire.pdf. Editors reserve the right to ask for the checklist and to reject submissions that do not adhere to these guidelines, to reject submissions based on ethical or animal welfare concerns or if the procedure described does not appear to be justified by the value of the work presented.

NSW Department of Primary Industries and Animal Research Review Panel. Three Rs. Available online: https://www.animalethics.org.au/three-rs

Home Office. Animals (Scientific Procedures) Act 1986. Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes. Available online: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/388535/CoPanimalsWeb.pdf

American Association for Laboratory Animal Science. The Scientific Basis for Regulation of Animal Care and Use. Available online: https://www.aalas.org/about-aalas/position-papers/scientific-basis-for-regulation-of-animal-care-and-use

European Animal Research Association. EU regulations on animal research. Available online: https://www.eara.eu/animal-research-law

Research Involving Cell Lines

Methods sections for submissions reporting on research with cell lines should state the origin of any cell lines. For established cell lines the provenance should be stated and references must also be given to either a published paper or to a commercial source. If previously unpublished *de novo* cell lines were used, including those gifted from another laboratory, details of institutional review board or ethics committee approval must be given, and confirmation of written informed consent must be provided if the line is of human origin.

An example of Ethical Statements:

The HCT116 cell line was obtained from XXXX. The $MLH1^{+}$cell line was provided by XXXXX, Ltd. The DLD-1 cell line was obtained from Dr. XXXX. The DR-GFP and SA-GFP reporter plasmids were obtained from Dr. XXX and the Rad51K133A expression vector was obtained from Dr. XXXX.

Research Involving Plants

Experimental research on plants (either cultivated or wild) including collection of plant material, must comply with institutional, national, or international guidelines. We recommend that authors comply with the Convention on Biological Diversity and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

For each submitted manuscript supporting genetic information and origin must be provided. For research manuscripts involving rare and non-model plants (other than, e.g., *Arabidopsis thaliana*, *Nicotiana benthamiana*, *Oryza sativa*, or many other typical model plants), voucher specimens must be deposited in an accessible herbarium or museum. Vouchers may be requested for review by future investigators to verify the identity of the material used in the study (especially if taxonomic rearrangements occur in the future). They should include details of the populations sampled on the site of collection (GPS coordinates), date of collection, and document the part(s) used in the study where appropriate. For rare, threatened or endangered species this can be waived but it is necessary for the author to describe this in the cover letter.

Editors reserve the rights to reject any submission that does not meet these requirements.

An example of Ethical Statements:

Torenia fournieri plants were used in this study. White-flowered Crown White (CrW) and violet-flowered Crown Violet (CrV) cultivars selected from 'Crown Mix' (XXX Company, City, Country) were kindly provided by Dr. XXX (XXX Institute, City, Country).

Arabidopis mutant lines (SALKxxxx, SAILxxxx,…) were kindly provided by Dr. XXX , institute, city, country).

Clinical Trials Registration

Registration

MDPI follows the International Committee of Medical Journal Editors (ICMJE) guidelines which require and recommend registration of clinical trials in a public trials registry at or before the time of first patient enrollment as a condition of consideration for publication.

Purely observational studies do not require registration. A clinical trial not only refers to studies that take place in a hospital or involve pharmaceuticals, but also refer to all studies which involve participant randomization and group classification in the context of the intervention under assessment.

Authors are strongly encouraged to pre-register clinical trials with an international clinical trials register and cite a reference to the registration in the Methods section. Suitable databases include clinicaltrials.gov, the EU Clinical Trials Register and those listed by the World Health Organisation International Clinical Trials Registry Platform.

Approval to conduct a study from an independent local, regional, or national review body is not equivalent to prospective clinical trial registration. MDPI reserves the right to decline any paper without trial registration for further peer-review. However, if the study protocol has been published before the enrolment, the registration can be waived with correct citation of the published protocol.

CONSORT Statement

MDPI requires a completed CONSORT 2010 checklist and as a condition of submission when reporting the results of a randomized trial. Templates for these can be found here or on the CONSORT website (http://www.consort-statement.org) which also describes several CONSORT checklist extensions for different designs and types of data beyond two group parallel trials. At minimum, your article should report the content addressed by each item of the checklist.

Sex and Gender in Research

We encourage our authors to follow the 'Sex and Gender Equity in Research – SAGER – guidelines' and to include sex and gender considerations where relevant. Authors should use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Article titles and/or abstracts should indicate clearly what sex(es) the study applies to. Authors should also describe in the background, whether sex and/or gender differences may be expected; report how sex and/or gender were accounted for in the design of the study; provide disaggregated data by sex and/or gender, where appropriate; and discuss respective results. If a sex and/or gender analysis was not conducted, the rationale should be given in the Discussion. We suggest that our authors consult the full guidelines before submission.

Borders and Territories

Potential disputes over borders and territories may have particular relevance for authors in describing their research or in an author or editor correspondence address, and should be respected. Content decisions are an editorial matter and where there is a potential or perceived dispute or complaint, the editorial team will attempt to find a resolution that satisfies parties involved.

MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Publication Ethics Statement

Insects is a member of the Committee on Publication Ethics (COPE). We fully adhere to its Code of Conduct and to its Best Practice Guidelines.

The editors of this journal enforce a rigorous peer-review process together with strict ethical policies and standards to ensure to add high quality scientific works to the field of scholarly publication. Unfortunately, cases of plagiarism, data falsification, image manipulation, inappropriate authorship credit, and the like, do arise. The editors of *Insects* take such publishing ethics issues very seriously and are trained to proceed in such cases with a zero tolerance policy.

Authors wishing to publish their papers in *Insects* must abide to the following:

Any facts that might be perceived as a possible conflict of interest of the author(s) must be disclosed in the paper prior to submission.

Authors should accurately present their research findings and include an objective discussion of the significance of their findings.

Data and methods used in the research need to be presented in sufficient detail in the paper, so that other researchers can replicate the work.

Raw data should preferably be publicly deposited by the authors before submission of their manuscript. Authors need to at least have the raw data readily available for presentation to the referees and the editors of the journal, if requested. Authors need to ensure appropriate measures are taken so that raw data is retained in full for a reasonable time after publication.

Simultaneous submission of manuscripts to more than one journal is not tolerated.

The journal accepts exact translations of previously published work. All submissions of translations must conform with our policies on translations.

If errors and inaccuracies are found by the authors after publication of their paper, they need to be promptly communicated to the editors of this journal so that appropriate actions can be taken. Please refer to our policy regarding Updating Published Papers.

Your manuscript should not contain any information that has already been published. If you include already published figures or images, please obtain the necessary permission from the copyright holder to publish under the CC-BY license. For further information, see the Rights and Permissions page.

Plagiarism, data fabrication and image manipulation are not tolerated.

Plagiarism is not acceptable in *Insects* submissions.

Plagiarism includes copying text, ideas, images, or data from another source, even from your own publications, without giving any credit to the original source.

Reuse of text that is copied from another source must be between quotes and the original source must be cited. If a study's design or the manuscript's structure or language has been inspired by previous works, these works must be explicitly cited.

All MDPI submissions are checked for plagiarism using the industry standard software iThenticate. If plagiarism is detected during the peer review process, the manuscript may be rejected. If plagiarism is detected after publication, an investigation will take place and action taken in accordance with our policies.

Image files must not be manipulated or adjusted in any way that could lead to misinterpretation of the information provided by the original image.

Irregular manipulation includes: 1) introduction, enhancement, moving, or removing features from the original image; 2) grouping of images that should obviously be presented separately (e.g., from different parts of the same gel, or from different gels); or 3) modifying the contrast, brightness or color balance to obscure, eliminate or enhance some information.

If irregular image manipulation is identified and confirmed during the peer review process, we may reject the manuscript. If irregular image manipulation is identified and confirmed after publication, we may correct or retract the paper.

Our in-house editors will investigate any allegations of publication misconduct and may contact the authors' institutions or funders if necessary. If evidence of misconduct is found, appropriate action will be taken to correct or retract the publication. Authors are expected to comply with the best ethical publication practices when publishing with MDPI.

Citation Policy

Authors should ensure that where material is taken from other sources (including their own published writing) the source is clearly cited and that where appropriate permission is obtained.

Authors should not engage in excessive self-citation of their own work.

Authors should not copy references from other publications if they have not read the cited work.

Authors should not preferentially cite their own or their friends', peers', or institution's publications.

Authors should not cite advertisements or advertorial material.

In accordance with COPE guidelines, we expect that "original wording taken directly from publications by other researchers should appear in quotation marks with the appropriate citations." This condition also applies to an author's own work. COPE have produced a discussion document on citation manipulation with recommendations for best practice.

Reviewer Suggestions

During the submission process, please suggest three potential reviewers with the appropriate expertise to review the manuscript. The editors will not necessarily approach these referees. Please provide detailed contact information (address, homepage, phone, e-mail address). The proposed referees should neither be current collaborators of the co-authors nor have published with any of the co-authors of the manuscript within the last five years. Proposed reviewers should be from different institutions to the authors. You may identify appropriate Editorial Board members of the journal as potential reviewers. You may suggest reviewers from among the authors that you frequently cite in your paper.

APPENDIX C

Instructions to authors: Biological Control (Elsevier)

INTRODUCTION

Biological Control promotes the science and technology of biological control through publication of original research articles and reviews of research and theory. The focus includes new and emerging trends in this field. Biological control is defined as the reduction or mitigation of pests and pest effects through the use of natural enemies. Biotechnologies dealing with the elucidation and use of genes or gene products for the enhancement of biological control agents are also of interest.

The journal encompasses biological control of viral, microbial, nematode, insect, mite, weed, and other invertebrate and vertebrate pests in agricultural, aquatic, forest, natural resource, stored products, and urban environments. Biological control of arthropod pests of human and domestic animals is also included. Ecological, behavioral, molecular, and biotechnological approaches to advancing the understanding of biological control agents are welcome.

The "rules of 6"

The Editors and Editorial Board have developed the "Rules of 6" for publishing in BCON. We have produced six clear criteria that each author needs to think about before submitting a manuscript and setting the whole process of editing and reviewing at work. Click here.

Types of paper

The following types of original papers only will be considered.

Regular research papers are hypothesis-driven projects in biological control as defined above under the "Subject areas of the journal". These papers constitute the majority of the articles published in the journal. However, papers which report on routine results of host specificity studies on new biological control agents, surveys for known or unknown biological control agents, or screening of natural enemies against a pest species will not be considered for publication unless the account describes unusual circumstances or novel methods, or unless the study is placed in a broader perspective. Research papers should have the following sections: a brief Abstract that contains a concise statement of the results obtained; Keywords listed immediately after the Abstract; Introduction; Materials and Methods; Results; Discussion; Acknowledgments; and References. Further details are provided below under "Preparation of manuscript".

Perspectives papers provide the authors with a forum for discussing topics and trends in biological control. These articles should raise interesting or unanswered questions, present arguments about the significance of recent findings, describe the application and limitations of new methods and technologies, or consider potential interfaces between biological control and other disciplines in the sciences. These manuscripts should include the format as listed for the **Regular research papers** or may deviate by having the following sections: Abstract, Introduction, appropriate headings, Conclusion, and References.

Review articles are intended to reach a broad audience of readers from investigators in the field to new graduate students learning the material for the first time. Review articles are subject to the same review process as original papers. Manuscripts should be prepared according to the general guidelines given below. The Materials and Methods, Results, and Discussion sections may be replaced with appropriate alternatives; an Abstract is still required. The editors invite inquiries and suggestions for timely and provocative review articles. In some cases, there may be a number of review articles (e.g., a symposium topic) in which case a special issue of the journal may be published. The special issue may include an invited "editor(s)" who invites the authors and selects the topics, provides the guidelines to the authors, sets deadlines, etc., and submits the manuscripts to the journal. The journal editor then handles the manuscripts following normal protocols.

Biological Control does not publish Short Communications or Research Notes.

Contact details for submission

Manuscripts should be written in grammatically correct English and should be submitted through the Web site at https://www.editorialmanager.com/bcon/default.aspx.

Submission checklist

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:

E-mail address

Full postal address

All necessary files have been uploaded:

Manuscript:

Include keywords

All figures (include relevant captions)

All tables (including titles, description, footnotes)

Ensure all figure and table citations in the text match the files provided

Indicate clearly if color should be used for any figures in print

Graphical Abstracts / Highlights files (where applicable)

Supplemental files (where applicable)

Further considerations

Manuscript has been 'spell checked' and 'grammar checked'

All references mentioned in the Reference List are cited in the text, and vice versa

Permission has been obtained for use of copyrighted material from other sources (including theInternet)

A competing interests statement is provided, even if the authors have no competing interests todeclare

Journal policies detailed in this guide have been reviewed

Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

Revised Submission

Please follow the Checklist for submission of revised manuscript for submitting the revised files.

BEFORE YOU BEGIN

Ethics in publishing

Please see our information on Ethics in publishing.

Policy and ethics

Ethics. Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, without the written consent of the copyright holder. Written authorization may be required at the discretion of the editors. Articles and any other material published in Biological Control represent the opinions of the authors and should not be construed to reflect the opinions of the editors or the publisher.

A submitted paper will be considered in violation of Elsevier's Ethics Guidelines, and thus potentially subject to rejection or retraction, in the event of the following: the study results are inaccurately or deceptively reported, the data from the study results cannot be produced, the paper submitted is not an original work or it has plagiarized (by copying or paraphrasing) another work, the paper has been submitted concurrently to another journal or is elsewhere published, other works discussed in the paper are improperly cited or un-cited, the list of co-authors is incomplete or contains those who have not contributed substantially to the work, any experiments involving human or animal subjects or hazardous chemicals are not ensured in the paper as having been conducted according to the appropriate guidelines, or financial or otherwise conflicts of interest are not disclosed. For a comprehensive explanation of Elsevier's Ethics Guidelines, please visit https://www.elsevier.com/ethicalguidelines.

Should questions of probity arise under extraordinary and controversial situations, the editors will reserve the right to subject the authors' data to independent scientific evaluation.

Upon acceptance of an article, authors will be asked to sign a Journal Publishing Agreement. (for more information on this and copyright see https://www.elsevier.com/copyright). Acceptance of the agreement will ensure the widest possible dissemination of information. An e-mail (or letter) will be sent to the corresponding author confirming receipt of the manuscript together with a "Journal Publishing Agreement" form or a link to the online version of this agreement. Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations (please consult https://www.elsevier.com/permissions). If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases: please consult https://www.elsevier.com/permissions.

Declaration of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Authors must disclose any interests in two places: 1. A summary declaration of interest statement in the title page file (if double anonymized) or the manuscript file (if single anonymized). If there are no interests to declare then please state this: 'Declarations of interest: none'. 2. Detailed disclosures as part of a separate Declaration of Interest form, which forms part of the journal's official records. It is important for potential interests to be declared in both places and that the information matches. More information.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyrightholder. To verify compliance, your article may be checked by Crossref Similarity Check and other originality or duplicate checking software.

Preprints

Please note that preprints can be shared anywhere at any time, in line with Elsevier's sharing policy. Sharing your preprints e.g. on a preprint server will not count as prior publication (see 'Multiple, redundant or concurrent publication' for more information).

Preprint posting on SSRN

In support of Open Science, this journal offers its authors a free preprint posting service. Preprints provide early registration and dissemination of your research, which facilitates early citations and collaboration.

During submission to Editorial Manager, you can choose to release your manuscript publicly as a preprint on the preprint server SSRN once it enters peer-review with the journal. Your choice will have no effect on the editorial process or outcome with the journal. Please note that the corresponding author is expected to seek approval from all co-authors before agreeing to release the manuscript publicly on SSRN.

You will be notified via email when your preprint is posted online and a Digital Object Identifier (DOI) is assigned. Your preprint will remain globally available free to read whether the journal accepts or rejects your manuscript.

For more information about posting to SSRN, please consult the SSRN Terms of Use and FAQs.

Use of inclusive language

Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Content should make no assumptions about the beliefs or commitments of any reader; contain nothing which might imply that one individual is superior to another on the grounds of age, gender, race, ethnicity, culture, sexual orientation, disability or health condition; and use inclusive language throughout. Authors should ensure that writing is free from bias, stereotypes, slang, reference to dominant culture and/or cultural assumptions. We advise to seek gender neutrality by using plural nouns ("clinicians, patients/clients") as default/wherever possible to avoid using "he, she," or "he/she." We recommend avoiding the use of descriptors that refer to personal attributes such as age, gender, race, ethnicity, culture, sexual orientation, disability or health condition unless they are relevant and valid. When coding terminology is used, we recommend to avoid offensive or exclusionary terms such as "master", "slave", "blacklist" and "whitelist". We suggest using alternatives that are more appropriate and (self-) explanatory such as "primary", "secondary", "blocklist" and "allowlist". These guidelines are meant as a point of reference to help identify appropriate language but are by no means exhaustive or definitive.

Author contributions

For transparency, we encourage authors to submit an author statement file outlining their individual contributions to the paper using the relevant CRediT roles: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing. Authorship statements should be formatted with the names of authors first and CRediT role(s) following. More details and an example.

Changes to authorship

Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors **after** the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Article transfer service

This journal uses the Elsevier Article Transfer Service to find the best home for your manuscript. This means that if an editor feels your manuscript is more suitable for an alternative journal, you might be asked to consider transferring the manuscript to such a journal. The recommendation might be provided by a Journal Editor, a dedicated Scientific Managing Editor, a tool assisted recommendation, or a combination. If you agree, your manuscript will be transferred, though you will have the opportunity to make changes to the manuscript before the submission is complete. Please note that your manuscript will be independently reviewed by the new journal. More information.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (see more information on this). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases.

For gold open access articles: Upon acceptance of an article, authors will be asked to complete a 'License Agreement' (more information). Permitted third party reuse of gold open access articles is determined by the author's choice of user license.

Author rights

As an author you (or your employer or institution) have certain rights to reuse your work. More information.

Elsevier supports responsible sharing

Find out how you can share your research published in Elsevier journals.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement, it is recommended to state this.

Open access

Please visit our Open Access page for more information.

Elsevier Researcher Academy

Researcher Academy is a free e-learning platform designed to support early and mid-career researchers throughout their research journey. The "Learn" environment at Researcher Academy offers several interactive modules, webinars, downloadable guides and resources to guide you through the process of writing for research and going through peer review. Feel free to use these free resources to improve your submission and navigate the publication process with ease.

Language (usage and editing services)

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's Author Services.

Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail.

PREPARATION

Queries

For questions about the editorial process (including the status of manuscripts under review) or for technical support on submissions, please visit our Support Center.

Use of word processing software

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.

To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision - numbered sections

Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

Theory/calculation

A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

Title. Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.

Author names and affiliations. Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lowercase superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.

Corresponding author. Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**

Present/permanent address. If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Highlights

Highlights are mandatory for this journal as they help increase the discoverability of your article via search engines. They consist of a short collection of bullet points that capture the novel results of your research as well as new methods that were used during the study (if any). Please have a look at the examples here: example Highlights.

Highlights should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

Graphical abstract

Although a graphical abstract is optional, its use is encouraged as it draws more attention to the online article. The graphical abstract should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. You can view Example Graphical Abstracts on our information site.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Consult the latest edition of the CBE Style Manual, Council of Biology Editors, Inc., for standard abbreviations, names, and symbols for units, as well as for informative suggestions about grammar, style, and usage. Nonstandard abbreviations should be minimal and should be defined at first mention. Follow the latest edition of "Webster's New International Dictionary" for spelling and division of words. Use numerals with standard units of measurement and for any number above nine. For the sake of consistency, the journal will use U.S. English. Manuscripts should be typed with the language set to U.S. English.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, it is recommended to include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

Nomenclature and units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other quantities are mentioned, give their equivalent in SI. You are urged to consult IUPAC: Nomenclature of Organic Chemistry for further information.

For organisms, the complete taxonomic name including the authority must be given at first mention in the text. For exceptions to this rule, such as names of bacteria, consult the editor. The names of insects will be in accordance with the Entomological Society of America. Wherever a common name for a pesticide exists, it should be used. The chemical name of the pesticide must be included in parentheses following the first mention of the common name. Most common names may be found in Guide to the Chemicals Used in Crop Protection by E.Y. Spencer, Agriculture Canada, 7th ed., 1982, and more recent entries are found in The Pesticide Manual-A World Compendium (C.R. Worthington, Ed.; S.B. Walker, Asst. Ed.), 8th ed., British Crop Protection Council, Binfield, Bracknell, Berks RG 125QE, England. In addition, common names of insecticides are listed from time to time by the Entomological Society of America; of herbicides, by the Weed Science Society of America; and of fungicides, by the American Phytopathological Society. For weed names, use the terminology approved by the Weed Science Society of America [Weed Science 32 (Suppl. 2), 1-137, 1984]. For enzymes, the systematic name and number given by the Enzyme Commission (EC) should be included at the first point of mention for each enzyme of importance in the paper. For EC numbers, consult

Recommendations (1984) of the Nomenclature Committee of the International Union of Biochemistry, 1984, Enzyme Nomenclature, Academic Press

Gene accession numbers refer to genes or DNA sequences about which further information can be found in the databases at the National Center for Biotechnical Information (NCBI) at the National Library of Medicine.

Authors are encouraged to check accession numbers used very carefully. An error in a letter or number can result in a dead link. Note that in the final version of the electronic copy, the accession number text will be linked to the appropriate source in the NCBI databases enabling readers to go directly to that source from the article.

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors can build footnotes into the text, and this feature may be used. Otherwise, please indicate the position of footnotes in the text and list the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Artwork

Electronic artwork General points

Make sure you use uniform lettering and sizing of your original artwork.

Embed the used fonts if the application provides that option.

Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, oruse fonts that look similar.

Number the illustrations according to their sequence in the text.

Use a logical naming convention for your artwork files.

Provide captions to illustrations separately.

Size the illustrations close to the desired dimensions of the published version.

Submit each illustration as a separate file.

Ensure that color images are accessible to all, including those with impaired color vision.

A detailed guide on electronic artwork is available.

You are urged to visit this site; some excerpts from the detailed information are given here. *Formats*

If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format.

Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

EPS (or PDF): Vector drawings, embed all used fonts.

TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.

TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi. TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.

Please do not:

Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have alow number of pixels and limited set of colors;

Supply files that are too low in resolution;

Submit graphics that are disproportionately large for the content.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article**. Please indicate your preference for color: in print or online only. Further information on the preparation of electronic artwork.

Figure captions

Ensure that each illustration has a caption. Supply captions on separate page(s) at the end of the manuscript. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

Please submit tables as editable text and not as images. Supply table captions on separate page(s) at the end at the end of the manuscript. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules.

References

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Reference links

Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, Crossref and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is highly encouraged.

A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. Journal of Geophysical Research, https://doi.org/10.1029/2001JB000884. Please note the format of such citations should be in the same style as all other references in the paper.

Web references

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

Data references

This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

Preprint references

Where a preprint has subsequently become available as a peer-reviewed publication, the formal publication should be used as the reference. If there are preprints that are central to your work or that cover crucial developments in the topic, but are not yet formally published, these may be referenced. Preprints should be clearly marked as such, for example by including the word preprint, or the name of the preprint server, as part of the reference. The preprint DOI should also be provided.

References in a special issue

Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference management software

Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support Citation Style Language styles, such as Mendeley. Using citation plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide. If you use reference management software, please ensure that you remove all field codes before submitting the electronic manuscript. More information on how to remove field codes from different reference management software.

Reference formatting

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/ book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference style

Text: All citations in the text should refer to:

Single author: the author's name (without initials, unless there is ambiguity) and the year of publication;

Two authors: both authors' names and the year of publication;

Three or more authors: first author's name followed by 'et al.' and the year of publication. Citations may be made directly (or parenthetically). Groups of references can be listed either first alphabetically, then chronologically, or vice versa.

Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999)…. Or, as demonstrated (Jones, 1999; Allan, 2000)… Kramer et al. (2010) have recently shown …'

List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.

Examples:

Reference to a journal publication:

Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci.

Commun. 163, 51–59. https://doi.org/10.1016/j.Sc.2010.00372.

Reference to a journal publication with an article number:

Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.

Reference to a book:

Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.

Reference to a chapter in an edited book:

Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

Reference to a website:

Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/ aboutcancer/statistics/cancerstatsreport/ (accessed 13 March 2003).

Reference to a dataset:

[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. https://doi.org/10.17632/ xwj98nb39r.1. Reference to software:

Coon, E., Berndt, M., Jan, A., Svyatsky, D., Atchley, A., Kikinzon, E., Harp, D., Manzini, G., Shelef, E., Lipnikov, K., Garimella, R., Xu, C., Moulton, D., Karra, S., Painter, S., Jafarov, E., & Molins, S., 2020. Advanced Terrestrial Simulator (ATS) v0.88 (Version 0.88). Zenodo. https://doi.org/10.5281/ zenodo.3727209.

Journal abbreviations source

Journal names should be abbreviated according to the List of Title Word Abbreviations.

Social Media

If your article is Accepted, we may promote your article on Elsevier's Twitter feed @ParasitologyELS. If you wish your article to be considered for promotion, please supply a short Tweet text that succinctly highlights the key research findings, and 2 or 3 hashtags. Please also include your own Twitter handle. You may also wish to include an image to accompany the Tweet (ideally 1200 x 675 pixel dimensions). For further promotion of your article we encourage you to tag @ParasitologyELS in any of your own Tweets of your accepted paper for greater dissemination.

Data visualization

Include interactive data visualizations in your publication and let your readers interact and engage more closely with your research. Follow the instructions here to find out about available data visualization options and how to include them with your article.

Supplementary material

Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.

Research data

This journal requires and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. When sharing data in one of these ways, you are expected to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Data linking

If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to underlying data that gives them a better understanding of the research described.

There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page.

For supported data repositories a repository banner will automatically appear next to your published article on ScienceDirect.

In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

Research Elements

This journal enables you to publish research objects related to your original research – such as data, methods, protocols, software and hardware – as an additional paper in Research Elements.

Research Elements is a suite of peer-reviewed, open access journals which make your research objects findable, accessible and reusable. Articles place research objects into context by providing detailed descriptions of objects and their application, and linking to the associated original research articles. Research Elements articles can be prepared by you, or by one of your collaborators.

During submission, you will be alerted to the opportunity to prepare and submit a Research Elements article.

More information can be found on the Research Elements page.

Data statement

To foster transparency, we require you to state the availability of your data in your submission if your data is unavailable to access or unsuitable to post. This may also be a requirement of your funding body or institution. You will have the opportunity to provide a data statement during the submission process. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page..

AFTER ACCEPTANCE

Online proof correction

To ensure a fast publication process of the article, we kindly ask authors to provide us with their proof corrections within two days. Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.

If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.

We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints

The corresponding author will, at no cost, receive a customized Share Link providing 50 days free access to the final published version of the article on ScienceDirect. The Share Link can be used for sharing the article via any communication channel, including email and social media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's Author Services. Corresponding authors who have published their article gold open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

AUTHOR INQUIRIES

Visit the Elsevier Support Center to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch.

You can also check the status of your submitted article or find out when your accepted article will be published.

APPENDIX D

Instructions to authors: International Journal of Tropical Insect Science (Springer)

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Online Submission

Please follow the hyperlink "Submit manuscript" and upload all of your manuscript files following the instructions given on the screen.

Source Files

Please ensure you provide all relevant editable source files at every submission and revision. Failing to submit a complete set of editable source files will result in your article not being considered for review. For your manuscript text please always submit in common word processing formats such as .docx or LaTeX.

Title Page

Title Page

Please make sure your title page contains the following information.

Title

The title should be concise and informative.

Author information

The name(s) of the author(s)

The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country

A clear indication and an active e-mail address of the corresponding author

If available, the 16-digit ORCID of the author(s)

If address information is provided with the affiliation(s) it will also be published.

For authors that are (temporarily) unaffiliated we will only capture their city and country of residence, not their e-mail address unless specifically requested.

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

For life science journals only (when applicable)

Trial registration number and date of registration for prospectively registered trials

Trial registration number and date of registration, followed by “retrospectively registered”, for retrospectively registered trials

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Statements and Declarations

The following statements should be included under the heading "Statements and Declarations" for inclusion in the published paper. Please note that submissions that do not include relevant declarations will be returned as incomplete.

Competing Interests: Authors are required to disclose financial or non-financial interests that are directly or indirectly related to the work submitted for publication. Please refer to “Competing Interests and Funding” below for more information on how to complete this section.

Please see the relevant sections in the submission guidelines for further information as well as various examples of wording. Please revise/customize the sample statements according to your own needs.

Text

Text Formatting

Manuscripts should be submitted in Word.

Use a normal, plain font (e.g., 10-point Times Roman) for text.

Use italics for emphasis.

Use the automatic page numbering function to number the pages.

Do not use field functions.

Use tab stops or other commands for indents, not the space bar.

Use the table function, not spreadsheets, to make tables.

Use the equation editor or MathType for equations.

Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX. We recommend using Springer Nature's LaTeX template.

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

References

Citation

Cite references in the text by name and year in parentheses. Some examples:

Negotiation research spans many disciplines (Thompson 1990).

This result was later contradicted by Becker and Seligman (1996).

This effect has been widely studied (Abbott 1991; Barakat et al. 1995a, b; Kelso and Smith 1998; Medvec et al. 1999, 2000).

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text.

Reference list entries should be alphabetized by the last names of the first author of each work. Please alphabetize according to the following rules: 1) For one author, by name of author, then chronologically; 2) For two authors, by name of author, then name of coauthor, then chronologically; 3) For more than two authors, by name of first author, then chronologically.

If available, please always include DOIs as full DOI links in your reference list (e.g. "https://doi.org/abc").

Journal article

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8

Ideally, the names of all authors should be provided, but the usage of "et al" in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

Article by DOI

Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. https://doi.org/10.1007/s001090000086

Book

South J, Blass B (2001) The future of modern genomics. Blackwell, London

Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

Dissertation

Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see

ISSN LTWA

If you are unsure, please use the full journal title.

Tables

All tables are to be numbered using Arabic numerals.

Tables should always be cited in text in consecutive numerical order.

For each table, please supply a table caption (title) explaining the components of the table.

Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.

Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Artwork and Illustrations Guidelines

Electronic Figure Submission

Supply all figures electronically.

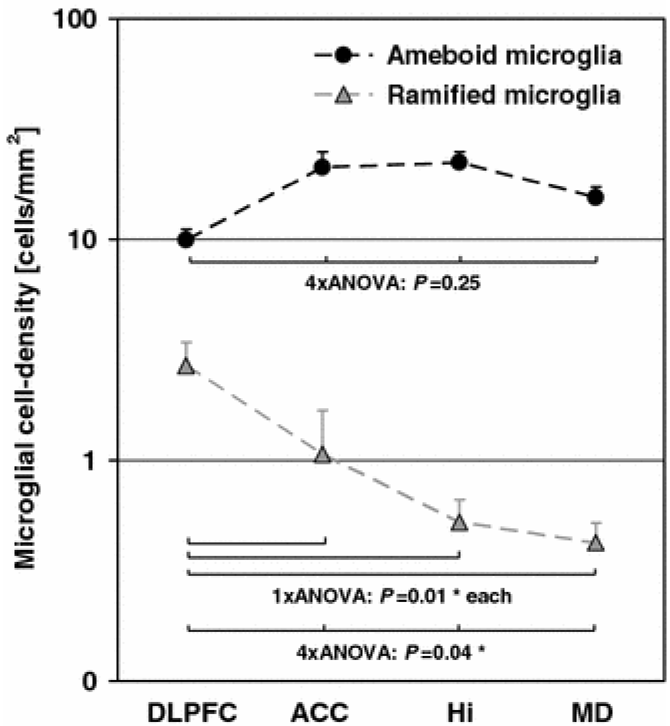
Indicate what graphics program was used to create the artwork.

For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.

Vector graphics containing fonts must have the fonts embedded in the files.

Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

Line Art



Definition: Black and white graphic with no shading.

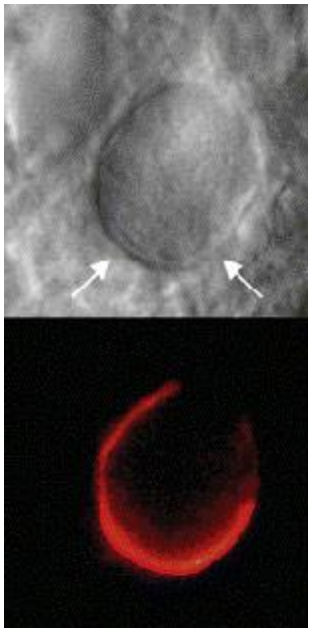
Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.

All lines should be at least 0.1 mm (0.3 pt) wide.

Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.

Vector graphics containing fonts must have the fonts embedded in the files.

Halftone Art

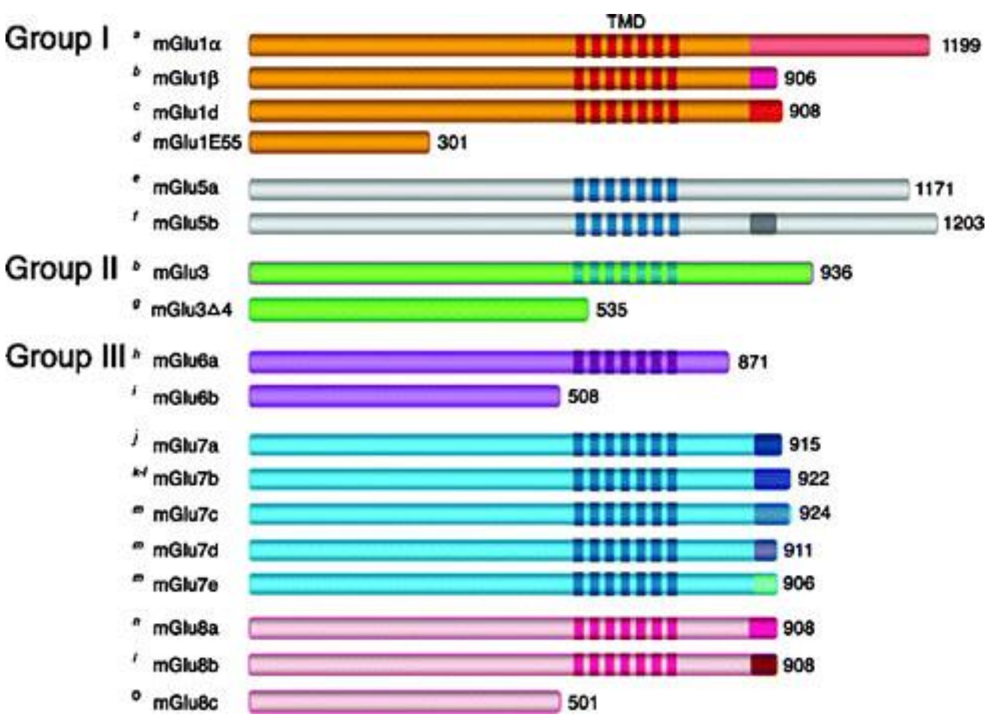


Definition: Photographs, drawings, or paintings with fine shading, etc.

If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.

Halftones should have a minimum resolution of 300 dpi.

Combination Art



Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.

Combination artwork should have a minimum resolution of 600 dpi.

Color Art

Color illustrations should be submitted as RGB (8 bits per channel).

Figure Lettering

To add lettering, it is best to use Helvetica or Arial (sans serif fonts).

Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).

Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.

Avoid effects such as shading, outline letters, etc.

Do not include titles or captions within your illustrations.

Figure Numbering

All figures are to be numbered using Arabic numerals.

Figures should always be cited in text in consecutive numerical order.

Figure parts should be denoted by lowercase letters (a, b, c, etc.).

If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures,"A1, A2, A3, etc." Figures in online appendices [Supplementary Information (SI)] should, however, be numbered separately.

Figure Captions

Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.

Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.

No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.

Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.

Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

Figures should be submitted within the body of the text. Only if the file size of the manuscript causes problems in uploading it, the large figures should be submitted separately from the text.

When preparing your figures, size figures to fit in the column width.

For large-sized journals the figures should be 84 mm (for double-column text areas), or 174 mm (for single-column text areas) wide and not higher than 234 mm.

For small-sized journals, the figures should be 119 mm wide and not higher than 195 mm.

Permissions

If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s). Please be aware that some publishers do not grant electronic rights for free and that Springer will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used.

Accessibility

In order to give people of all abilities and disabilities access to the content of your figures, please make sure that

All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)

Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)

Any figure lettering has a contrast ratio of at least 4.5:1

Supplementary Information (SI)

Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.

Before submitting research datasets as Supplementary Information, authors should read the journal’s Research data policy. We encourage research data to be archived in data repositories wherever possible.

Submission

Supply all supplementary material in standard file formats.

Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.

To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.

High resolution (streamable quality) videos can be submitted up to a maximum of 25GB; low resolution videos should not be larger than 5GB.

Audio, Video, and Animations

Aspect ratio: 16:9 or 4:3

Maximum file size: 25 GB for high resolution files; 5 GB for low resolution files

Minimum video duration: 1 sec

Supported file formats: avi, wmv, mp4, mov, m2p, mp2, mpg, mpeg, flv, mxf, mts, m4v, 3gp

Text and Presentations

Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability.

A collection of figures may also be combined in a PDF file.

Spreadsheets

Spreadsheets should be submitted as .csv or .xlsx files (MS Excel).

Specialized Formats

Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

Collecting Multiple Files

It is possible to collect multiple files in a .zip or .gz file.

Numbering

If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.

Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", “... additional data are given in Online Resource 4”.

Name the files consecutively, e.g. “ESM_3.mpg”, “ESM_4.pdf”.

Captions

For each supplementary material, please supply a concise caption describing the content of the file.

Processing of supplementary files

Supplementary Information (SI) will be published as received from the author without any conversion, editing, or reformatting.

Accessibility

In order to give people of all abilities and disabilities access to the content of your supplementary files, please make sure that

The manuscript contains a descriptive caption for each supplementary material

Video files do not contain anything that flashes more than three times per second (so that users prone to seizures caused by such effects are not put at risk)

Ethical Responsibilities of Authors

This journal is committed to upholding the integrity of the scientific record. As a member of the Committee on Publication Ethics (COPE) the journal will follow the COPE guidelines on how to deal with potential acts of misconduct.

Authors should refrain from misrepresenting research results which could damage the trust in the journal, the professionalism of scientific authorship, and ultimately the entire scientific endeavour. Maintaining integrity of the research and its presentation is helped by following the rules of good scientific practice, which include*:

The manuscript should not be submitted to more than one journal for simultaneous consideration.

The submitted work should be original and should not have been published elsewhere in any form or language (partially or in full), unless the new work concerns an expansion of previous work. (Please provide transparency on the re-use of material to avoid the concerns about text-recycling ('self-plagiarism').

A single study should not be split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (i.e. 'salami-slicing/publishing').

Concurrent or secondary publication is sometimes justifiable, provided certain conditions are met. Examples include: translations or a manuscript that is intended for a different group of readers.

Results should be presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation (including image based manipulation). Authors should adhere to discipline-specific rules for acquiring, selecting and processing data.

No data, text, or theories by others are presented as if they were the author's own ('plagiarism'). Proper acknowledgements to other works must be given (this includes material that is closely copied (near verbatim), summarized and/or paraphrased), quotation marks (to indicate words taken from another source) are used for verbatim copying of material, and permissions secured for material that is copyrighted.

Important note: the journal may use software to screen for plagiarism.

Authors should make sure they have permissions for the use of software, questionnaires/(web) surveys and scales in their studies (if appropriate).

Research articles and non-research articles (e.g. Opinion, Review, and Commentary articles) must cite appropriate and relevant literature in support of the claims made. Excessive and inappropriate self-citation or coordinated efforts among several authors to collectively self-cite is strongly discouraged.

Authors should avoid untrue statements about an entity (who can be an individual person or a company) or descriptions of their behavior or actions that could potentially be seen as personal attacks or allegations about that person.

Research that may be misapplied to pose a threat to public health or national security should be clearly identified in the manuscript (e.g. dual use of research). Examples include creation of harmful consequences of biological agents or toxins, disruption of immunity of vaccines, unusual hazards in the use of chemicals, weaponization of research/technology (amongst others).

Authors are strongly advised to ensure the author group, the Corresponding Author, and the order of authors are all correct at submission. Adding and/or deleting authors during the revision stages is generally not permitted, but in some cases may be warranted. Reasons for changes in authorship should be explained in detail. Please note that changes to authorship cannot be made after acceptance of a manuscript.

*All of the above are guidelines and authors need to make sure to respect third parties rights such as copyright and/or moral rights.

Upon request authors should be prepared to send relevant documentation or data in order to verify the validity of the results presented. This could be in the form of raw data, samples, records, etc. Sensitive information in the form of confidential or proprietary data is excluded.

If there is suspicion of misbehavior or alleged fraud the Journal and/or Publisher will carry out an investigation following COPE guidelines. If, after investigation, there are valid concerns, the author(s) concerned will be contacted under their given e-mail address and given an opportunity to address the issue. Depending on the situation, this may result in the Journal's and/or Publisher's implementation of the following measures, including, but not limited to:

If the manuscript is still under consideration, it may be rejected and returned to the author.

If the article has already been published online, depending on the nature and severity of the infraction:

- an erratum/correction may be placed with the article

- an expression of concern may be placed with the article

- or in severe cases retraction of the article may occur.

The reason will be given in the published erratum/correction, expression of concern or retraction note. Please note that retraction means that the article is maintained on the platform, watermarked "retracted" and the explanation for the retraction is provided in a note linked to the watermarked article.

The author's institution may be informed

A notice of suspected transgression of ethical standards in the peer review system may be included as part of the author's and article's bibliographic record.

Fundamental errors

Authors have an obligation to correct mistakes once they discover a significant error or inaccuracy in their published article. The author(s) is/are requested to contact the journal and explain in what sense the error is impacting the article. A decision on how to correct the literature will depend on the nature of the error. This may be a correction or retraction. The retraction note should provide transparency which parts of the article are impacted by the error.

Suggesting / excluding reviewers

Authors are welcome to suggest suitable reviewers and/or request the exclusion of certain individuals when they submit their manuscripts. When suggesting reviewers, authors should make sure they are totally independent and not connected to the work in any way. It is strongly recommended to suggest a mix of reviewers from different countries and different institutions. When suggesting reviewers, the Corresponding Author must provide an institutional email address for each suggested reviewer, or, if this is not possible to include other means of verifying the identity such as a link to a personal homepage, a link to the publication record or a researcher or author ID in the submission letter. Please note that the Journal may not use the suggestions, but suggestions are appreciated and may help facilitate the peer review process.

Compliance with Ethical Standards

To ensure objectivity and transparency in research and to ensure that accepted principles of ethical and professional conduct have been followed, authors should include information regarding sources of funding, potential conflicts of interest (financial or non-financial), informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals.

Authors should include the following statements (if applicable) in a separate section entitled “Compliance with Ethical Standards” when submitting a paper:

Disclosure of potential conflicts of interest

Research involving Human Participants and/or Animals

Informed consent

Please note that standards could vary slightly per journal dependent on their peer review policies (i.e. single or double blind peer review) as well as per journal subject discipline. Before submitting your article check the instructions following this section carefully.

The corresponding author should be prepared to collect documentation of compliance with ethical standards and send if requested during peer review or after publication.

The Editors reserve the right to reject manuscripts that do not comply with the above-mentioned guidelines. The author will be held responsible for false statements or failure to fulfill the above-mentioned guidelines.

Competing Interests

Authors are requested to disclose interests that are directly or indirectly related to the work submitted for publication. Interests within the last 3 years of beginning the work (conducting the research and preparing the work for submission) should be reported. Interests outside the 3-year time frame must be disclosed if they could reasonably be perceived as influencing the submitted work. Disclosure of interests provides a complete and transparent process and helps readers form their own judgments of potential bias. This is not meant to imply that a financial relationship with an organization that sponsored the research or compensation received for consultancy work is inappropriate.

Editorial Board Members and Editors are required to declare any competing interests and may be excluded from the peer review process if a competing interest exists. In addition, they should exclude themselves from handling manuscripts in cases where there is a competing interest. This may include – but is not limited to – having previously published with one or more of the authors, and sharing the same institution as one or more of the authors. Where an Editor or Editorial Board Member is on the author list they must declare this in the competing interests section on the submitted manuscript. If they are an author or have any other competing interest regarding a specific manuscript, another Editor or member of the Editorial Board will be assigned to assume responsibility for overseeing peer review. These submissions are subject to the exact same review process as any other manuscript. Editorial Board Members are welcome to submit papers to the journal. These submissions are not given any priority over other manuscripts, and Editorial Board Member status has no bearing on editorial consideration.

Interests that should be considered and disclosed but are not limited to the following:

Funding: Research grants from funding agencies (please give the research funder and the grant number) and/or research support (including salaries, equipment, supplies, reimbursement for attending symposia, and other expenses) by organizations that may gain or lose financially through publication of this manuscript.

Employment: Recent (while engaged in the research project), present or anticipated employment by any organization that may gain or lose financially through publication of this manuscript. This includes multiple affiliations (if applicable).

Financial interests: Stocks or shares in companies (including holdings of spouse and/or children) that may gain or lose financially through publication of this manuscript; consultation fees or other forms of remuneration from organizations that may gain or lose financially; patents or patent applications whose value may be affected by publication of this manuscript.

It is difficult to specify a threshold at which a financial interest becomes significant, any such figure is necessarily arbitrary, so one possible practical guideline is the following: "Any undeclared financial interest that could embarrass the author were it to become publicly known after the work was published."

Non-financial interests: In addition, authors are requested to disclose interests that go beyond financial interests that could impart bias on the work submitted for publication such as professional interests, personal relationships or personal beliefs (amongst others). Examples include, but are not limited to: position on editorial board, advisory board or board of directors or other type of management relationships; writing and/or consulting for educational purposes; expert witness; mentoring relations; and so forth.

Primary research articles require a disclosure statement. Review articles present an expert synthesis of evidence and may be treated as an authoritative work on a subject. Review articles therefore require a disclosure statement. Other article types such as editorials, book reviews, comments (amongst others) may, dependent on their content, require a disclosure statement. If you are unclear whether your article type requires a disclosure statement, please contact the Editor-in-Chief.

Please note that, in addition to the above requirements, funding information (given that funding is a potential competing interest (as mentioned above)) needs to be disclosed upon submission of the manuscript in the peer review system. This information will automatically be added to the Record of CrossMark, however it is not added to the manuscript itself. Under ‘summary of requirements’ (see below) funding information should be included in the ‘Declarations’ section.

Summary of requirements

The above should be summarized in a statement and placed in a ‘Declarations’ section before the reference list under a heading of ‘Funding’ and/or ‘Competing interests’. Other declarations include Ethics approval, Consent, Data, Material and/or Code availability and Authors’ contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

When all authors have the same (or no) conflicts and/or funding it is sufficient to use one blanket statement.

Examples of statements to be used when funding has been received:

Partial financial support was received from [...]

The research leading to these results received funding from […] under Grant Agreement No[…].

This study was funded by […]

This work was supported by […] (Grant numbers […] and […]

Examples of statements to be used when there is no funding:

The authors did not receive support from any organization for the submitted work.

No funding was received to assist with the preparation of this manuscript.

No funding was received for conducting this study.

No funds, grants, or other support was received.

Examples of statements to be used when there are interests to declare:

Financial interests: Author A has received research support from Company A. Author B has received a speaker honorarium from Company Wand owns stock in Company X. Author C is consultant to company Y.

Non-financial interests: Author C is an unpaid member of committee Z.

Financial interests: The authors declare they have no financial interests.

Non-financial interests: Author A is on the board of directors of Y and receives no compensation as member of the board of directors.

Financial interests: Author A received a speaking fee from Y for Z. Author B receives a salary from association X. X where s/he is the Executive Director.

Non-financial interests: none.

Financial interests: Author A and B declare they have no financial interests. Author C has received speaker and consultant honoraria from Company M and Company N. Dr. C has received speaker honorarium and research funding from Company M and Company O. Author D has received travel support from Company O.

Non-financial interests: Author D has served on advisory boards for Company M, Company N and Company O.

Examples of statements to be used when authors have nothing to declare:

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

The authors have no competing interests to declare that are relevant to the content of this article.

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

The authors have no financial or proprietary interests in any material discussed in this article.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Research involving human participants, their data or biological material

Ethics approval

When reporting a study that involved human participants, their data or biological material, authors should include a statement that confirms that the study was approved (or granted exemption) by the appropriate institutional and/or national research ethics committee (including the name of the ethics committee) and certify that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. If doubt exists whether the research was conducted in accordance with the 1964 Helsinki Declaration or comparable standards, the authors must explain the reasons for their approach, and demonstrate that an independent ethics committee or institutional review board explicitly approved the doubtful aspects of the study. If a study was granted exemption from requiring ethics approval, this should also be detailed in the manuscript (including the reasons for the exemption).

Retrospective ethics approval

If a study has not been granted ethics committee approval prior to commencing, retrospective ethics approval usually cannot be obtained and it may not be possible to consider the manuscript for peer review. The decision on whether to proceed to peer review in such cases is at the Editor's discretion.

Ethics approval for retrospective studies

Although retrospective studies are conducted on already available data or biological material (for which formal consent may not be needed or is difficult to obtain) ethics approval may be required dependent on the law and the national ethical guidelines of a country. Authors should check with their institution to make sure they are complying with the specific requirements of their country.

Ethics approval for case studies

Case reports require ethics approval. Most institutions will have specific policies on this subject. Authors should check with their institution to make sure they are complying with the specific requirements of their institution and seek ethics approval where needed. Authors should be aware to secure informed consent from the individual (or parent or guardian if the participant is a minor or incapable) See also section on Informed Consent.

Cell lines

If human cells are used, authors must declare in the manuscript: what cell lines were used by describing the source of the cell line, including when and from where it was obtained, whether the cell line has recently been authenticated and by what method. If cells were bought from a life science company the following need to be given in the manuscript: name of company (that provided the cells), cell type, number of cell line, and batch of cells.

It is recommended that authors check the NCBI database for misidentification and contamination of human cell lines. This step will alert authors to possible problems with the cell line and may save considerable time and effort.

Further information is available from the International Cell Line Authentication Committee (ICLAC).

Authors should include a statement that confirms that an institutional or independent ethics committee (including the name of the ethics committee) approved the study and that informed consent was obtained from the donor or next of kin.

Research Resource Identifiers (RRID)

Research Resource Identifiers (RRID) are persistent unique identifiers (effectively similar to a DOI) for research resources. This journal encourages authors to adopt RRIDs when reporting key biological resources (antibodies, cell lines, model organisms and tools) in their manuscripts.

Examples:

Organism: $Filip1^{tm1a(KOMP)Wtsi}$ RRID:MMRRC_055641-UCD

Cell Line: RST307 cell line RRID:CVCL_C321

Antibody: Luciferase antibody DSHB Cat# LUC-3, RRID:AB_2722109

Plasmid: plasmid RRID:Addgene_104005

Software: ImageJ Version 1.2.4 RRID:SCR_003070

RRIDs are provided by the Resource Identification Portal. Many commonly used research resources already have designated RRIDs. The portal also provides authors links so that they can quickly register a new resource and obtain an RRID.

Clinical Trial Registration

The World Health Organization (WHO) definition of a clinical trial is "any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes". The WHO defines health interventions as “A health intervention is an act performed for, with or on behalf of a person or population whose purpose is to assess, improve, maintain, promote or modify health, functioning or health conditions” and a health-related outcome is generally defined as a change in the health of a person or population as a result of an intervention.

To ensure the integrity of the reporting of patient-centered trials, authors must register prospective clinical trials (phase II to IV trials) in suitable publicly available repositories. For example www.clinicaltrials.gov or any of the primary registries that participate in the WHO International Clinical Trials Registry Platform.

The trial registration number (TRN) and date of registration should be included as the last line of the manuscript abstract.

For clinical trials that have not been registered prospectively, authors are encouraged to register retrospectively to ensure the complete publication of all results. The trial registration number (TRN), date of registration and the words 'retrospectively registered’ should be included as the last line of the manuscript abstract.

Standards of reporting

Springer Nature advocates complete and transparent reporting of biomedical and biological research and research with biological applications. Authors are recommended to adhere to the minimum reporting guidelines hosted by the EQUATOR Network when preparing their manuscript.

Exact requirements may vary depending on the journal; please refer to the journal's Instructions for Authors.

Checklists are available for a number of study designs, including:

Randomised trials (CONSORT) and Study protocols (SPIRIT)

Observational studies (STROBE)

Systematic reviews and meta-analyses (PRISMA) and protocols (Prisma-P)

Diagnostic/prognostic studies (STARD) and (TRIPOD)

Case reports (CARE)

Clinical practice guidelines (AGREE) and (RIGHT)

Qualitative research (SRQR) and (COREQ)

Animal pre-clinical studies (ARRIVE)

Quality improvement studies (SQUIRE)

Economic evaluations (CHEERS)

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Ethics approval'.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

Examples of statements to be used when ethics approval has been obtained:

• All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of the Medical University of A (No. ...).

• This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University B (Date.../No. ...).

• Approval was obtained from the ethics committee of University C. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

• The questionnaire and methodology for this study was approved by the Human Research Ethics committee of the University of D (Ethics approval number: ...).

Examples of statements to be used for a retrospective study:

• Ethical approval was waived by the local Ethics Committee of University A in view of the retrospective nature of the study and all the procedures being performed were part of the routine care.

• This research study was conducted retrospectively from data obtained for clinical purposes. We consulted extensively with the IRB of XYZ who determined that our study did not need ethical approval. An IRB official waiver of ethical approval was granted from the IRB of XYZ.

• This retrospective chart review study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Human Investigation Committee (IRB) of University B approved this study.

Examples of statements to be used when no ethical approval is required/exemption granted:

• This is an observational study. The XYZ Research Ethics Committee has confirmed that no ethical approval is required.

• The data reproduced from Article X utilized human tissue that was procured via our Biobank AB, which provides de-identified samples. This study was reviewed and deemed exempt by our XYZ Institutional Review Board. The BioBank protocols are in accordance with the ethical standards of our institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

After acceptance

Upon acceptance, your article will be exported to Production to undergo typesetting. Once typesetting is complete, you will receive a link asking you to confirm your affiliation, choose the publishing model for your article as well as arrange rights and payment of any associated publication cost.

Once you have completed this, your article will be processed and you will receive the proofs.

Article publishing agreement

Depending on the ownership of the journal and its policies, you will either grant the Publisher an exclusive licence to publish the article or will be asked to transfer copyright of the article to the Publisher.

Creative Commons Attribution-NonCommercial 4.0 International License

Offprints

Offprints can be ordered by the corresponding author.

Color illustrations

Online publication of color illustrations is free of charge.

Proof reading

The purpose of the proof is to check for typesetting or conversion errors and the completeness and accuracy of the text, tables and figures. Substantial changes in content, e.g., new results, corrected values, title and authorship, are not allowed without the approval of the Editor.

After online publication, further changes can only be made in the form of an Erratum, which will be hyperlinked to the article.

Online First

The article will be published online after receipt of the corrected proofs. This is the official first publication citable with the DOI. After the issue-publication, the paper can also be cited by issue and page numbers.

Open access publishing

To find out more about publishing your work Open Access in *International Journal of Tropical Insect Science*, including information on fees, funding and licenses, visit our Open access publishing page.

APPENDIX E

Instructions to authors: Entomologia Generalis (Schweizerbart Science Publishers)

Aims and Scope (see more details in *Desneux & Biondi, 2018, Entomologia Generalis 37(1), 1–5*)

Entomologia Generalis welcomes high-quality contributions from the field of basic and applied ecology of arthropods, insects and mite pests, as well as their natural enemies and pollinators. Articles published in *Entomologia Generalis* should not be descriptive, but should bring novel findings on topics of current importance.

Article types

Original Research Articles

Such articles are high quality research articles on advances in knowledge on fields covered by the journal (see scope). They should bring valuable and original insights in key research areas and they are expected to have a broad and rapid impact on the scientific community working in the fields of entomology and ecology of arthropods.

Review Articles

They should provide significant developments in the field of entomology and ecology of arthropods. Although review papers are usually solicited by members of the editorial board, non-solicited review articles may be considered for publication in Entomologia Generalis. Please send proposals to the Editor-in-Chief (Dr. Nicolas Desneux, nicolas.desneux@univ-cotedazur.fr) for preliminary assessment by the editorial board team before formal submission to the journal.

Letters and Short Notes

Short notes are short documents providing original research results, mini-reviews, perspectives or letters, which report novel findings addressing topics of major importance and that need to be made available to the scientific community quickly.

Comments

The journal also publishes comments and rebuttals on papers previously published in Entomologia Generalis. Such documents should first be discussed with the editors and are not to be submitted directly to the journal.

Article length

Word counts given below include the abstract (250 words maximum), text, acknowledgments, references (30 references accounting for 900 words), tables and figures (half page table or figure accounting for 450 words and full-page figure or table accounting for 900 words).

Research article: maximum 7000 words.

Review articles: maximum 13000 words.

Letters and Short notes: maximum 3000 words.

Comments: maximum 2500 words.

Manuscript Formatting

Manuscripts should be submitted in MS word format i.e. .doc or.docx.

Acceptable files: main document: .doc and .docx; tables: editable tables at the end of the main document as doc and .docx; figures: jpg, tif, pdf or any vectorized file format.

Page and line numbering (MS Word numbering option) must be used continuously throughout the entire manuscript.

Please use double-line spacing for the text.

12-point Times Roman should be used throughout the whole manuscript.

Page format should be set up at 2.5 cm margins in left and right sides as well as top and bottom of the page.

The text of the entire manuscript must be left justified.

Use tab stops or other commands for indents, not spaces.

Up to three levels of headings are accepted.

Use the MS equation editor or MathType for equations.

Figure legends must be numbered and provided at the end of the main document. Make sure to cite each table and figure at least once in the main text using the wording Fig. and Table

Tables should be prepared using the table function in MS Word, do not use not spreadsheets.

Genus and species names must be written in italics. Spell out the genus name only at the first mentioning in the main text and also when appearing at the beginning of a sentence throughout the manuscript.

Abbreviations can be used pending they are defined at first time they appear, abbreviations should be used consistently throughout the manuscripts.

Sentences with personal reference to the authors (I, We, Our) should be avoided, passive voice is preferred.

Footnotes are not allowed in main text, still they can be used in Tables.

Language

All contributions should be written in either American or British English language. The English type should be consistent throughout the entire manuscript.

Authors for whom English is a second language should have their manuscript edited before submission, either by a professional service or with the help of a native English speaker. Manuscripts with poor English not understandable for reviewers will be returned to authors without review. Accepted manuscripts may still have to be polished for English before final publication; Schweizerbart Science Publishers can provide such service at reasonable costs.

Manuscript structure

Title page (page 1)

Title: must be concise and informative. For organism names, please give the common or latin name but no authority or order and family. Do not use capital letters for the first letter or each word of the title.

A short title (max. 45 characters) must be added below the full title.

Author(s) name(s): Include first the given name, the initial(s) of the middle name(s) then the family name. Check that all names are accurately spelled.

Authors shall provide their affiliation(s) and full address(es), a valid e-mail address and telephone number(s).

Abstract (page 2)

Provide an abstract of up to 250 words. The abstract should be self-explaining and should summarize the conceptual framework and aim of the work, the main results and conclusions. Moreover, it should not contain any undefined abbreviations or unspecified references. All Latin names should be provided with the correct authority and if applicable with (Order: Family).

Keywords (page 2)

Provide a minimum of 5 keywords which can be used for indexing purposes. Avoid words already present in the title and include the family of the most important organism(s) in the paper (e.g., those referred to in the title).

Main body for Research Articles (starting page 3)

Divide your manuscript into clearly divided sections following strictly the order: Introduction, Materials and methods, Results, Discussion, Acknowledgments, References, Figure legends, Tables (one per page), Figures (one figure per page or one figure panel per page). Sections should not be numbered.

Each heading should appear on its own separate line.- Subsections (subheadings) can be used only in Materials and methods and Results sections. Subsections should have a brief title and no more than two sublevels are allowed.

Introduction: Provide an adequate background, formulate the hypothesis(es), state the objectives of the work. Avoid a detailed literature survey or a summary of the results.

Material and methods: Provide clear details to enable the work to be reproduced or expanded. Methods already published should be indicated by reference(s), and mostly relevant modifications should be described.

Results: Be clear and concise. Do not repeat and/or list all the data presented in figures and/or in tables, but mention and describe the significant and striking ones. Ideally, the results should have subsections matching those of the Material and methods section.

Discussion: The text should explore and discuss thoroughly the significance of the results. Do not repeat the results and do not list studies from other authors without deep integrative text legitimating the citations. Do not over-speculate on results.

Combining Results and Discussion sections is not accepted in Entomologia Generalis. Ideally, the main conclusions of the study should be presented in a short paragraph at the end of the Discussion section.

Acknowledgements should be given as a brief statement following the Discussion section. They may refer to any technical, scientific or linguistic help received for the work by colleagues, and/or by professionals and/or by the editor(s) and anonymous referees. Authors should use this section to acknowledge any funding, citing the funding source, the project title/acronym and grant number (if any).

Citation in the text. Cite references in the text by family name of the first author and year in parentheses, and use the format provided here as template. Examples: “Several studies supported this hypothesis (Abbott 1991; Smith et al. 2001; 2002a; 2002b; Thompson et al. 2010). The samples were analyzed using the procedures by Heimpel et al. (2012) and modified by Peterson (2015). Plant belonging to the Rubus genus are considered the main hosts for this insect (Wang & Lee 2016).”

Reference list. The list of references should only include works that are cited in the text and that have been published, in press or accepted for publication. Personal communications and unpublished works are generally not accepted in the text and thus should not be given as references. Reference list entries should be alphabetized by the last names of the first author of each work. List multi-author publications of the same first author in chronological order. When authors cite multiple papers authored by the same first author and published in the same year, the year should be followed by a letter, using “a” for the first cited paper, “b” for the second and so on. Ideally, the names of all authors should be provided, the usage of "et al" in long author lists (longer than six authors) can also be used. We strongly recommend authors to use the APA 6 reference style to format references. EndNote users can download the EndNote style file here: www.schweizerbart.de/resources/downloads/style-files/endnote_style_schweizerbart_apa6.ens. Authors not using EndNote shall follow the APA 6 reference formatting style. If possible, please also supply the Endnote reference file.

Sample references

Journal Article:

Ito, L., Omori, T., Yoneda, M., Yamaguchi, T., Kobayashi, R., & Takahashi, Y. (2018). Origin and migration of trace elements in the surface sediments of Majuro Atoll, Marshall Islands. *Chemosphere*, 202, 65–75. https://doi.org/10.1016/j.chemosphere.2018.03.083

Book:
Aitken, C. G. G., & Taroni, F. (2004). *Statistics and the Evaluation of Evidence for Forensic Scientists.* J. Wiley & Sons Ltd. https://doi.org/10.1002/0470011238

Book chapter:

Wantzen, K. M., Callil, C., & Butakka, C. M. M. (2011). Benthic invertebrates of the Pantanal and its tributaries. In W. J. Junk, C. J. Silva, C. N. Cunha, & K. M. Wantzen (Eds.), *The Pantanal: Ecology, biodiversity and sustainable management of a large neotropical seasonal wetland* (pp. 127–141). Sofia, Moscow: Pensoft.

Thesis:
Králík, M. (2004). *Paleodermatoglyphics. Analysis of fingerprints on ancient ceramics: theoretical basis, methodological issues and practical recommendations.* PhD thesis, Masaryk University in Brno.

Web:
Haq, B. U. (2018): Jurassic Sea-Level Variations: A Reappraisal. *GSA Today.* https://doi.org/10.1130/GSATG359A.1

Other:
Rivaldería, N., Expósito, N., Gómez-Herrero, F. J., Juanino, R. M., Cruz, R., Hernández-Hurtado, L. E., . . . Alonso-Rodríguez, C. G. R. (2017). Diferencias intra e interpoblacionales en la frecuencia de apar-ición de los distintos tipos de deltas (o trirradii) dactilares. *XX Congreso Sociedad Española de Antropología Física.*

Tables should be presented following the list of references, they must be numbered and provided with a concise heading.

Figures should be presented at the end of the manuscript, they should be provided with an explanatory short heading. A main figure may be divided in several subfigures that need to be named with capital letters in round brackets, likewise to be repeated in the legend. All figures should be ready for reproduction with clear lettering in a suitable size and of excellent quality.

Article Processing Charge (APC); optional Open Access

For all accepted papers an APC charge of € 600.– (plus taxes, if applicable) will apply.

Furthermore we offer a flat Open Access fee of € 1.890.– + taxes for a CC-BY-NC Open Access license. The Open Access license fee *includes* the above-mentioned APC charges as well as color publishing charges.

Please indicate, if you opt for Open access publication of your article against the mentioned fees.

Manuscript submission

Submission of a manuscript implies that (i) the work described has not been published before, (ii) it is not under consideration for publication anywhere else, (iii) its publication has been approved by all co-authors. The publisher will not be held legally responsible should there be any claims for compensation.

Authors should submit their manuscript as a single MS word document (including all tables and figures) AND as a matching single pdf file to https://www.schweizerbart.de/submit/entomologia First time author have to register to the journals submission system (link see above).

Submitted documents will be considered for publication and forwarded to the referees only when they fully meet all above mentioned preconditions