

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

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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 *Stenomesus* 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 *Stenomesus* 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 *Stenomesus* 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	<i>Biocontrol Science and Technology</i> (Taylor & Francis)
Chapter 4	Article 2: Published	<i>Insects</i> (MDPI)
Chapter 5:	Article 3: Submitted	<i>Biological control</i> (Elsevier)
Chapter 6:	Article 4: Submitted	<i>International Journal of Tropical Insect Science</i> (Springer)
Chapter 7:	Article 5: Published	<i>Biology</i> (MDPI)
Chapter 8:	Article 6: Prepared for submission	<i>Entomologia Generalis</i> (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.

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CHAPTER 1: INTRODUCTION

1.1. Introduction

The invasive tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) invaded areas outside its 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 southern 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 parasitized 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 initiated, 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 *Stenomesus* 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. gelechiidivoris*.
- ii. Both native parasitoids, *B. nigricans* and *Stenomesus* 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 *Stenomesus* 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*.

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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 simplexella* (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; Krechmer and Foerster, 2015; Machezano *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).

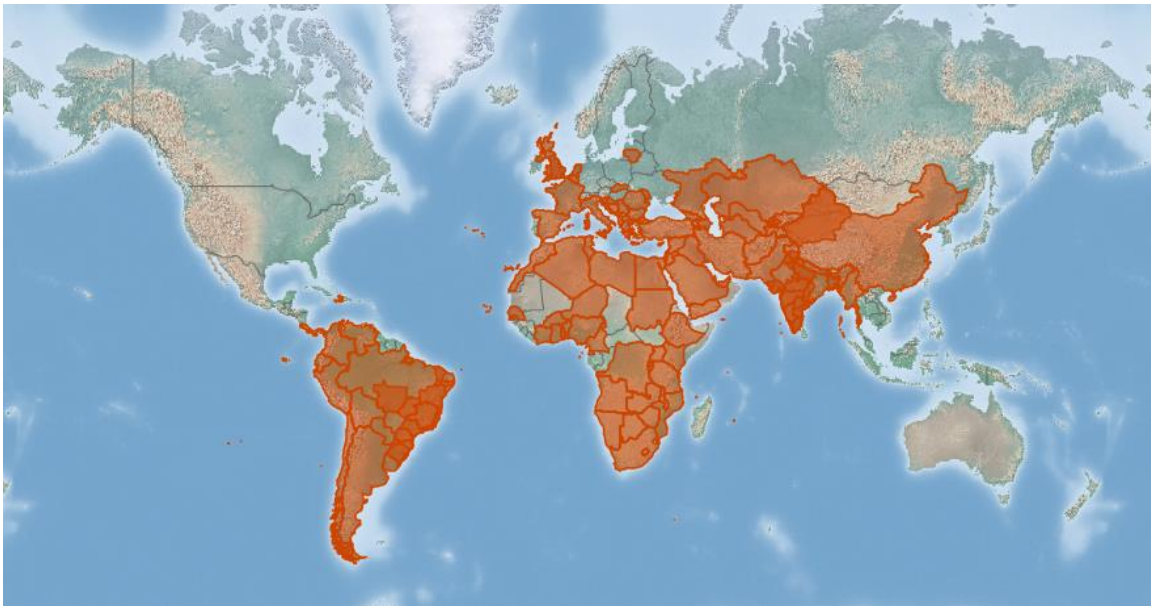


Figure 2.3: Distribution of *T. absoluta* in the world. **Source:** CABI, accessed 28 august 2022 at <https://www.cabi.org/isc/datasheet/49260#toDistributionMaps>

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 parasitoids of *T. absoluta* also (Idriss *et al.*, 2018). These parasitoids are generalist parasitoids of Lepidoptera species, such as *Lobesia botrana* (Denis & Schiffermüller) (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	<i>Chelonus</i> sp.	Braconidae	Larvae	0.64	N/A	F	Senegal	Seydi <i>et al.</i> (2021)
	<i>Apanteles litae</i> (Dixon)	Braconidae	Larvae	0.27				
	<i>Cotesia vestalis</i> (Haliday)	Braconidae	Larvae	0.04				
	<i>Meteorus laphygmarum</i> (Brues)	Braconidae	Larvae	0.04				
	<i>Diadegma insulare</i> (Cresson)	Ichneumonidae	Larvae	0.02				
	<i>Pristomerus pallidus</i> (Kriechbaumer)	Ichneumonidae	Larvae	0.02				
	<i>Hockeria</i> sp.	Chalcididae	Larvae	13				
	<i>Brachymeria</i> sp.	Chalcididae	Larvae	10	N/A	F	Kenya	Kinyanjui <i>et al.</i> (2021)
	<i>Bracon</i> sp.	Braconidae	Larvae	6				
	<i>Neochrysocharis formosa</i> (Westwood)	Eulophidae	Larvae	5				
	<i>Goniozus</i> sp.	Bethylidae	Larvae	5				
	<i>Diglyphus isaea</i> (Walker)	Eulophidae	Larvae	4				
	<i>Stenomesus rufescens</i> (Retzius)	Eulophidae	Larvae	4				
	<i>Bracon nigricans</i> (Szépligeti)	Braconidae	Larvae	21	N/A	F	Kenya	Mama Sambo <i>et al.</i> (2022c)
<i>Stenomesus</i> sp. near <i>japonicus</i> (Ashmead)	Eulophidae	Larvae	17					

	<i>Dolichogenidea appellator</i> (Telenga)	Braconidae	Larvae	60	24h	L	Sudan	Idriss <i>et al.</i> (2018)
	<i>Bracon nigricans</i>	Braconidae	Larvae	54				
Exotic parasitoid	<i>Dolichogenidea gelechiidivoris</i> (Marsh)	Braconidae	Larvae	55	24h	L	Kenya	Aigbedion-Atalor <i>et al.</i> (2020)
	<i>D. gelechiidivoris</i>	Braconidae	Larvae	59	24h	L	Kenya	Mama Sambo <i>et al.</i> (2022b)
Native predators	<i>Nesidiocoris tenuis</i> Reuter	Miridae	Eggs	51	24h	L	Senegal	Sylla <i>et al.</i> (2016)
	<i>N. tenuis</i>		Larvae	14	24h	L	Senegal	Sylla <i>et al.</i> (2016)
	<i>N. tenuis</i>		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
	<i>N. tenuis</i>		Eggs and larvae	N/A	N/A	F	Burkina Faso	Sawadogo <i>et al.</i> (2022)
	<i>N. tenuis</i>		Eggs	75	24h	L	Burkina Faso	Sawadogo <i>et al.</i> (2022)
	<i>Macrolophus pygmaeus</i>	Miridae	Eggs	N/A	N/A	L	Senegal	Sylla <i>et al.</i> (2016)
	<i>M. pygmaeus</i>		Eggs and larvae	N/A	N/A	F	Kenya	Kinyanjui <i>et al.</i> (2021)
IPM	<i>D. gelechiidivoris</i> + <i>N. tenuis</i>		Eggs and larvae	83	24h	L	Kenya	Aigbedion-Atalor <i>et al.</i> (2021)
	<i>D. gelechiidivoris</i> + <i>S. sp. nr. japonicus</i>		Larvae	80	24h	L	Kenya	Mama Sambo <i>et al.</i> Unpublished data
	<i>D. gelechiidivoris</i> + <i>M. anisopliae</i> ICIPE 20		Larvae	78	24h	L	Kenya	Mama Sambo <i>et al.</i> (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 <i>et al.</i> (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 LT ₅₀ = 3.17 LT ₅₀ = 2.38	Kenya	Akutse <i>et al.</i> (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 <i>et al.</i> (2021)
					ICIPE 18	91%,	LT ₅₀ = 1.41		
					ICIPE 20	90%	LT ₅₀ = 1.48		
					ICIPE 665	75%	LT ₅₀ = 2.92		
	<i>Beauveria bassiana</i> (Bals.) Vuill., strain BB02	Real Beauveria	Infected soil - laboratory	Pupal mortality	Real Beauveria	98%	LT ₅₀ < 1	South Africa	Erasmus <i>et al.</i> (2021)
	<i>B. bassiana</i> (Bals.) Vuill., strain R444	Eco-Bb			Real Metarhizium 69	90%	LT ₅₀ < 1		
	<i>Metarhizium anisopliae</i> (Metsch.) Sorok., strain ICIPE 69	Real Metarhizium 69							
	<i>M. anisopliae</i> (Metsch.) Sorok., strain E9	Metarril WP E9			Metarril WP E9	89%	LT ₅₀ = ~5		
	<i>M. anisopliae</i> (Metsch.) Sorok.,	Real Metarhizium 69	Open field experimental	Infested fruit	Real Metarhizium	42% < control	N/A	Tanzania	Zekeya <i>et al.</i> (2022)

	strain ICIPE 69		plot spray		69				
	<i>M. anisopliae</i> , Strain FCM Ar 23B3, 5×10^9 CFU/g <i>B. bassiana</i> , 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 <i>et al.</i> (2020)
	<i>Aspergillus oryzae</i> (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	<i>A. oryzae</i> at 30.4°C and 70%RH 1.0×10^8	77%	LT ₁₀₀ = 3.5	Tanzania	Zekeya <i>et al.</i> (2019)
Bacteria	<i>Bacillus thuringiensis</i> var. kurstaki	BatikWG	Open field - spray	Number of leaflets mined	<i>Bacillus thuringiensis</i> var. kurstaki	79% < control	N/A	Senegal	Sarr <i>et al.</i> (2021)

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

*All studies with no LT indicated, were field studies

2.4 Structure of the thesis

Chapter 1 formulates the rationale 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 *Stenomesus* sp. near *japonicus* and predicted areas suitable for persistence of *Stenomesus* 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. gelechiidivoris* and the native *B. nigricans* and recommends on release of the exotic parasitoid, taking into consideration the presence of this native parasitoid.

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 establishment 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.

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CHAPTER 3

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






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RESEARCH ARTICLE



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

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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, *Dolichogenidea 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.

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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;

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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; Gervasio 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 microgastrinae 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 EZAD 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 (density²) 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 \pm SE of number of parasitised and percentage of superparasitised *T. absoluta* larvae at different host and parasitoid densities.

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

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

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.

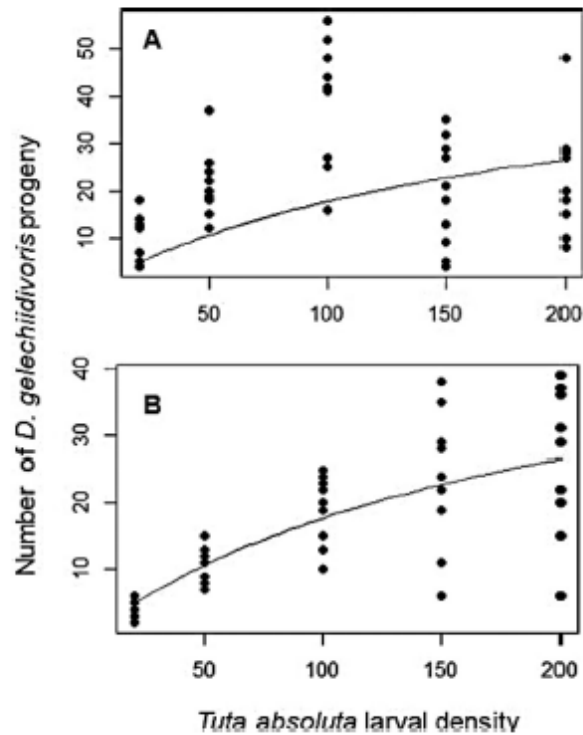


Figure 1. Type II functional response of *D. gelechiidivoris* to *T. absoluta* larval density; (A) Exposure to one couple (single foraging), and (B) Interaction effect (one female response in group of three foraging)

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.

Host Densities	Single female foraging (1♀:1♂)		Group of females foraging (3♀:3♂)	
	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*, *Stenomesus* 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.

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Disclosure statement


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CHAPTER 4

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

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Article

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

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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, *Stenomesus* sp. near *japonicus* and *Bracon nigricans*, with up to 17% and 21% parasitism respectively. *Stenomesus* 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 *Stenomesus* 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

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



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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 Trichromatid, *Trichogramma bourarachae* Pintureau & Babault, the Patygastrid, *Telenomus* sp., and the Torymid, *Hemiptarsenus zilahisebessi* Erdős 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 cademat* (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; $65 \pm 5\%$ 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 bar-coding primers LCO1490 (5'GGTCAACAAATCATAAAGATATTGG3'), and HCO2198 (5'TAAACTTCAAGGGTGACCAAAAATCA) for arthropods identification [46], DDH2O and hot start. Each sample was prepared with a 48 μ L master mix solution in a PCR tube and 2 μ L 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 72.5 [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) $\times 100$ [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).

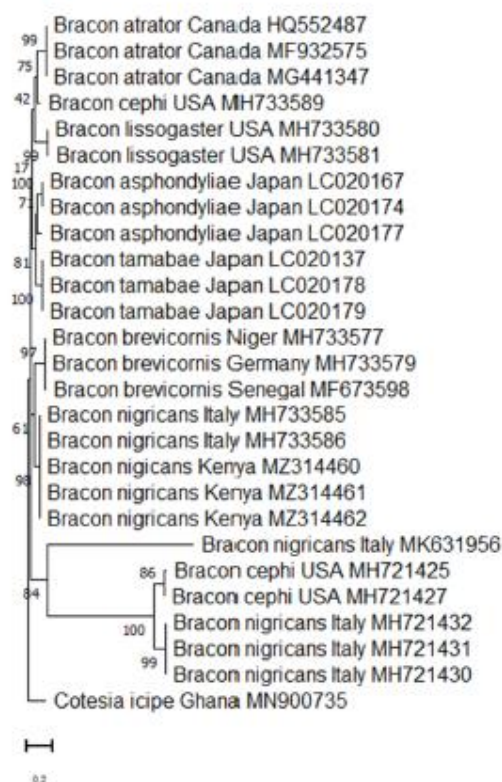


Figure 1. Maximum likelihood phylogenetic tree for the mitochondrial COI sequences of *Bracon* species from GenBank together with the specimen *Bracon nigricans* identified in our study.

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 *Stenomomesius* sp. and some closely related sequences in the GenBank. Sequences from specimens of the current study are in the same clade as *Stenomomesius Hansson*

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

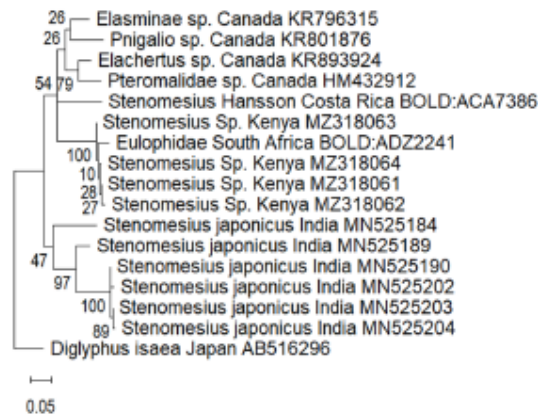


Figure 2. Maximum likelihood phylogenetic tree for the mitochondrial COI sequences of *Stenomesus* species from GenBank and BOLD together with the specimen identified in our study.

3.1.2. Morphological and Ecological Identification

Morphological identification was done for the Eulophidae, *Stenomesus* 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 *Stenomesus* 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 *Stenomesus* genus.

Three species of the genus *Stenomesus* have been reported in the Afrotropical region, namely *Stenomesus elegantulus* (Risbec) (Hymenoptera: Eulophidae) in Cameroon and Senegal and *S. japonicus* in the Afrotropical, Palaearctic, Indo-Malaya, and Australian regions [63,64] while *Stenomesus 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]. *Stenomesus 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 *Stenomesus* 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). *Stenomesus 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*.

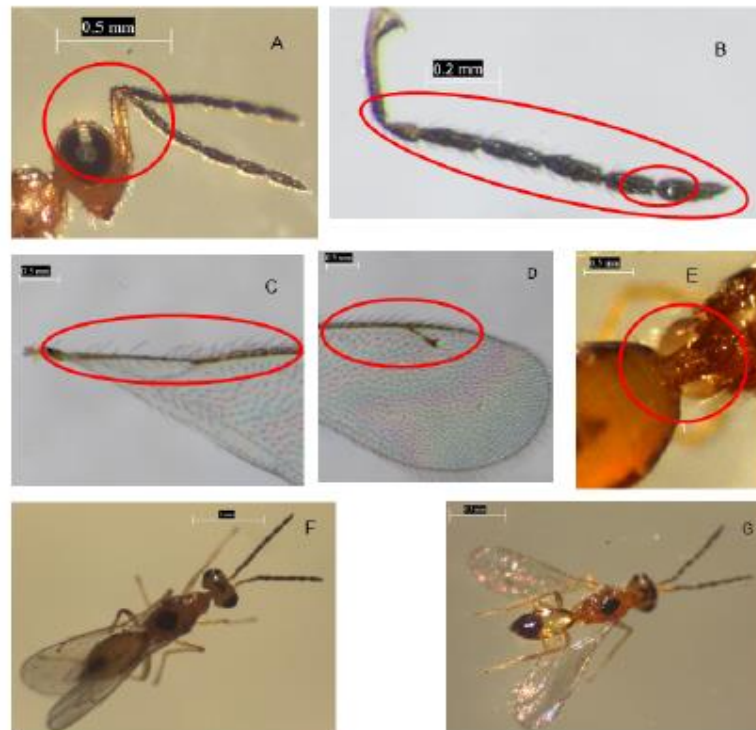


Figure 3. The collected *Stenomesius* sp. near *japonicus* description: (A): Scape and vertex, (B): Female antenna with scape slender and funicle 4-segmented, (B): The flagellum with 1–2 anelli, (C): Forewing with submarginal vein with more than 3: setae, (D): Postmarginal vein at least 1.5 times the 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).

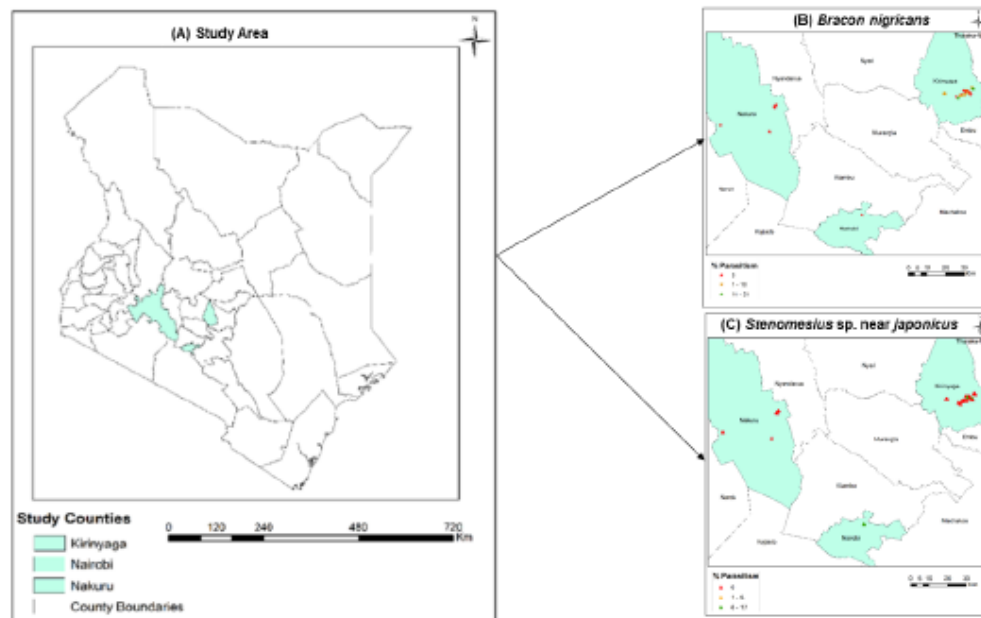


Figure 4. Maps showing (A) the study area, (B) *Bracon nigricans* parasitism rate, and (C) *Stenomesius* sp. nr. *japonicus* parasitism rate, within the sampling sites.

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	<i>T. absoluta</i> Infestation/kg of Leaves (No.)	<i>B. nigricans</i> (%)	<i>S. sp. nr japonicus</i> (%)
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)
<i>B. nigricans</i>				
(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 <i>N. tenuis</i>	2.50	0.83	3.02	0.002
Medium level of <i>N. tenuis</i>	0.04	1.26	0.03	0.97
High level of <i>N. tenuis</i>	1.33	1.23	1.08	0.28
<i>S. sp. nr japonicus</i>				
(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 <i>N. tenuis</i>	−0.27	1.06	−0.25	0.80
Medium level of <i>N. tenuis</i>	0.26	0.39	0.65	0.52
High level of <i>N. tenuis</i>	−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 *Stenomiesius 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

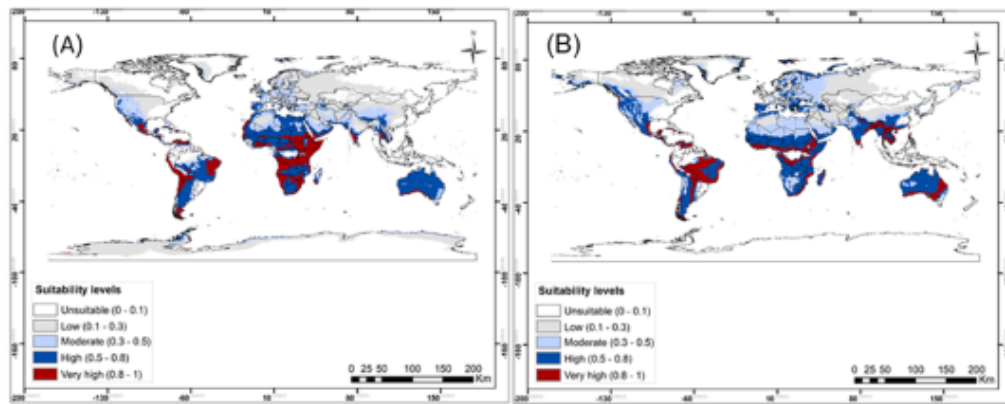


Figure 5. Probable bioclimatic suitability of *Bracon nigricans* under (A) current, and (B) representative concentration pathways (RCP2.5) of 2050 climate scenarios.

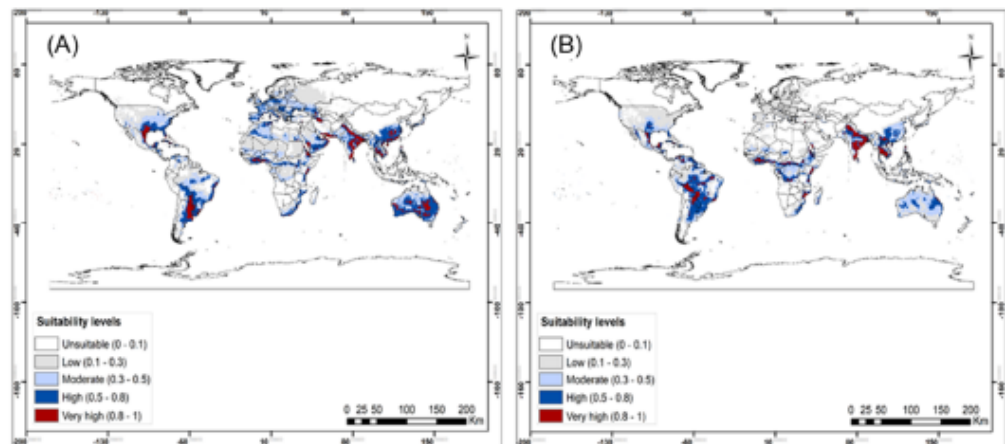


Figure 6. Probable bioclimatic suitability of *Stenomiesius sp. near japonicus* under (A) current, and (B) representative concentration pathways (RCP2.5) of 2050 climate scenario.

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].

Stenomiesius spp. are generalist ectoparasitoids from several Lepidoptera families, such as Pyralidae, Noctuidae, Gelechiidae, Tortricidae, Lyonetiidae, Glyphipterygidae [78], but also from Diptera pest [79]. *Stenomiesius* sp. nr *japonicus* has been associated with *Approaerema 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, *Stenomiesius* 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]. *Stenomiesius* sp. near *japonicus* was also recorded from *T. absoluta*-infested tomato plants in Spain and France [51,72,74]. *Stenomiesius* sp. has also been reported on *T. absoluta* in Algeria [26], while *S. rufescens* was previously identified in Kenya on the same host [65]. *Stenomiesius* 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% and 100%, 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 (41 adults) 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 *Stenomestus 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 *Dalichogenidea 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.

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CHAPTER 5

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

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1 **Interaction between two parasitoids of *Tuta absoluta*: the exotic *Dolichogenidea***
2 ***gelechiivoris* and the indigenous *Stenomesus* sp. near *japonicus***

3
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13 **Abstract**

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

25 *Dolichogenidea gelechiidivoris* preferred 1st-instar *T. absoluta* larvae. More eggs were laid in
26 1st-, compared to 3rd-instar larvae, and more *D. gelechiidivoris* wasps emerged from 1st-instar
27 larvae. *Stenomesus* sp. near *japonicus* laid eggs in both the 1st- and 3rd-larval instars, without
28 any preference, but the highest wasp emergences were recorded from 3rd instar larvae. The
29 combined parasitism by the two parasitoids simultaneously released, resulted in up to 77%
30 pest parasitism. The co-existence of both parasitoid species can therefore significantly
31 contribute to *T. absoluta* control, with minimal effect on each other.

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

34 Introduction

35 Invasive insect pests often enter into new areas with no associated natural enemies in these
36 areas, which allows for rapid increases in pest populations. The Afro-Eurasian supercontinent
37 invasion by the destructive *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), presents a
38 big challenge for tomato production (Desneux et al., 2010; Tumuhaise et al., 2016; Karlsson
39 et al., 2018, Mansour and Biondi, 2021). It is a cosmopolitan pest, causing huge economic
40 losses in many counties of the world (Biondi et al., 2018; Mansour et al., 2018; Han et al.,
41 2019; Rwomushana et al., 2019). When no control measures are applied, total yield losses
42 due to the direct feeding of the larvae on the leaves, stems and fruit can occur (Chermiti et al.,
43 2009). The main management practice for *T. absoluta*, is the application of synthetic
44 insecticides (Rwomushana et al., 2019; Aigbedion-Atalor et al., 2019; Chepchirchir et al.,
45 2021). However, this pest has developed resistance to various insecticide classes such as
46 pyrethroids, spinosyns and diamides, which presents an additional challenge in its
47 management (Roditakis et al., 2015; Guedes et al., 2019). To improve on the management
48 options currently available for *T. absoluta*, new sustainable management strategies are
49 needed with minimum adverse effects on the environment, users, and consumers.

50 Biological control using natural enemies is a sustainable management option used in
51 integrated pest management (IPM). Several indigenous parasitoids belonging to various
52 families including Braconidae, Eulophidae, Chalcididae, Torymidae, Bethylidae and
53 Trichogrammatidae have been identified as natural control agents of this pest in invaded
54 areas (Abbes et al., 2013; Mansour et al., 2018; Desneux et al., 2022; van Lenteren et al.,
55 2021). For example, in East Africa, the Braconidae parasitoids, *Bracon nigricans* Szépligeti,
56 *Dolichogenidea appellator* (Telenga), and an unidentified *Bracon* sp. were reported to
57 parasitise different larval stages of *T. absoluta* (Idriss et al., 2018; Kinyanjui et al., 2021).
58 However, these parasitoids are not highly effective and are therefore not able to maintain the
59 damage levels below the economic threshold. In response, the International Centre of Insect
60 Physiology and Ecology (*icipe*), in Kenya introduced the exotic parasitoid *Dolichogenidea*
61 *gelechiidivoris* Marsh (Hymenoptera: Braconidae) from Peru, for classical biological control
62 of *T. absoluta* in Africa (Shiraku, 2020).

63 *Dolichogenidea gelechiidivoris* is a specialist solitary endoparasitoid (Fernandez-Triana et
64 al., 2020; Mama Sambo et al., 2022a) that parasitises all larval stages of *T. absoluta*, but with
65 a preference for 1st- and 2nd-instar larvae (Aigbedion-Atalor et al., 2020). Parasitism levels of
66 approximately 45, 4, 12, and 5% were reported on 1st-, 2nd-, 3rd- and 4th-instar larvae, of *T.*
67 *absoluta*, respectively in a previous study. In its aboriginal area, more than 60% parasitism by
68 *D. gelechiidivoris* was reported in a field study (Morales et al., 2014). However, the
69 establishment and efficiency of an introduced parasitoid is usually influenced by the nature of
70 its competition with the native parasitoids that are associated with the pest (Wang et al.,
71 2008; Cabello et al., 2011). For example, species might seek to exclusively use hosts
72 resulting in fierce competition (Wang et al., 2008). This competition might lead to
73 competitive exclusion with the introduced parasitoid species displacing the native species,
74 hence resulting in biodiversity imbalances (Bennett, 1993; Sorribas et al., 2010).

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

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

89 2. Materials and methods

90 2.1. Host plants

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

95 2.2. Insects

96 2.2.1. *Tuta absoluta*

97 *Tuta absoluta*-infested tomato plants were sampled from Naivasha (36° 12' 52.1" E, 0° 46'
98 22.5" S), Nairobi (36°53'48.1" E, 1°13'18.3" S), and Kirinyaga County (037°22'299" E,

99 00°36'992" S), Kenya to initiate a rearing colony. The colony was kept in an insectary at
100 ambient conditions of 25 ± 2 °C; $70 \pm 5\%$ Relative humidity (RH); 12hL:12hD photoperiod
101 and reared following the protocol described by Mama Sambo et al. (2022a). The moths used
102 in the current study were from the F₅-generation after field collections.

103 2.2.2. *Dolichogenidea gelechiidivoris*

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

109 2.2.3. *Stenomesus sp. near japonicus*

110 The ectoparasitoid *S. sp. nr. japonicus* colony originated from samples of *T. absoluta*-infested
111 tomato collected from an experimental screenhouses at *icipe*, Nairobi (1°13'18.3" S,
112 36°53'48.1" E, and altitude of 1604 m). For laboratory rearing of *S. sp. nr. japonicus*, potted
113 tomato plants were exposed to *T. absoluta* in a perspex cage (50×40×40 cm) for two days.
114 Thereafter, the plants were removed and kept at ambient temperature (25 ± 2 °C), $70 \pm 5\%$
115 RH and 12hL:12hD photoperiod for nine days, until the larvae reached the 3rd-instar. The
116 plants were then introduced into a cage (50×40×40 cm) containing 100 *S. sp. nr. japonicus*
117 pairs. The plants were left in the cage for two days, to allow the parasitoids to oviposit,
118 whereafter the plants were removed. All the leaves with larvae were incubated in a plastic
119 lunch box (21 x 15 x 16 cm), covered with a sheet of paper towel. The larvae were regularly
120 supplied with uninfested tomato leaflets until the wasps emerged. These wasps were
121 transferred daily to a clean cage containing *T. absoluta*-infested plants and kept under
122 laboratory conditions. Droplets of 80% honey solution were applied on the inside of the top

123 of the cages with a camel-hair brush to serve as food for the wasps. The parasitoids were
124 reared for seven generations before the onset of the experiment.

125 2.3. Interaction between *D. gelechiidivoris* and *S. sp. nr. japonicus*

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

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

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

141

142

143 2.3.1. Extrinsic competition

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

154 2.3.2. Intrinsic competition

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

164 2.5. Data analysis

165 A two-way repeated measures Analysis of variance (ANOVA) was used to analyse the
166 number of probing and oviposition events executed on *T. absoluta* larvae for each larval stage

167 and for the respective parasitoid treatment combinations. A Generalized Linear Model
168 (GLM) with negative binomial distribution was used to assess the effect of larval instar and
169 parasitoid combinations on the number of eggs laid by each parasitoid species, percentage of
170 multi-parasitised host larvae, percentage parasitism by each species, percentage parasitism by
171 both species, and percentage of female wasps that eclosed. The developmental times for each
172 parasitoid species in the respective treatment combinations in 1st and 3rd-instar *T. absoluta*
173 larvae were analysed using GLM with gamma distribution. All tests were followed by
174 Tukey's multiple comparison of means post hoc test, whenever a significant difference was
175 detected. Data were analysed with R software (R Core Team, 2018).

176 3. Results

177 3.1. Extrinsic competition

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

188

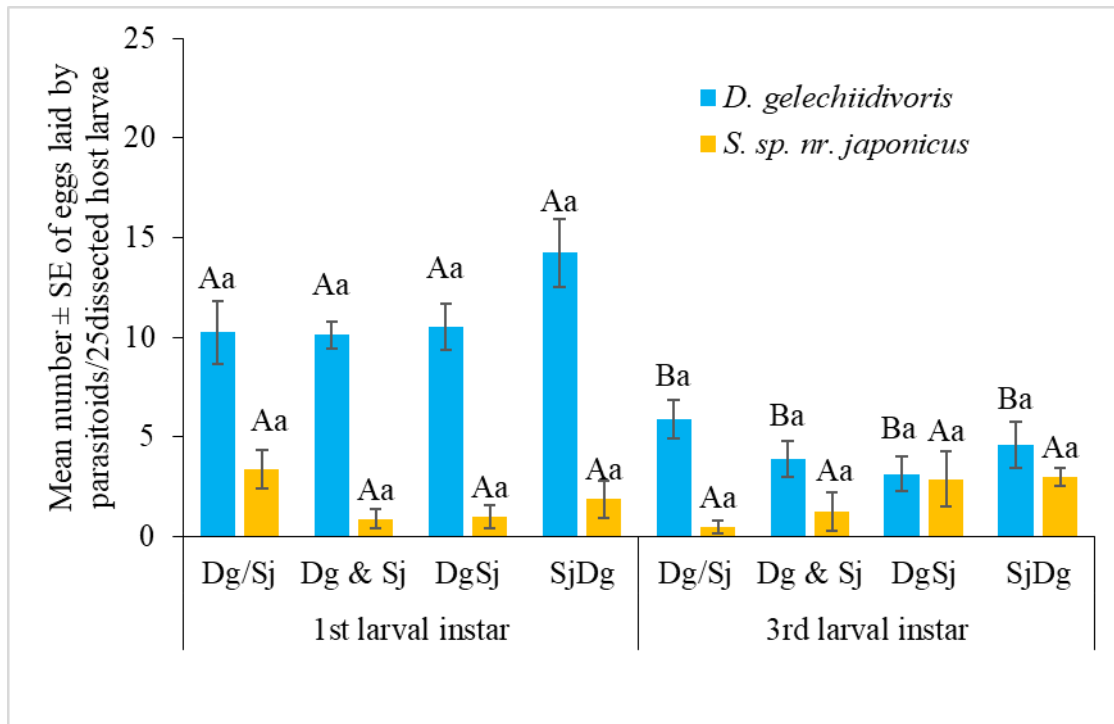
189 The number of eggs laid by *D. gelechiidivoris* was significantly affected by *T. absoluta* larval
190 instars ($F_{1, 59} = 81.41$, $P < 0.001$), but not by the parasitoid combinations ($F_{3, 59} = 2.28$, $P =$

191 0.09). The highest number of eggs laid by *D. gelechiidivoris* was in 1st-instar larvae,
192 compared to 3rd- instar larvae (Fig. 1). However, the number of eggs laid by *S. sp. nr.*
193 *japonicus* was not affected by the larval instars ($F_{1, 59} = 2.01$, $P = 0.16$) as well as by the
194 parasitoid combinations ($F_{3, 59} = 1.10$, $P = 0.36$). The highest percentage multiparasitism of 5
195 $\pm 3\%$ on 1st-instar, and $3 \pm 1\%$ on 3rd-instar *T. absoluta* larvae were recorded when *S. sp. nr.*
196 *japonicus* was released first for parasitism of *T. absoluta* larvae, followed by release of *D.*
197 *gelechiidivoris*. However, no multiparasitism was recorded for the scenario where *D.*
198 *gelechiidivoris* was released before *S. sp. nr. japonicus* for both larval instars. When both
199 parasitoid species were released simultaneously, 1% of 1st-instar larvae, but no 3rd-instar
200 larvae were multiparasitised.

201 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
 202 (Dg/Sj), simultaneous release (Dg & Sj), and sequential releases either of the parasitoids, *D. gelechiidivoris* (DgSj) or *S. sp. nr. japonicus*
 203 released first (SjDg).

<i>T. absoluta</i> larval instar	Parasitoid combinations	Mean no. of probes in 5 min		Mean no. of eggs in 5 min	
		<i>D. gelechiidivoris</i>	<i>S. sp. nr. japonicus</i>	<i>D. gelechiidivoris</i>	<i>S. sp. nr. japonicus</i>
1 st	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
3 rd	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

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



208

209 Fig. 1. Mean number of eggs laid by *D. gelechiidivoris* (Dg) and *S. sp. nr. japonicus* (Sj) in
 210 1st- and 3rd-instar *T. absoluta* larvae following a single release (Dg/Sj), simultaneous release
 211 (Dg & Sj), and sequential releases, with either *D. gelechiidivoris* (DgSj) or *S. sp. nr.*
 212 *japonicus* released first (SJDg). Means with different uppercase letters indicate significant
 213 differences in the number of eggs laid by the respective parasitoid species in 1st- and 3rd-
 214 instar larvae. Means with different lowercase letters indicate significant differences in the
 215 number of eggs laid by the respective parasitoids released in different combinations for 1st-
 216 and 3rd-instar *T. absoluta* larvae (Tukey's HSD test, $\alpha = 0.05$).

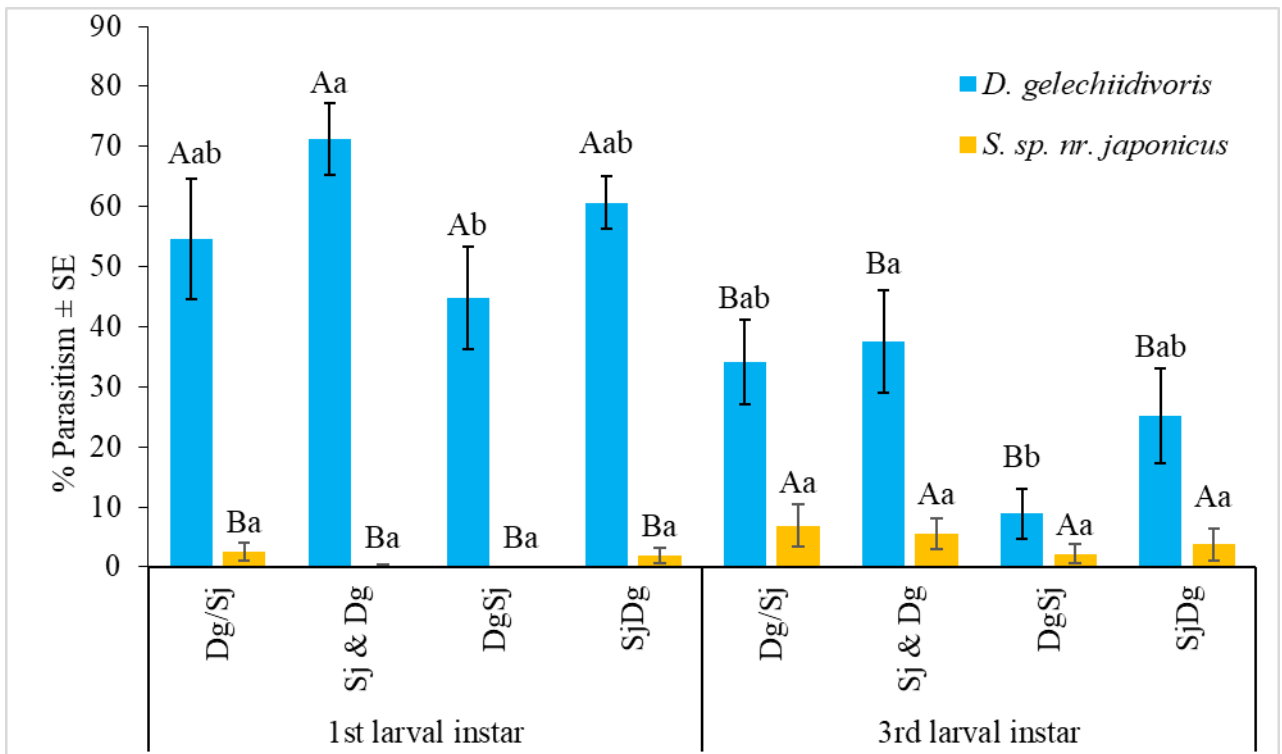
217

218 3.2. Intrinsic competition

219 3.2.1. Percentage parasitism by the respective parasitoid species

220 *Dolichogenidea gelechiidivoris* parasitism of *T. absoluta* larvae varied significantly between
221 the larval instars ($F_{1, 59} = 37.41$, $P < 0.001$) as well as between the parasitoid combinations
222 ($F_{3, 59} = 4.95$, $P = 0.004$). The highest parasitism by *D. gelechiidivoris* was reported on 1st-
223 instar larvae (Fig. 2). Parasitism by *S. sp. nr. japonicus* was also significantly affected by host
224 larval instars ($F_{1, 59} = 6.19$, $P = 0.02$), but not by the parasitoid combinations ($F_{3, 59} = 1.17$, $P =$
225 0.34). The 3rd-instar larvae were the most parasitised by *S. sp. nr. japonicus* (Fig. 2). The
226 proportion of *D. gelechiidivoris* females in the progeny was neither affected by host larval
227 instars ($F_{1, 50} = 0.15$, $P = 0.70$), nor by parasitoid combinations ($F_{3, 50} = 0.55$, $P = 0.65$).
228 However, the proportion of *S. sp. nr. japonicus* females that emerged, was affected by host
229 larval instars ($F_{1, 14} = 11.15$, $P = 0.004$), as well as by parasitoid combinations ($F_{3, 10} = 4.34$, P
230 $= 0.03$). Overall, *D. gelechiidivoris* that emerged from 3rd-instar larvae, was male biased in all
231 the scenarios where the two species were combined either simultaneously or in sequential
232 release (Table 3).

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Fig. 2. Percentage parasitism by *D. gelechiidivoris* (Dg) and *S. sp. nr. japonicus* (Sj) in 1st- and 3rd-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 between 1st- and 3rd-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 1st and 3rd-instar *T. absoluta* larvae (Tukey's HSD test, $\alpha = 0.05$).

244 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-
 245 and 3rd-instar *T. absoluta* larvae following a single release (Dg/Sj), simultaneous release (Dg & Sj), and sequential releases of either of the
 246 parasitoid species, *D. gelechiidivoris* (DgSj) or *S. sp. nr. japonicus* (SjDg) released first.

<i>T. absoluta</i> larval instar	Parasitoid combinations	% <i>D. gelechiidivoris</i> females	% <i>S. sp. nr. japonicus</i> females
1 st	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
3 rd	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

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

250 3.2.2. Parasitoid development time

251 Development time of *D. gelechiidivoris* males and females varied significantly according to
252 the instar of the host larvae being parasitised (males: $F_{1, 124} = 48.20$, $P < 0.001$ and females:
253 $F_{1, 100} = 33.01$, $P < 0.001$), as well as for the parasitoid combinations used (males: $F_{3, 124} =$
254 8.91 , $P < 0.001$ and females: $F_{3, 100} = 8.20$, $P < 0.001$). For *S. sp. nr. japonicus*, host larval
255 stages affected female and male developmental time ($F_{1, 13} = 11.74$, $P = 0.004$) and ($F_{1, 13} =$
256 52.08 , $P < 0.001$) respectively, while parasitoid combinations affected only female
257 developmental time ($F_{3, 13} = 5.63$, $P = 0.01$) but not the male development time ($F_{1, 13} = 2.86$,
258 $P = 0.08$). Developmental time of *S. sp. nr. japonicus* was shorter on 3rd-instar larvae (Table
259 4).

260

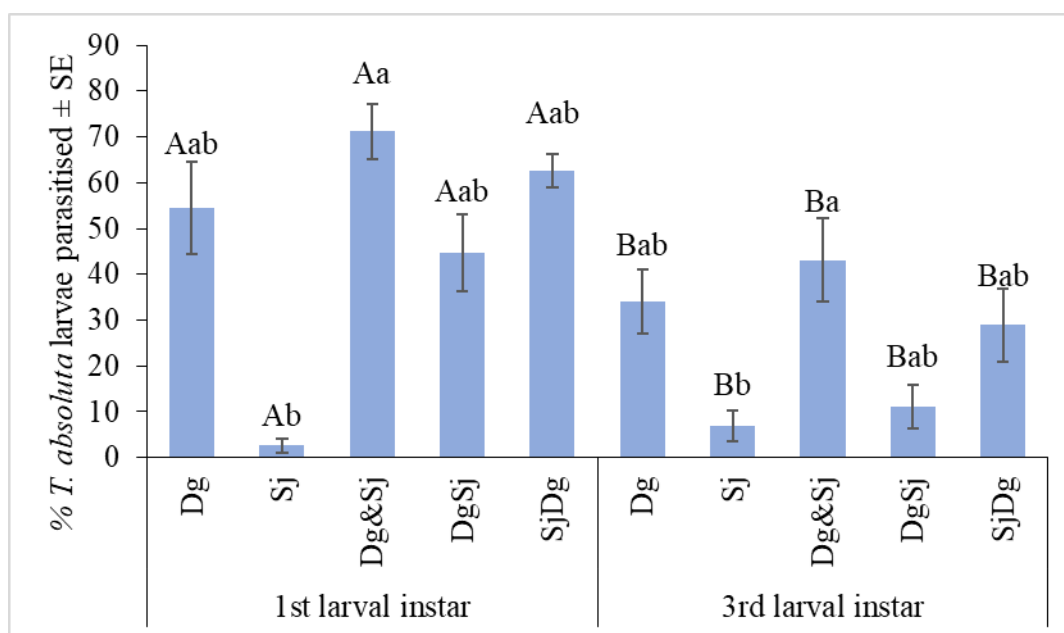
261 Table 4. Effect of the interaction between *D. gelechiidivoris* and *S. sp. nr. japonicus* on the developmental time from egg to adult (days) of the
 262 respective parasitoids from *T. absoluta* larvae parasitised while in the 1st- and 3rd-instars following a single release (Dg/Sj), simultaneous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

<i>T. absoluta</i> larval instar	Parasitoid combinations	<i>D. gelechiidivoris</i>		<i>S. sp. nr. japonicus</i>	
		♂	♀	♂	♀
1 st	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	*
3 rd	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

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

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

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



274
 275 Fig. 3. Percentage *T. absoluta* larvae parasitised by *D. gelechiidivoris* (Dg) and *S. sp. nr.*
 276 *japonicus* (Sj), when the parasitoids were released simultaneously (Dg & Sj), in sequence
 277 with either of the parasitoid species, *D. gelechiidivoris* release first (DgSj) or *S. sp. nr.*
 278 *japonicus* (SjDg), released first. Means with different uppercase letters indicate significant
 279 differences in parasitism between 1st and 3rd instar *T. absoluta* larvae. Means with different
 280 lowercase letters indicate significant differences in parasitism by the respective parasitoids
 281 released either alone, or in combinations (Tukey's HSD test, $\alpha = 0.05$).

282 Discussion

283 The introduction of a parasitoid species that can be useful for classical biocontrol may have a
284 negative impact on other native species (Kenis et al., 2019). *Stenomesus* sp. nr. *japonicus* is a
285 native ectoparasitoid, which attacks the different larval instars of *T. absoluta* with a high
286 killing rate in addition to parasitism (Chailleux et al., 2014). *Dolichogenidae gelechiidivoris*
287 was released in Kenya, where a native parasitoid, *S.* sp. nr. *japonicus* widely occurs and
288 where it has formed a new association with the invasive *T. absoluta* (Mama Sambo et al.,
289 2022b; Shiraku, 2020).

290 4.1. Extrinsic competition

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

307 100 first-instar larvae were offered to the same parasitoid. A much lower number of two to
308 five progenies per day was recorded by Chailleux et al. (2014) for *S. sp. nr. japonicus*. The
309 number of eggs laid by *D. gelechiidivoris* was the highest in 1st-instar larvae, which is also its
310 preferred host stage (Aigbedion-Atalor et al., 2020).

311 *Stenomesus sp. nr. japonicus* discriminated against already parasitised larvae during
312 oviposition, since multiparasitism only occurred when this parasitoid was released before *D.*
313 *gelechiidivoris*, or when the *T. absoluta* larvae were exposed to the two species
314 simultaneously. Similarly, Savino et al. (2016) also reported the highest searching time for *T.*
315 *absoluta* larvae by the ectoparasitoid *D. phthorimaeae*, when the endoparasitoid *P. dignus*
316 was present. However, it differs from other larval parasitoids, for example, the ectoparasitoid,
317 *Spathius agrili* Yang (Hymenoptera: Braconidae) that parasitised *Agrilus planipennis*
318 (Fairmaire) (Coleoptera: Buprestidae) larvae, which were previously parasitised by the
319 endoparasitoid, *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae) (Ulyshen et al.,
320 2010). The *S. agrili* progeny did, however, not develop on hosts previously parasitised by *T.*
321 *planipennisi* (Ulyshen et al., 2010). On the same pest, the endoparasitoid, *Dinarmus basalis*
322 (Rond.) (Hymenoptera: Pteromalidae), also discriminated against pulse beetle larvae earlier
323 parasitised by the ectoparasitoid, *Eupelmus vuilleti* Crawford (Hymenoptera: Eupelmidae)
324 (van Alebeek et al., 1993).

325 *Intrinsic competition*

326 The percentage parasitism by *D. gelechiidivoris* was significantly affected by *T. absoluta*
327 larval instar. It confirmed the results of Aigbedion-Atalor et al. (2020), who reported that the
328 highest number of *D. gelechiidivoris* cocoons formed in earlier instar larvae (1st- and 2nd-
329 instar), compared to late instar *T. absoluta* larvae (3rd- and 4th-instar). The percentage
330 parasitism by *D. gelechiidivoris* was, however, significantly lower when *S. sp. nr. japonicus*
331 was released after *D. gelechiidivoris* has already been present. The killing ability of the

332 ectoparasitoid *S. sp. nr. japonicus* released after *D. gelechiidivoris*, slightly affected the
333 emergence of the endoparasitoid also. Chailleux et al. (2014) estimated about two larvae
334 killed per day per female by this ectoparasitoid. Although the percentage parasitism of *S. sp.*
335 *nr. japonicus* was higher in 3rd-instar larvae as demonstrated by Chailleux et al. (2014). The
336 highest parasitism by this parasitoid occurred when it operated alone, in the absence of the
337 exotic parasitoid. The exotic *D. gelechiidivoris* therefore dominated the native *S. sp. nr.*
338 *japonicus*.

339 Developmental time of *D. gelechiidivoris* and *S. sp. nr. japonicus* was found to be host larval
340 instar dependent in this study. It is in contrast to the findings of Aigbedion-Atalor et al.
341 (2020) who reported no difference in developmental time of *D. gelechiidivoris* in the four
342 host larval instars. These authors also reported no difference in the proportion of male and
343 female parasitoids that eclosed, which is also in contrast to the findings of this study. The sex
344 ratio of *D. gelechiidivoris* progeny was male biased when it co-occurred with *S. sp. nr.*
345 *japonicus* on 3rd-instar larvae. Similarly, Mama Sambo et al. (2022a) also reported male
346 biased progeny for this parasitoid, while Aigbedion-Atalor et al. (2020) the progeny to be
347 female biased. However, abiotic factors affect parasitoid performance, including development
348 time and sex of the parasitoids (Bajonero et al., 2008; Dannon et al., 2010), which may
349 explain the differences found between the respective studies. The study of Aigbedion-Atalor
350 et al. (2020) was done at 26 ± 4 °C and $60 \pm 5\%$ RH, while this study was conducted at $21 \pm$
351 2 °C and $70 \pm 5\%$ RH. Developmental time of *D. gelechiidivoris* was longer than that of *S.*
352 *sp. nr. japonicus*, and almost similar to the developmental time of *D. gelechiidivoris* reported
353 by Bajonero et al. (2008) and Aigbedion-Atalor et al. (2020), as well as the developmental
354 time of *S. sp. nr. japonicus* reported by Chailleux et al. (2014). If only the shorter duration of
355 the life cycle of *S. sp. nr. japonicus* is considered, this parasitoid can be regarded as the more
356 preferred species, compared to *D. gelechiidivoris* (Chailleux et al., 2014).

357 The parasitism of *T. absoluta* larvae by both *D. gelechiidivoris* and *S. sp. nr. japonicus* varied
358 depending on the larval instar been parasitised and the combinations in which the two
359 parasitoids were used, with more than 75% parasitism when both species were released
360 simultaneously. This is in agreement with approximately 70% *T. absoluta* parasitism reported
361 for *D. gelechiidivoris* by Morales et al. (2014) and 75% that was reported by Bajonero et al.
362 (2008). Several parameters such as the host-parasitoid ratio, parasitoid age and temperature
363 during exposure, have an effect on the parasitism efficacy of *D. gelechiidivoris* (Bajonero et
364 al., 2008; Aigbedion-Atalor et al., 2020; Mama Sambo et al., 2022a). To the best of our
365 knowledge this is the first report on parasitism by *D. gelechiidivoris* in combination with
366 another parasitoid, but combinations of this parasitoid with the predator *Nesidiocoris tenuis*
367 (Reuter) (Hemiptera: Miridae) was highly effective (Aigbedion-Atalor et al., 2021). Up to
368 90% *T. absoluta* mortality was recorded, which was higher than *T. absoluta* mortality caused
369 by either the parasitoid or the predator alone. Although parasitism by *S. sp. nr. japonicus* was
370 low on 3rd-instar larvae, it still contributed towards control of the pest in this life stage. A
371 low efficacy in control of *T. absoluta* by a related species, *Stenomesus rufescens* (Retzius)
372 (Hymenoptera: Eulophidae), also a native parasitoid of *T. absoluta* has also been reported by
373 Kinyanjui et al. (2021). However, Chailleux et al. (2017) reported similar control by *S. sp. nr.*
374 *japonicus* and *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) from the fourth week
375 after release of the parasitoid.

376 Conclusion

377 *Dolichogenidea gelechiidivoris* performed better than *S. sp. nr. japonicus* in terms of
378 percentage parasitism. Although both species oviposit in the two *T. absoluta* larval instars, *D.*
379 *gelechiidivoris* preferred 1st-instar larvae, while *S. sp. nr. japonicus* preferred 3rd-instars. It is
380 possible that this preference may become more delineated with time, if the two species
381 segregate their niches as a survival mechanism. Developmental time and sex ratio of the

382 progenies of both species were not affected by their interspecific interaction. To deepen the
383 understanding of this interaction, chemical cues released by the host when parasitised by a
384 specific species should be investigated. Additionally, further studies should assess the
385 combined use of these two parasitoids species in commercial greenhouses and in open-field
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CHAPTER 6

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

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

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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.

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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; Abbas

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 *Stenomesus japonicus* Ashmead (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. absoluta* by *B. nigricans* was reported from open field tomato in central Kirinyaga county, Kenya where the exotic *D. gelechiidivoris* 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, *T. 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 \approx 1200 msl and 00°37'922" S, 37°22'794" E, Elevation \approx 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 ($25 \pm 1^\circ\text{C}$, 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 ($25 \pm 1^\circ\text{C}$, 70 ± 5 RH, and 12:12 L:D of photoperiod). The experiment was conducted in cages (30 cm (W) \times 30 cm (H) \times 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).

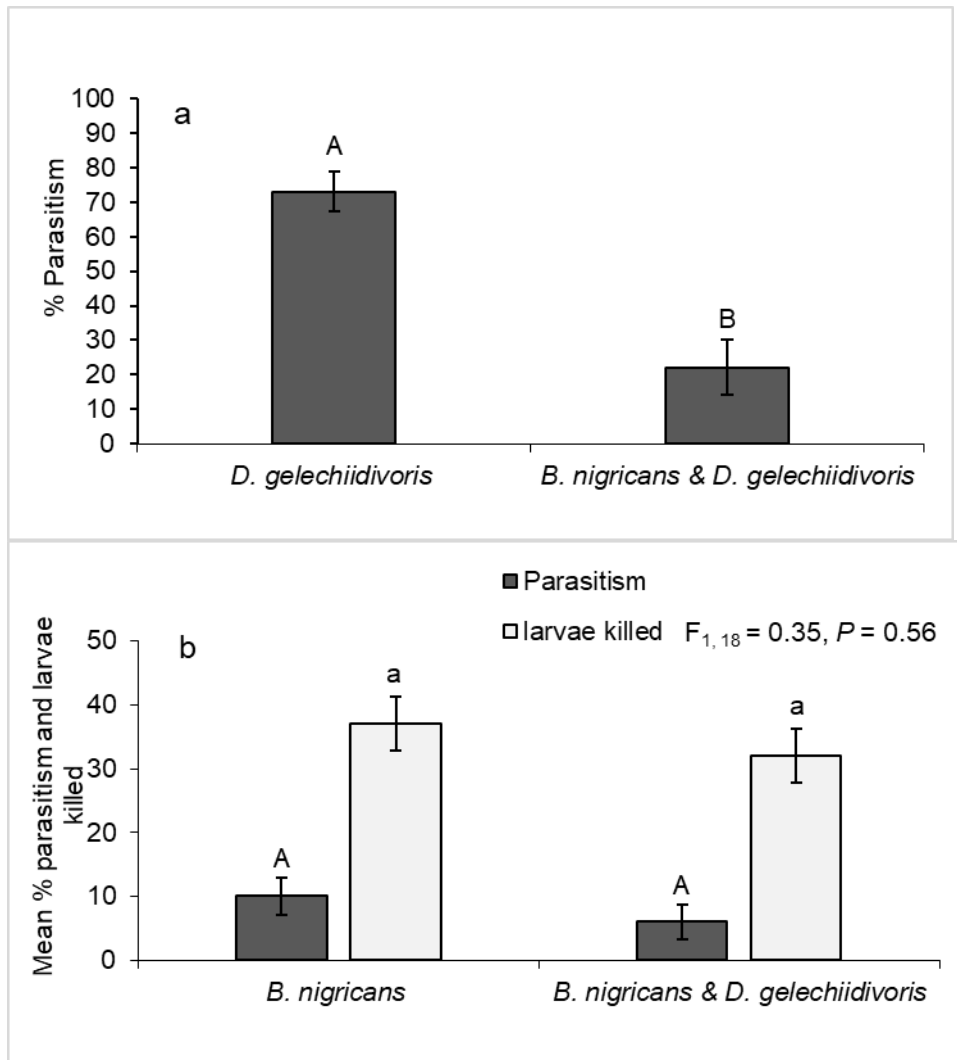


Fig. 1 (a) Mean percentage (\pm SE) *T. absoluta* larvae parasitised by *D. gelechiidivoris* and (b) Mean percentage *T. absoluta* larvae parasitised and killed by *B. nigricans* when released as a standalone treatment as well as in combination with *D. gelechiidivoris*. Bars capped with the same upper 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 <i>D. gelechiidivoris</i>	<i>D.</i>	(Intercept)	5.08	14.91	0.34
		<i>D. gelechiidivoris</i>	41.62	7.31	5.70
Number of <i>B. nigricans</i>	<i>B.</i>	(Intercept)	6.36	3.26	1.95
		<i>B. nigricans</i> and <i>D. gelechiidivoris</i>	3.46	1.94	1.79
Number of <i>T. absoluta</i> progenies (eggs and larvae)		(Intercept)	41.80	28.47	1.47
		<i>B. nigricans</i> and <i>D. gelechiidivoris</i>	-8.66	32.45	-0.27
		<i>D. gelechiidivoris</i>	23.44	34.72	0.67
		Control (no parasitoids)	482.66	177.39	2.72

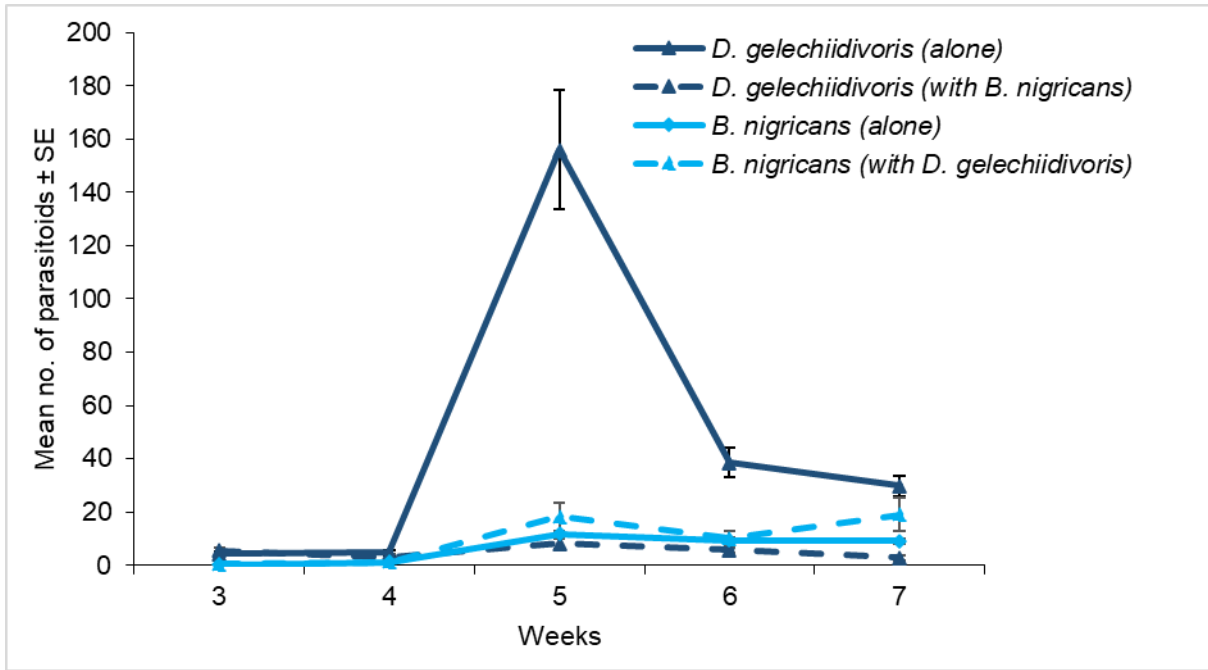


Fig. 2 Mean number (\pm SE) of *D. gelechiidivoris* and *B. nigricans* adults, per week in the respective treatments over time.

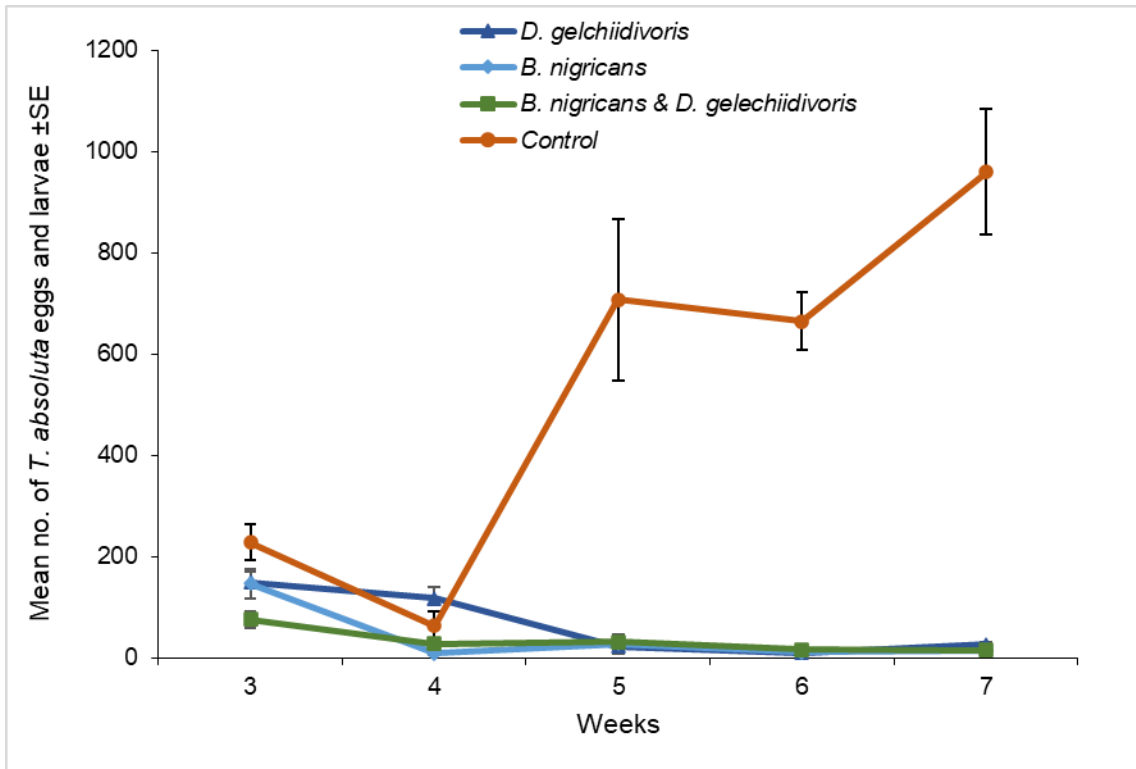


Fig. 3 Mean number (\pm SE) of *T. absoluta* progeny per week in the respective treatments over time.

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 when 1st-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, $60 \pm 10\%$ 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.

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CHAPTER 7

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

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Article

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

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Simple Summary: The theory of beneficial species association in a cropping system can sustain ecosystem services and reduce pest pressure 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 synergistic, 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*

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.) produc-

tion 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 devastating 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 organisms 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 ($50 \times 50 \times 60$ cm). The incubated larvae were supplied with clean tomato leaves, sourced from an insecticide-free greenhouse 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 greenhouse, 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 ($40 \times 20 \times 50$ 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 10⁸ 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 enclosed *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-enclosed 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 first-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.

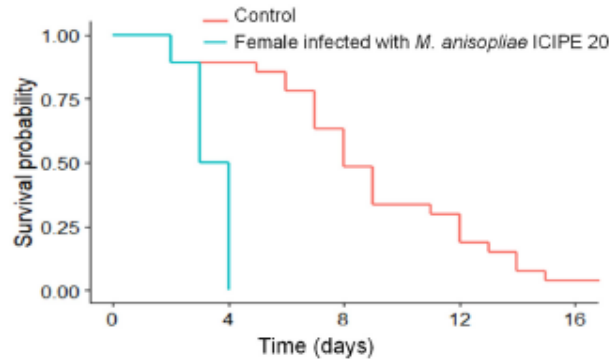


Figure 1. Effect of *M. anisopliae* ICIPE 20 (direct infection with dry conidia) on *D. gecheiivovis* survival.

The performance of *M. anisopliae* ICIPE 20 infected and uninfected *D. gecheiivovis* 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 ($87.79 \pm 6.32\%$) for uninfected wasps. Nonetheless, no transmission of ICIPE 20 conidia by infected *D. gecheiivovis* 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).

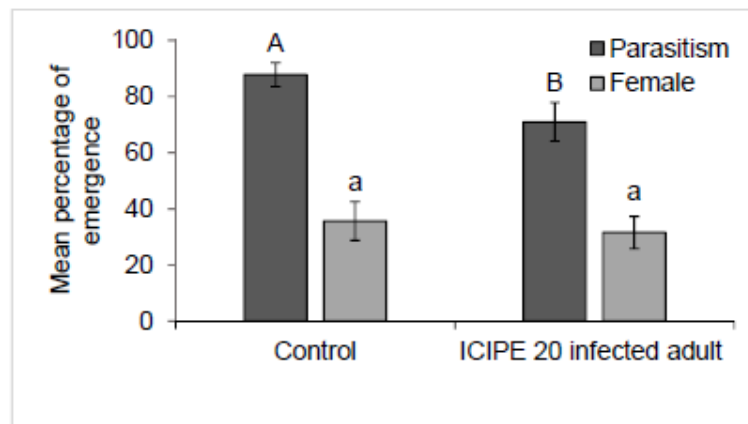


Figure 2. Performance of *M. anisopliae* ICIPE 20 infected and uninfected *D. gecheiivovis* on *T. absoluta*. Bars capped with the same upper/lower case letters are not significantly different.

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. gecheiivovis* 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 \pm SE) of *D. gelechiidivoris* on *M. anisopliae* ICIPE 20 treated and untreated *T. absoluta* larvae.

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

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

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 ($5 \pm 1.67\%$) for one day old post-exposure to the fungus (Figure 3).

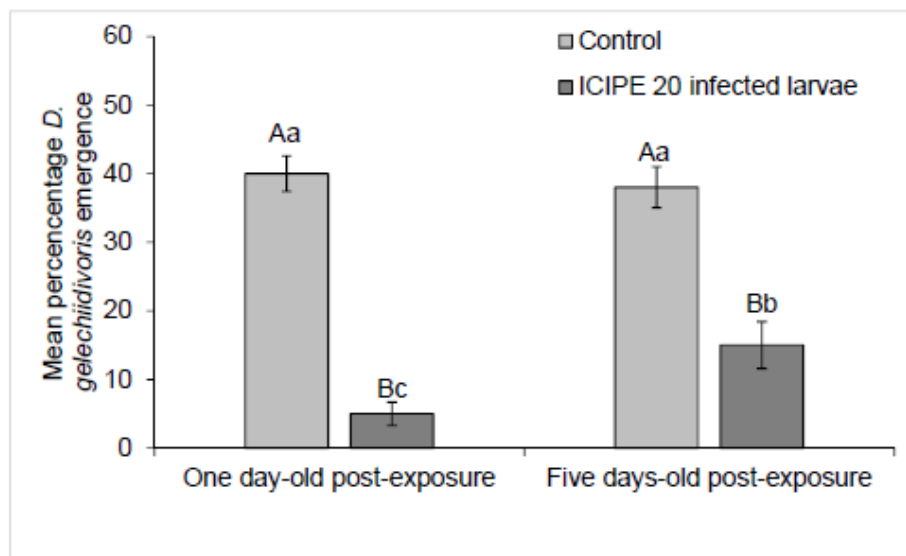


Figure 3. Percentage of *D. gelechiidivoris* that emerged from larvae exposed to the parasitoids infected with *M. anisopliae* ICIPE 20 at one day and five days post-exposure. Bars capped with the same uppercase letter indicate no difference between control and treatment within the same age exposed-larvae,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

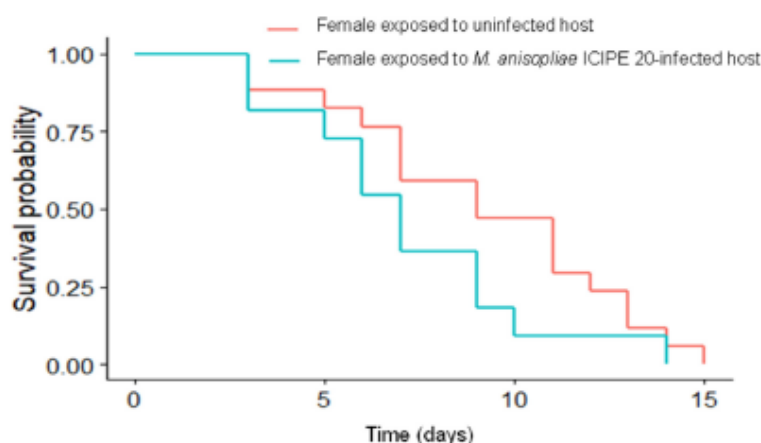
3.3. *Dolichogenidea gelechiidivoris* Preference for and Performance on *M. anisopliae* ICIPE 20-Sprayed and *T. absoluta* Infested Host Plants

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

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

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

The longevity of *D. gecheiivivoris* 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. gecheiivivoris* 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. gecheiivivoris* 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. gecheiivivoris* that foraged on fungal sprayed host plants, 77% were infected with *M. anisopliae* ICIPE 20.

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

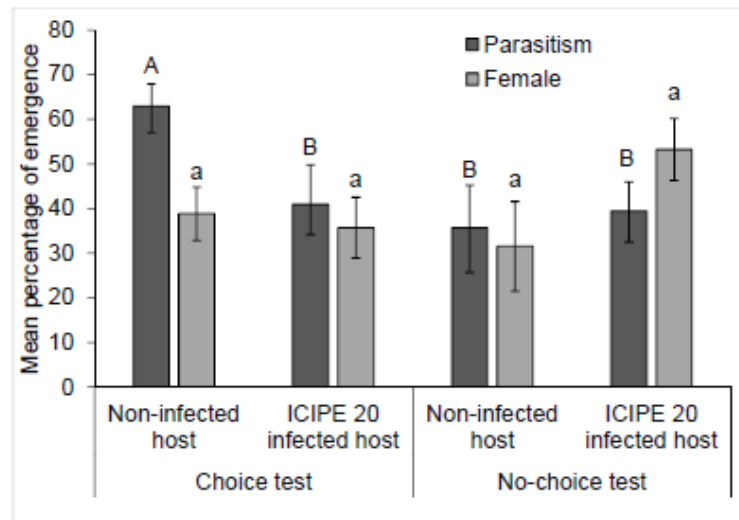


Figure 5. Performance of *D. gelechiidivoris* on *M. anisopliae* ICIPE 20 sprayed and unsprayed plants. Bars capped with same upper/lower case letters are not significantly different.

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).

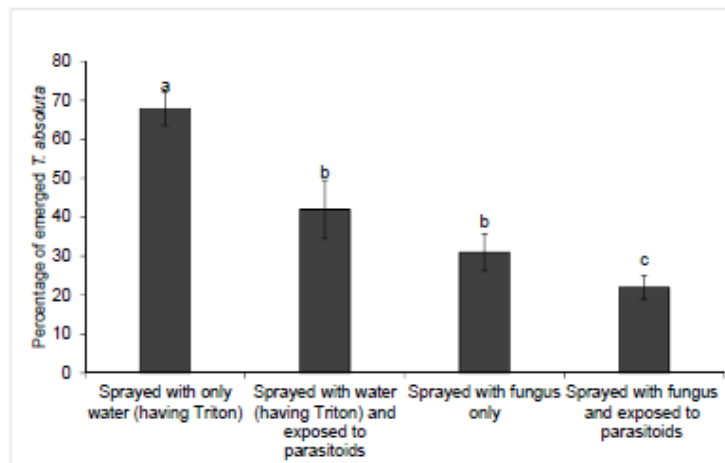


Figure 6. Effect of sole and combined use of two biocontrol agents on *Tuta absoluta* emergence. Bar capped with the same letters are not significantly different, (Tukey's HSD, $\alpha = 0.05$).

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. gelechiivivora* 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 gelechiivivora* 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. gelechiivivora*, 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. gelechiivivora* 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. gelechiivivora* 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. gelechiivivora* 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. gelechiivivora* 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 leaf-worm, *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 1.5×10^5 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 an 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.

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CHAPTER 8

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

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

3 Short title: Exotic parasitoid field effectiveness

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22 **Abstract**

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

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

46 **1 Introduction**

47 Tomato production has been threatened by the tomato leafminer *Tuta absoluta* (Meyrick)
48 (Lepidoptera: Gelechiidae) in its invaded areas since 2006 (Biondi et al. 2018). Tomato
49 production is drastically reduced by the larvae consuming the mesophyll of leaves, stems, and
50 fruits, resulting in fruit that are unsuitable for trade (Desneux et al. 2011). Endemic natural
51 enemy species of *T. absoluta* have been investigated but economic thresholds using these species
52 have not been determined for the control of this pest (Mansour et al. 2018; Mama Sambo, et al.
53 2022a). The performance of *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) under
54 greenhouse tomato production has been evaluated in different invaded areas (Chailleux et al.
55 2013; Zouba et al. 2013; Cherif et al. 2019). *Trichogramma* spp. are, however, generalist
56 parasitoids of various lepidopteran species (Brotodjojo & Walter, 2006; Laurentis et al. 2019),
57 with varying performance due to the learning capability of these species to new cues (Gonthier et
58 al. 2022).

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

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

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

84
85 Biological control can be strengthened by complementarity among natural enemies, e.g. if
86 multiple enemies attack a pest during different periods of its occurrence in the field, or different
87 stages of the lifecycle (Jonsson et al. 2017). The selection of the most appropriate combination of
88 arthropods as natural enemies for *T. absoluta* control in tomato fields will depend on their pest
89 suppression capacity when used alone, or in an integrated pest management (IPM) scenario. *Tuta*

90 *absoluta* is also susceptible to various entomopathogenic fungi (Agbessenou et al. 2021; Akutse
91 et al. 2020; Ndereyimana et al. 2019). The entomopathogenic fungus, *Metarhizium anisopliae* is
92 pathogenic to both the adult and larval stages of this pest (Akutse et al. 2020). An additive effect
93 in the control of *T. absoluta* was reported by Mama Sambo et al. (2022b) with the combined use
94 of *D. gelechiidivoris* and *M. anisopliae*. The technique to auto-disseminate the *M. anisopliae*-
95 infection in a pest population through a trap containing the fungus and the pest being lured to a
96 trap was described by (Migiro et al. 2010). It can be applied to *T. absoluta* also, but the attraction
97 of *D. gelechiidivoris* females to the *T. absoluta* lure (Ayelo et al. 2021) is unknown. It may have
98 a negative effect on the performance of the parasitoid. Thus, the aim of this study was to develop
99 an efficient method to control *T. absoluta* by releasing *D. gelechiidivoris* at different frequencies,
100 using a lure trap contaminated with fungus, as well as combined use of the parasitoid and the lure
101 trap contaminated with fungus in greenhouses. Additionally, the parasitoid dispersion in an open
102 field area where it was released was assessed.

103

104 **2 Material and methods**

105 **2.1 Tomato planting**

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

111

112 **2.2 Insect production**

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

120

121 **2.3 *Metarhizium anisopliae* ICIPE 20 mass production**

122 *Metarhizium anisopliae* ICIPE 20 was mass-produced on a rice substrate. A liquid media was
123 prepared with 2% dextrose, 1% peptone, 0.25% yeast extract, and 250 ml distilled water in a 1L
124 conical flask. The flasks containing the mixture were then sterilized at 121°C for 15-20 min in an
125 autoclave. After cooling at room temperature, 0.02% antibacterial chloramphenicol followed by
126 0.05 g of *M. anisopliae* ICIPE 20 dry conidia were added to the broth, and the flasks were
127 continuously shaken for 4 days in a refrigerated orbital shaker (New Brunswick Scientific™
128 Innova™ 44, Germany) at $25 \pm 2^\circ\text{C}$ and 100 rpm. Pishori rice (2.5 kg) was washed for 30-45
129 minutes until all the starch was removed. The water was drained from the rice for 10-15 minutes
130 before the rice was placed into Milner bags (60 cm long \times 35 cm wide), which were filled with
131 air and sealed with an electric sealer following the technique described by Maniania, (1998).
132 These rice-containing bags were autoclaved and sterilized at 121°C for 1 hour and cooled down
133 at room temperature (Opisa et al. 2019). A small cut was made in each bag and 5 ml of the
134 mycelia solution was added. The bags were re-sealed using an electric sealer before they were

135 vigorously shaken to homogeneously mix the mycelia with rice grains (Jenkins et al. 1998). The
136 inoculated bags were placed in a tray at 20–26°C and 40%–70% relative humidity (RH) and
137 shaken and manipulated 48-hourly until the fungus matured. Conidia from these bags were
138 harvested 30 days after inoculation. Conidial viability was determined before application in the
139 field, by spreading and incubation on Sabouraud Dextrose Agar media plates as described by
140 Goettel and Inglis (1997). The viability of the harvested conidia was more than 98%. The dry
141 conidia were stored at 4 °C for the period of application in the greenhouses.

142

143 **2.4 Application of the treatment in the greenhouse**

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

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

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

163

164 **2.5 Data collection**

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

176

177 **2.6 *Dolichogenidea gelechiidivoris* dispersion**

178 In October 2020, 500 *D. gelechiidivoris* couples were transported to a farmer field in Kirinyaga
179 south subcounty (S 00.63222°, E 037.38222°, Alt 1194 m; 1 ha in size) in a net cage with honey
180 droplets provided as food. The parasitoids were released at the center of the farms. The tomato

181 farm was an *icipes* demonstration plot at flowering stage where IPM strategies were used to
182 control *T. absoluta* infestation. Moreover, before the release, the infested material collection was
183 done regularly from that farm and all the tomato farms in that region to supplement laboratory
184 colonies, pest density monitoring, and native parasitoids monitoring. *Dolichogenidea*
185 *gelechiidivoris* was not recorded at this site prior to this study. Five months after release, the
186 first random sampling of infested tomato leaves was done in the county where the parasitoids
187 were released. For each sampling time, the Global Positioning System (GPS) coordinate of each
188 collection point was recorded. The collected leaves were incubated per collection point, and *D.*
189 *gelechiidivoris* that emerged from infested *T. absoluta* were recorded. A second sampling was
190 done one year after the parasitoids were released.

191

192 **2.7 Data analysis**

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

203 **3 Results**

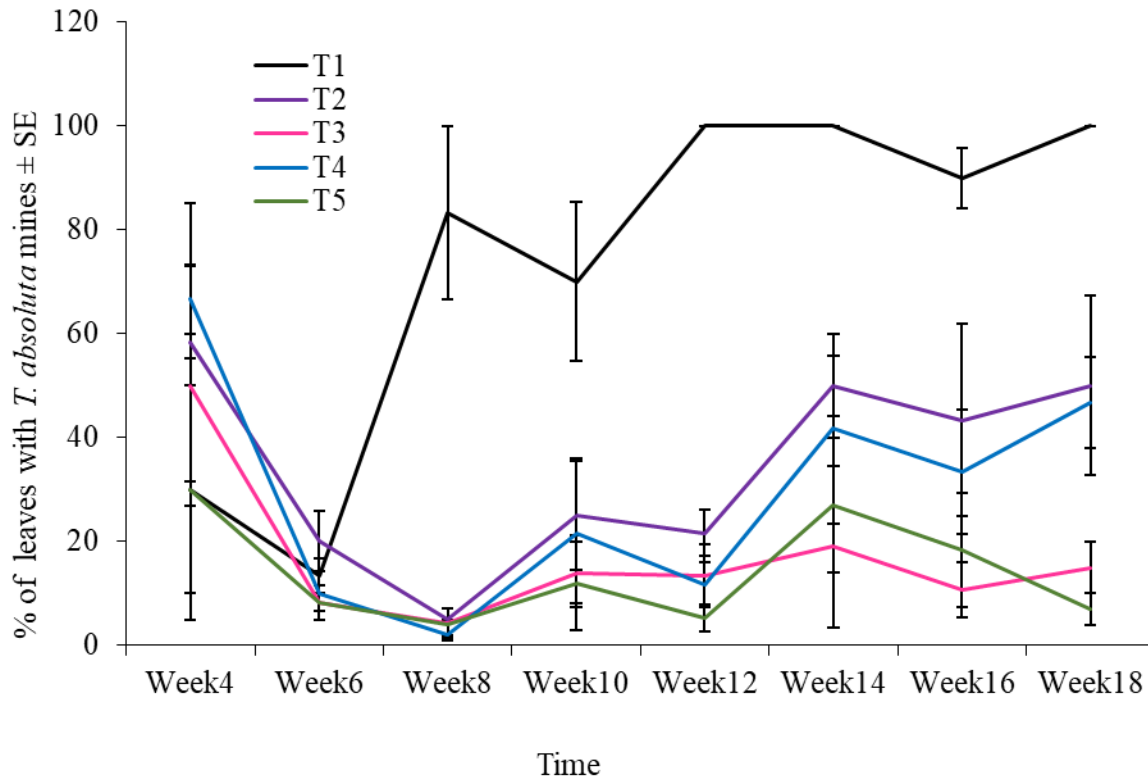
204 **3.1 Effectiveness of the treatments**

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

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

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

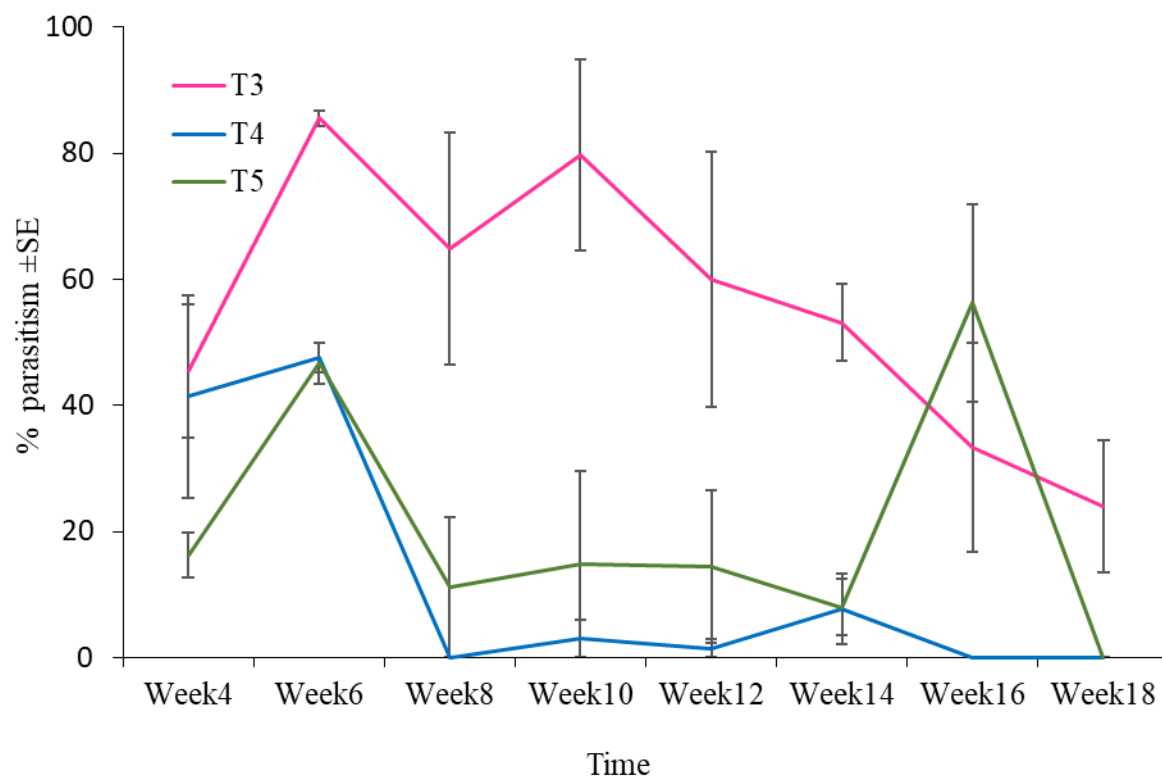
222 A negative t-value indicates a negative correlation.



223

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

232



233

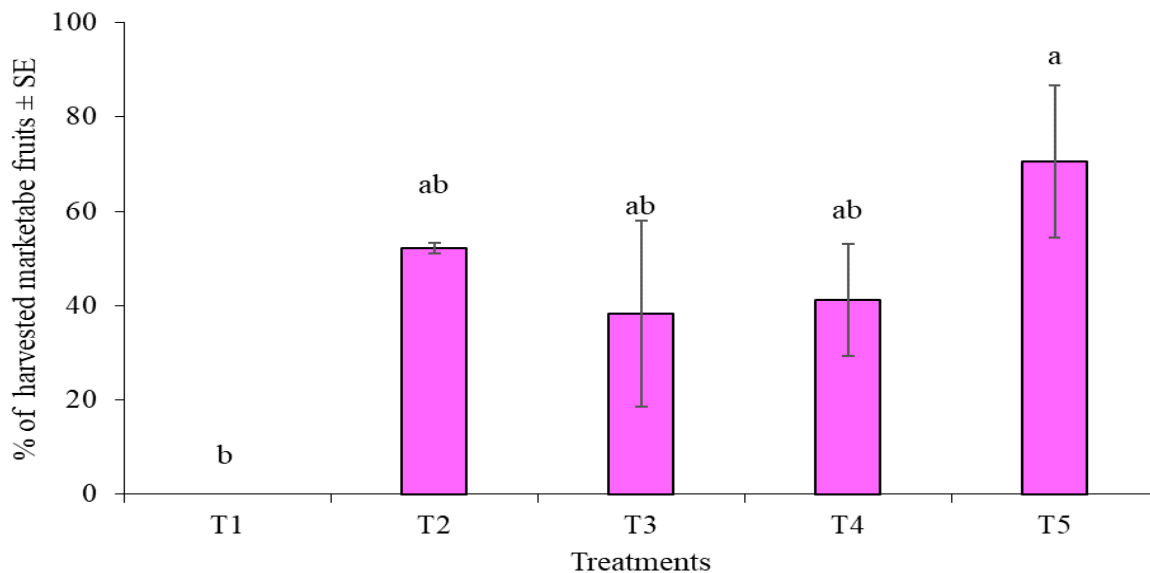
234

235 **Fig. 2.** Percentage *Tuta absoluta* larval parasitism by *Dolichogenidea gelechiidivoris* ± SE
 236 following application of the respective treatments: T3 = Weekly release of *D. gelechiidivoris* for
 237 12 weeks, T4 = Monthly release of *D. gelechiidivoris* for a period of four months, and T5 =
 238 Monthly release of *D. gelechiidivoris* in combination with the use of a pheromone trap
 239 contaminated with dry *M. anisopliae* ICIPE 20 conidia, which was replaced every month for a
 240 period of four months. Means followed by the same letters did not differ significantly (Tukey's
 241 HSD, $\alpha = 0.05$).

242

243 The percentage marketable fruit estimated from the number of fruits harvested and the *T.*
 244 *absoluta* infested fruit varied significantly between the treatments ($F_{4, 10} = 6.07, P = 0.001$). The

245 highest percentage yield was achieved with the combination of *D. gelechiidivoris* released and
246 placement of a pheromone trap contaminated with dry *M. anisopliae* ICIPE 20 conidia (Fig. 3).

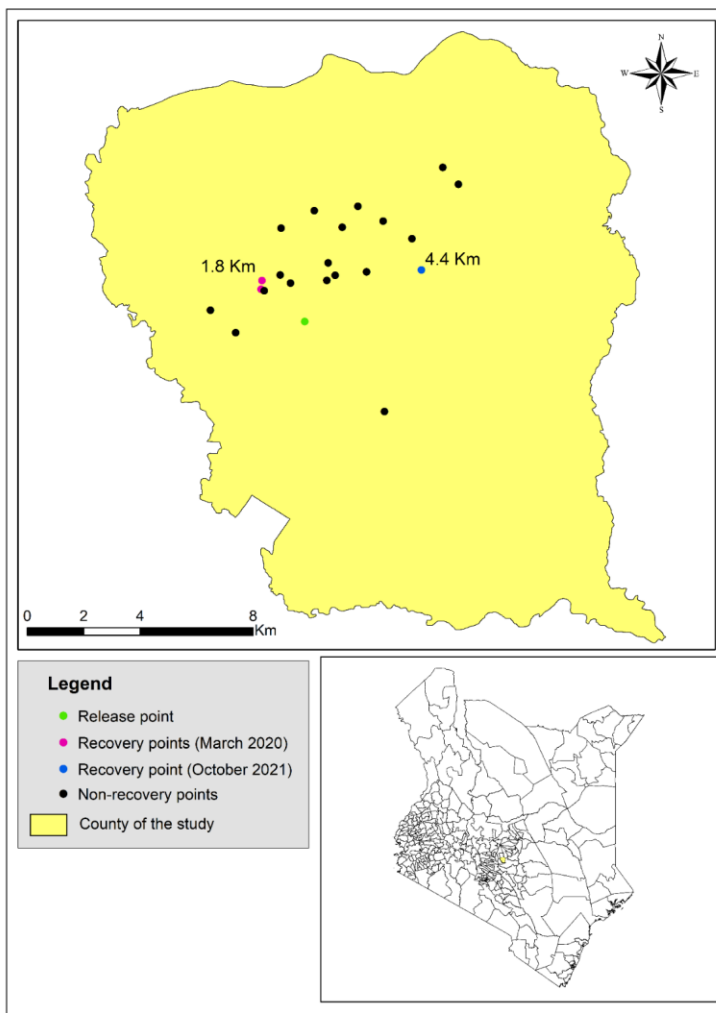


247 **Fig. 3.** Percentage of non-infested tomato fruit harvested from greenhouses following application
248 of the respective treatments: T1 = untreated, T2 = Pheromone trap contaminated with dry *M.*
249 *anisopliae* ICIPE 20 conidia, which was replaced every month for a period of four months, T3 =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

255

256 **3.2 *Dolichogenidea gelechiidivoris* dispersion**

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



262 .
263 **Fig. 4.** Recovery of *D. gelechiidivoris* five months and one year after release at Kirinyaga south
264 subcounty, Kenya.

265 4 Discussion

266 Our findings confirmed the success of *D. gelechiidivoris* as a biological control agent of *T.*
267 *absoluta* as well as its compatibility with, and use in combination with the entomopathogenic
268 fungus, *M. anisopliae* ICIPE 20 contaminated to a pheromone trap. The successful establishment
269 of *D. gelechiidivoris* in Kenya was also confirmed. Several cases of successful establishment of
270 other introduced natural enemies and their success as biocontrol agents in the areas invaded by
271 co-evolved pests have been reported (Cock, 2016; Mohamed et al. 2022; Ndiaye et al. 2015).
272 However, a single natural enemy of a pest often fails to reduce the pest to levels below the
273 economic injury level (Chailleux et al. 2013; Farrar et al. 2016; Labbé et al. 2009; Mas et al.
274 2019). A variety of control measures, combined in an IPM strategy for control of *T. absoluta*
275 should be promoted.

276 In this study, *D. gelechiidivoris* release frequencies and the combination of parasitoid releases
277 with the use of a *T. absoluta* pheromone trap contaminated with *M. anisopliae* ICIPE 20, did not
278 affect the percentage of *T. absoluta* infested leaves in general. Morales et al. (2014) reported
279 similar results, with the number of mined leaves, which were similar with the release of *D.*
280 *gelechiidivoris* only, and where *D. gelechiidivoris* parasitoids were released in combination with
281 *T. absoluta* pheromone traps. Weekly releases of 20 and 30 *Trichogramma cacoeciae* (Marchal)
282 (Hymenoptera: Trichogrammatidae)/plant was equally effective and significantly decreased *T.*
283 *absoluta* densities in greenhouses (Cherif et al., 2019). Successful control of *T. absoluta* with
284 *Trichogramma* spp. was also reported by Zouba et al. (2013), with a reduction in leaf damage of
285 78.89% and 87.62% by *T. cacoeciae* and *Trichogramma bourarachae* Pintureau & Babault,
286 (Hymenoptera: Trichogrammatidae) respectively after inundative releases of 25000
287 parasitoids/week in a greenhouse. A positive correlation was also reported between the number

288 of *Trichogramma euproctidis* Girault (Hymenoptera: Trichogrammatidae) or *T. achaeae*
289 Nagaraja & Nagarkatti parasitoids released at 25, 50, and 75 parasitoids/m² and the level of *T.*
290 *absoluta* control (El-Arnaouty et al., 2014).

291 In the present study, the highest percentage parasitism rate by *D. gelechiidivoris* (24-86%) was
292 recorded when the parasitoids were released every week for a period of 12 weeks. Monthly
293 parasitoid releases for a period of four months, were similar for parasitoids released in
294 association with or without the *M. anisopliae* ICIPE 20-contaminated trap. Several authors
295 reported on the performance of *D. gelechiidivoris* in controlling Gelechiidae species (Bajonero
296 et al. 2008; Morales et al. 2014, Aigbedion-Atalor et al. 2020; Mama Sambo et al. 2022c).
297 Morales et al. (2014) recorded up to 86.38 % *T. absoluta* larval parasitism with *Apanteles*
298 *gelechiidivoris* (Hymenoptera: Braconidae) when this parasitoid was used in combination with a
299 pheromone trap. Similarly, Mama Sambo et al. (2022b) reported 86% of 1st-instar larvae
300 parasitism by *D. gelechiidivoris* under laboratory conditions. Various factors affect parasitism
301 performance of parasitoids, which was evident from the lower percentage parasitism of *T.*
302 *absoluta* larvae by *D. gelechiidivoris* in laboratory studies, reported by Bajonero et al. (2008)
303 (75%), Aigbedion-Atalor et al. (2020) (50%), and Mama Sambo et al. (2022c) (59%) under
304 scenarios that included differences in temperature, larval stages, host densities, and parasitoid
305 density. However, Morales et al. (2013) reported more than 90% parasitism with 4, 8, and 12 *D.*
306 *gelechiidivoris* females exposed to a *T. absoluta*-infested plant under laboratory conditions.

307 The release of *D. gelechiidivoris* in a greenhouse, in combination with a *T. absoluta* pheromone
308 trap contaminated with *M. anisopliae* ICIPE 20, resulted in higher marketable tomato yield. A
309 similar trend was reported by Mama Sambo et al. (2022b) where the combined use of the two
310 biocontrol agents, *M. anisopliae* ICIPE 20 applied as a spray onto a *T. absoluta* infested host

311 plant, followed by the release of *D. gelechiidivoris*, resulted in higher mortality of this pest,
312 compared to use of the two agents individually. In contrast, the combination of *T. bactrae*
313 released and mass trapping of *T. absoluta* resulted in higher tomato yields compared to a
314 scenario where biopesticides (Biotrine and Fytomax) were used in combination with mass
315 trapping, and with an insecticide application (Goda et al. 2015). Other combinations of
316 biocontrol agents of *T. absoluta* under protected cropping that influenced tomato yield, included
317 a combination of parasitoids and predators. For example, better *T. absoluta* control was achieved
318 with a combination of *Macrolophus pygmaeus* Rambur (Hemiptera: Meridae) and the release of
319 *T. achaeae* compared to the individual use of the respective agents (Chailleux et al. 2013). More
320 uninfested fruit were harvested in a greenhouse where *T. evanescens* and *Nesidiocoris tenuis*
321 (Reuter) (Hemiptera: Meridae) were released, compared to *Trichogramma evanescens*
322 Westwood (Hymenoptera: Trichogrammatidae) alone and *N. tenuis* alone (Öztemiz, 2013).
323 However, Mirhosseini et al. (2019), found the highest percentage of undamaged fruits, with *N.*
324 *tenuis* regardless of the application of *Trichogramma brassicae* Bezdenko (Hymenoptera:
325 Trichogrammatidae).

326 *Dolichogenidea gelechiidivoris* was recovered a year after its first release in Kenya, at a distance
327 of 4.4 km from the release area. Dispersion might have been achieved through wind or
328 transportation of infested material. *Dolichogenidea gelechiidivoris* has also recently been
329 recovered in the palearctic region (Denis et al. 2022; Krache et al. 2021). A steady increase in
330 endoparasitism levels of *T. absoluta* larvae occurred during the tomato production season in
331 2020, in samples collected from tomato fields in northeastern Spain. By October, 21.8% of *T.*
332 *absoluta* larvae were parasitized by Braconidae, all identified as *D. gelechiidivoris* (Denis et al.
333 2022). Although the speed of dispersal and spread of several introduced parasitoid species are

334 available (Baoua et al. 2018; Bokonon-Ganta et al. 2013; Salazar-mendoza et al. 2020; Sallam et
335 al. 2001), it has not been published for *D. gelechiidivoris*. The establishment of
336 *Diachasmimorpha kraussii* (Fullaway) (Hymenoptera: Braconidae) in Hawaii for the control of
337 *Bactrocera latifrons* (Hendel) (Diptera: Tephritidae) infesting turkey berry, *Solanum torvum*
338 Swartz, (Solanaceae) was confirmed after three years, 5 km away from the release point
339 (Bokonon-Ganta et al. 2013). The exotic *Cotesia flavipes* Cameron (Hymenoptera: Braconidae),
340 a larval endoparasitoid of stemborers, was recorded 64 m away from the point of its release after
341 four days, with the number of parasitised larvae that decreased with distance (Sallam et al. 2001).
342 In contrast, parasitism by both *Tetrastichus julis* (Walker) of *Oulema melanopus* (Linnaeus)
343 (Coleoptera: Chrysomelidae) (Hymenoptera: Eulophidae) and *Bathyplectes curculionis*
344 (Thomson) (Hymenoptera: Ichneumonidae) of *Hypera postica* (Gyllenhal) (Coleoptera:
345 Curculionidae) did not change with increasing distance (Evans, 2018). Parasitism by *Telenomus*
346 *remus* Nixon (Hymenoptera: Platygasteridae), a native parasitoid of *Spodoptera frugiperda* (J.E.
347 Smith) (Lepidoptera: Noctuidae) eggs in Brazil, also declined linearly with increasing distance
348 from the parasitoid release point, 48 hours post-release (Salazar-Mendoza et al. 2020). Parasitism
349 levels of *Heliocheilus albipunctella* de Joannis (Lepidoptera: Noctuidae) larvae by *Habrobracon*
350 *hebetor* Say (Hymenoptera: Braconidae) after augmentative releases in Burkina Faso and Niger,
351 were initially higher at the site of dissemination compared to sites at 3 km and 5 km away.
352 However, 5 weeks after release, parasitism of *H. albipunctella* 3 km away, was similar to those
353 at the release point (Baoua et al. 2018). These findings indicate that for optimization of *D.*
354 *gelechiidivoris* distribution in control of *T. absoluta*, the distance between release points needs to
355 be taken into account. Since the parasitoid has now spread in Kenya. Decision-makers in the

356 inhabited area could consider this parasitoid for classical biological control programs as an eco-
357 friendly and economically sustainable management tool for the control of *T. absoluta*.

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

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

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570

GENERAL CONCLUSIONS AND RECOMMENDATIONS

573 Classical biological control (CBC) has been defined as “the intentional introduction of an exotic
574 biological control agent for permanent establishment and long-term pest control” (Eilenberg *et*
575 *al.*, 2001). Success of CBC can be defined ecologically as the establishment of the introduced
576 natural enemy, and economically as the reduction of the pest population to such an extent that it
577 is economical valuable (Hokkanen and Sailer, 1985). Known successes in the field of CBC in
578 Africa are those of cassava mealybug, mango mealybug, fruit flies, and *Liriomyza* leafminer
579 (Cock, 2016; Gnanvossou *et al.*, 2016; Mohamed *et al.*, 2022). Ekesi *et al.* (2011) did, however,
580 warn that although biocontrol-based integrated pest management (IPM) is successful in some
581 cases, long-term sustainability might be adversely affected by emerging issues such as climate
582 change, occurrence of more invasive species, changing consumer demands related to quality and
583 standards, sanitary and phytosanitary requirements, and trade issues. Integrated pest management
584 are therefore widely recommended for control of insect pests (Ekesi *et al.*, 2011; Niassy *et al.*,
585 2022), including *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Desneux *et al.*, 2022).
586 *Trichogramma* spp. have been tested in Chile for classical biocontrol of *T. absoluta* (Desneux *et*
587 *al.*, 2010), while *D. gelechiidivoris* have been released as classical biological agent in Colombia
588 (Rojas, 1997), from where establishment and spread to areas such as Chile was expected. This
589 introduction resulted in establishment, and it is now considered as one of the most important
590 parasitoids of *T. absoluta* in Chile (Desneux *et al.*, 2010). The aim of this project was to
591 investigate the management of *T. absoluta* with introduced and native biocontrol agents in
592 Kenya. The aim was divided into objectives that were addressed and provided as chapters in this
593 thesis. The outcome of this study and recommendations for future research will be discussed per
594 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 597 production of *Dolichogenidea gelechiidivoris*.

598 The International Center for Insect Physiology and Ecology (icipe) imported the microgastrinae
599 koinobiont endoparasitoid, *Dolichogenidea gelechiidivoris* Masch (Syn.: *Apanteles*

600 *gelechiidivoris* Marsh) (Hymenoptera: Braconidae) from Peru and introduced it into Kenya
601 (Aigbedion-Atalor *et al.*, 2020). It is important to be able to mass produce the parasitoid and
602 release it in numbers to achieve a significant reduction in *T. absoluta* populations. The results in
603 chapter 3 of this thesis, indicated that host and parasitoid density affected superparasitism.
604 Intraspecific competition enhanced the parasitism rate of *D. gelechiidivoris* females, but their
605 performance was reduced with intraspecific competition. The efficiency in control of *T. absoluta*
606 by *D. gelechiidivoris* was evident from the type II functional response and the high percentage of
607 wasps that emerged from larvae exposed to the parasitoid. Based on these results a ratio of one
608 *D. gelechiidivoris* female to 100 first instar *T. absoluta* larvae are recommended to optimize
609 mass rearing of the parasitoid. The first hypothesis is therefore accepted.

610 **Recommendations for future research**

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

618 **9.1.2 Hypothesis 2: Two native parasitoids, *B. nigricans* and *Stenomesus* sp. near** 619 ***japonicus* present in Kenya, could establish in sub-Saharan Africa and can be** 620 **implemented as biocontrol agents in IPM programs for control of *T. absoluta* in** 621 **Africa.**

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

629 when both natural enemies are significantly less effective than the most effective species alone
630 (Bilu and Coll, 2007; Turner *et al.*, 2010).

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

640 **9.1.3 Hypothesis 3: The exotic *Dolichogenidea gelechiidivoris* can be used together**
641 **with the native *Stenomesus* sp. nr. *japonicus* and *Bracon nigricans* for control of**
642 ***Tuta absoluta*.**

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

646 The same level of pest control was achieved where *D. gelechiidivoris* and *B. nigricans* co-
647 occurred as well as where each species of parasitoid was individually present. The native
648 parasitoid *B. nigricans* did, however, reduce the population growth of the exotic *D.*
649 *gelechiidivoris*. Major parts of sub-Saharan Africa are suited for establishment of *B. nigricans*.
650 Hypothesis 3 is partly accepted since *D. gelechiidivoris* and *Stenomesus* sp. nr. *japonicus* can be
651 released together to achieve better control of *T. absoluta*. A reduction in population growth of *D.*
652 *gelechiidivoris* as a result of its co-existence with *B. nigricans*, was, however, reported in chapter
653 6 of this study, resulting in a partly rejection of hypothesis 3 pending future research.

654 **Recommendations for future research**

655 Results from this study reported on the performance and population growth of parasitoid species
656 that co-existed at one specific ratio. Future research should be elaborated to also include co-
657 existence of parasitoids at different ratios. Parasitism performance and population growth of *B.*
658 *nigricans* and *D. gelechiidivoris* in co-existence might also be influenced when multiple

659 lepidopteran pests from different families are present on the same crop or, alternatively, where
660 tomato is intercropped with another crop hosting an additional host of *B. nigricans*. For example,
661 *B. nigricans* acting as an ectoparasitic idiobiont species of *Spodoptera littoralis* (Boisduval)
662 (Lepidoptera: Noctuidae) (Becchimanzi *et al.*, 2017), and *D. gelechiidivoris*, as a koinobiont
663 solitary larval endoparasitoid of *T. absoluta*. Tomato planted together with a host plant of the
664 pest, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), may provide for an
665 alternative host of *B. nigricans* (Loni *et al.*, 2016). These aspects warrant future research. The
666 possibility of using the venom of an ectoparasitoid such as *B. nigricans* to develop new
667 biopesticides lines could also be explored and host finding by parasitoids may be enhanced by
668 pheromones and plant kairomones that attract females. Future research on these aspects may
669 improve parasitism by parasitoids.

670 **9.1.4 Hypothesis 4: The combined use of *Dolichogenidea gelechiidivoris* and**
671 ***Metarhizium anisopliae* ICIPE 20 has an additive effect in biocontrol of *T. absoluta*.**

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

688

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 gelechiidivoris* 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 *Stenomesus* 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.

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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.

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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.

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No tables or figures are allowed and there is a limit of 4 references.

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Please use single quotation marks, except where 'a quotation is "within" a quotation'. Please note that long quotations should be indented without quotation marks.

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Instructions to authors: Insects and Biology (MDPI)

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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.

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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](#).

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These sections should appear in all manuscript types

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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

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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.

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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.

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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.

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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.

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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.

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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.animaethics.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>

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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).

Arabidopsis 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

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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.

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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.

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Image files must not be manipulated or adjusted in any way that could lead to misinterpretation of the information provided by the original image.

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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

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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

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A competing interests statement is provided, even if the authors have no competing interests to declare

Journal policies detailed in this guide have been reviewed

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BEFORE YOU BEGIN

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Policy and ethics

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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.

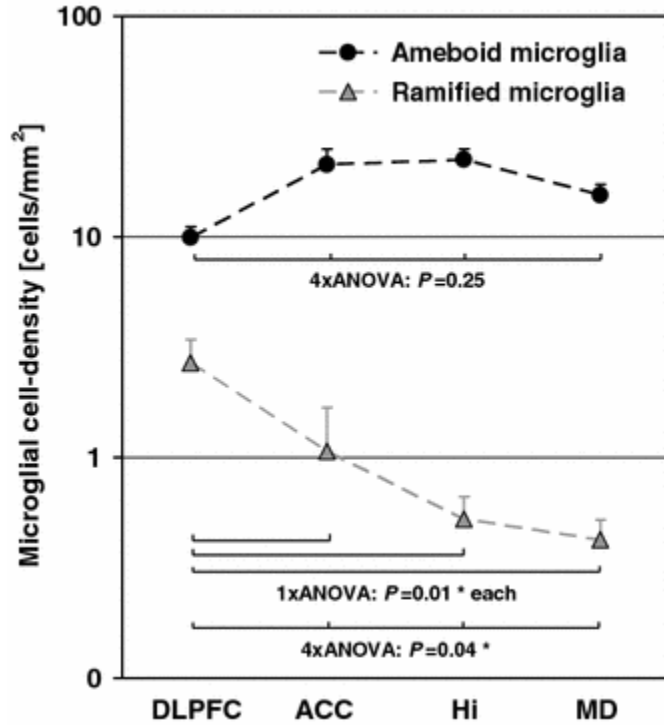
Indicate what graphics program was used to create the artwork.

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.

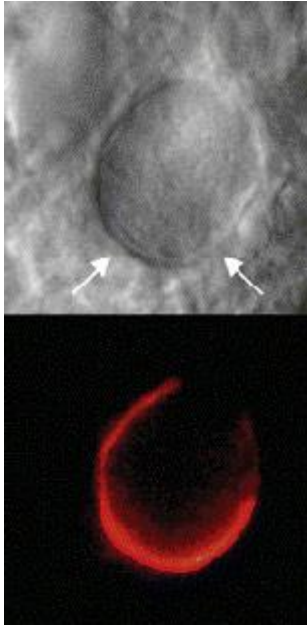
Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.

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

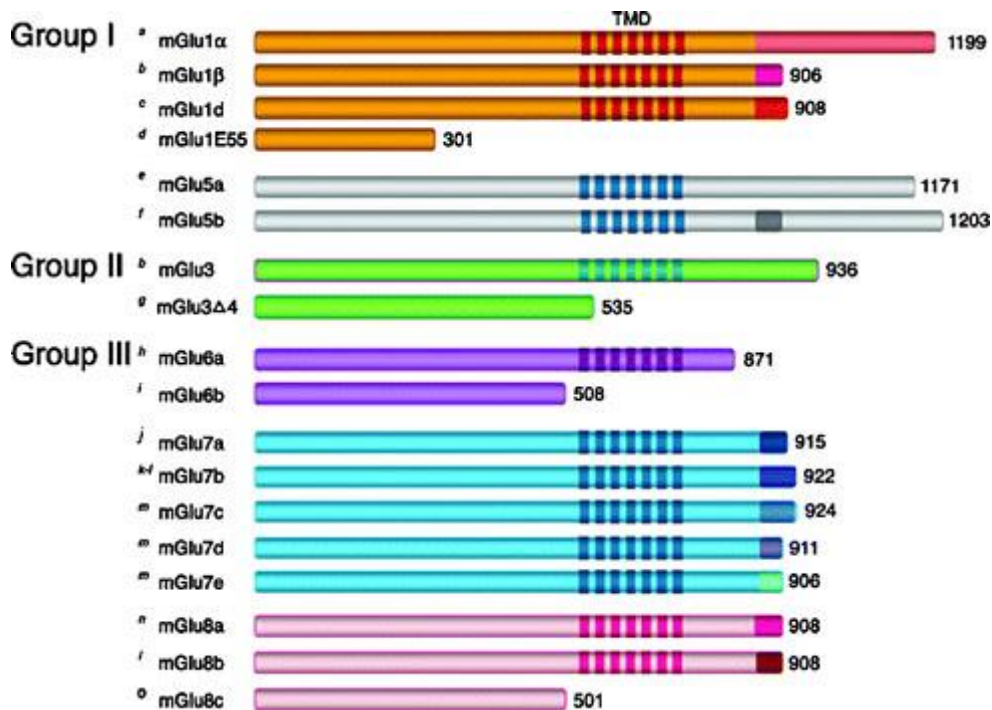


Definition: Photographs, drawings, or paintings with fine shading, etc.

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.

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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.

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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.

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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.

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- an expression of concern may be placed with the article
- or in severe cases retraction of the article may occur.

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The author's institution may be informed

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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

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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.

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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 aparición 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