Bio-ecological studies of *Tuta absoluta* in Sudan

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DEDICATION

This thesis is dedicated to the soul of my father and my aunt

May God bless them

DECLARATION BY THE CANDIDATE

This research project is entirely my original work and has not been accepted for the award of any other degree in any other university.

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Approval by supervisors

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ABSTRACT

Production of tomato crops in Sudan is threatened by the invasion of *Tuta absoluta* (Meyrick). This pest develops and disperses rapidly and it is also known to develop resistance to insecticides. Knowledge of its bio-ecology and genetic diversity in its newly invaded areas can be used to develop sustainable and effective IPM strategies. Results of field surveys conducted in the major tomato producing States of Sudan showed that the highest abundance of *T. absoluta* occurs in winter and early summer seasons (December-April). However, its population densities declined in the late summer season and remained very low during the fall season (July-October). In the field, *T. absoluta* was found infesting only tomato (*Solanum lycopersicum*), eggplant (*S. melongena*), black nightshade (*S. nigrum*) and gubbain (*S. dibium* Fr.). The *T. absoluta* highest infestation level was on tomato, followed by eggplant, gubbain and black nightshade. Six hymenopteran parasitoids and two predatory species were found at the surveyed sites. The parasitoid species are *Bracon nigircans* Szepligeti (Braconidae), *Bracon hebetor* (Say) (Braconidae), *Dolichogenidea appellator* (Telenga) (Braconidae), *Eupelmus* sp. (Eupelmidae), and two species belonging to Pteromalidae and Ichneumonidae families. The predators found were *Nesidiocoris tenuis* (Nesibug) (Miridae) and *Chrysoperla* sp. (Chrysopidae). Infestation of *T. absoluta* appears approximately two months after seeding of tomato and eggplant during the winter seasons in Gezira State. Pest incidence and infestation rate on tomato were significantly higher than on eggplant.

In no-choice tests under laboratory conditions, *T. absoluta* laid eggs on tomato, black nightshade, French bean (*Phaseolus vulgaris*) and pepper (*Capsicum annuum*) with a significantly higher number of eggs laid on tomato. In choice tests, females laid eggs only on tomato and black nightshade with a higher number of eggs laid on tomato. In the larval performance test, 88.5%, 68.5% and 3% of inoculated neonate larvae survived to the adult stage on tomato, black nightshade and French bean, respectively. Rearing host plants did not significantly affect female fecundity. Offspring (F1) of *T. absoluta* reared on tomato and French bean maintained a strong ovipositional preference towards tomato. However, offspring F1, F2 and F3 reared on nightshade showed a comparable preference to oviposit on both tomato and black nightshade.

The gregarious ectoparasitoid *B. nigricans* strongly accepted fourth instar *T. absoluta* larvae for oviposition followed by the third instar larvae in laboratory experiments. Fourth instar larvae also yielded a higher number of parasitoid offspring compared to third instar larvae. The performance of *D. appellator* in terms of the total number of offspring produced and female progeny were similar for second and third instar larvae of *T. absoluta*. The preimaginal developmental time for both parasitoid species did not vary with either instar of the larval host or sex of the parasitoid. *Bracon nigricans* adult longevity was similar for both sexes, while the longevity of *D. appellator* females was longer than for males. Molecular analysis showed a high genetic homogeneity in *T. absoluta* populations collected from Sudan, Uganda, Senegal and Tanzania.

Keywords: *Bracon nigricans*, *Dolichogenidea appellator,* natural enemies, parasitoids, oviposition, preference, *Tuta absoluta*

ABSTRACT

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CHAPTER 1

General Introduction

1.1. Introduction

Tomato (*Solanum lycopersicum* L.) (Solanaceae) is one of the most consumed and widely grown vegetables in the world, second to potato *Solanum tuberosum* (Desneux *et al.,* 2011; Nelson, 2008). The global production is approximately 177 million tons of fresh fruit produced on 4.8 million hectares (FAO, 2016). Tomato production generates a high income for farmers, it contributes to national economies and it creates employment for rural populations and other communities along the tomato value chain in Sudan (Ahmed, 1994). Tomato also provides vital vitamins and minerals which contribute to the improvement of human health (Bhowmik *et al.*, 2012). In Sudan, the most valuable vegetable crop is onion (*Allium cepa*), followed by tomato which is planted on 28% of the total area under vegetable production (Ahmed, 1994). The FAO (2016) reported a yield of 617 400 tons of tomatoes harvested from 46 746 hectares in Sudan with an average yield of 13.2 t/ha. The production is, however, hampered by insect pests, diseases and nematodes (Ahmed, 2000).

The South American tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) which originated from South America (Arnó and Gabarra, 2011; Zolf and Suffert, 2012), is the most destructive insect pest of tomato crops in both open fields and greenhouses (Hajj *et al.*, 2017). The pest was detected beyond the borders of South America for the first time in eastern Spain in 2006 (Urbaneja *et al.*, 2009). However, within three years, the pest quickly spreads across Europe, North Africa and the Middle East (Tropea Garzia *et al.*, 2012) and the pest is now present in almost all countries of Europe, Africa, the Middle East and India (EPPO, 2017). *Tuta absoluta* larvae damage tomato from the seedling stage to mature plants (Urbaneja *et al.,* 2012). Immediately after hatching, neonate larvae mine into the leaves, stems, apical buds and fruits where they feed and develop (Moreno *et al.*, 2012; Sánchez *et al.*, 2009; Urbaneja *et al.*, 2012; Zolf and Suffert, 2012). Without appropriate control measures, *T. absoluta* can cause tomato crop losses of up to 100% (Arnó and Gabarra, 2011; Gabarra *et al.*, 2014; Potting *et al.*, 2013; Urbaneja *et al.*, 2012).

In 2010, this pest was reported for the first time in Sudan in greenhouse tomato where it had caused severe losses in Khartoum State (Mohamed *et al.,* 2012). Since then it has spread to several States which include Al Gezira, North Kordofan, Red Sea, River Nile, White Nile, and Blue Nile (Mohamed *et al.*, 2015). *Tuta absoluta* has been reported to develop on cultivated plants from the solanaceous family, namely eggplant (*Solanum melongena* L.), sweet pepper (*S. muricatum* L.), potato (*S. tuberosum* L.), tobacco (*Nicotiana tabacum* L.), cape gooseberry (*Physalus peruviana* L.) as well as on uncultivated Solanaceae such as nightshade (*S. nigrum* L.) and other plants naturally available *viz*. devil's apple (*Datura stramonium* L.) (Desneux *et al.,* 2010; Mohamed *et al.,* 2012). In Sudan, tomato yield losses of 53% and 80% were reported in greenhouses and open fields, respectively. *Tuta absoluta* damage of up to 50% was also reported on potato foliage (Mohamed *et al.,* 2012).

In South America, insecticides of different classes have been used intensively for more than four decades to control this pest. Consequently, development of resistance by *T. absoluta* to various insecticides has been reported (Siqueira *et al.*, 2001; Lietti *et al.*, 2005). Chemical control in Europe has resulted in resistant populations (Haddi *et al.,* 2012; Roditakis *et al.,* 2013). The use of synthetic insecticides also negatively affect the natural enemies and pollinators (Campos *et al*., 2017) as well as human health (Desneux *et al.*, 2010). This scenario can also happen in Africa if *T. absoluta* will be mainly controlled with synthetic insecticides. The bio-ecology and genetic diversity of this pest in its invaded range should therefore be studied and Integrated Pest Management (IPM) strategies should be developed which are environmentally sustainable, affordable and effective.

1.2. Problem Statement

In Sudan, tomato production is constrained by various pests such as the African bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae), aphids *Aphis gossypii* (Homoptera: Aphididae), the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) and the leafminers *Liriomyza* spp. (Diptera: Agromyzidae) (Ahmed, 2000). Added to this pest complex is the South American micro lepidopteran, *T. absoluta* which invaded Sudan in 2010, and causes a substantial reduction in tomato yields since then (Mohamed *et al.,* 2012). In areas where this pest is present, production costs increased due to *T. absoluta* management, as well as the reliance on pesticides for its control (Desneux *et al.,* 2011; Speranza and Sannino 2012; Urbaneja *et al.,* 2012). Extensive and inappropriate use of pesticides may also affect the natural enemies and may lead to resistance problems, as occurred in the area of origin of this pest (Desneux *et al.,* 2011) and Europe (Roditakis *et al.,* 2013). Therefore, understanding the bio-ecology and population genetic structure of the pest in its invaded area is crucial to the development of sustainable, affordable and effective control strategies.

1.3. General objective

The objective of this study was to investigate the bio-ecology and genetic diversity of *T. absoluta* for the development of sustainable and effective IPM strategies in Sudan.

1.3.1. Specific objectives

The specific objectives of the study were:

a) To determine the geographic abundance/distribution, host plant range and damage level of *T. absoluta.*
b) To study the population incidence of *T. absoluta* on tomato (*S. lycopersicum*) and eggplant (*S. melongena*) crops.
c) To investigate the oviposition preference and larval performance of *T. absoluta* on selected host plants.
d) To characterize the indigenous natural enemies and their performance on *T. absoluta*.
e) To determine the phylogeography of *T. absoluta* populations through DNA barcoding.

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CHAPTER 2

Literature Review

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is commonly known as the tomato leafminer, tomato borer, South American tomato moth and South American tomato pinworm. The genus of this species had been changed a few times over the years. It was first described by Meyrick as *Phthorimaea absoluta* (Meyrick, 1917), followed by *Gnorimoschema absoluta* (Clarke, 1962), *Scrobipalpula absoluta* (Povolny, 1964), *Scrobipalpuloides absoluta* (Povolny, 1987) and finally as *Tuta absoluta* (Povolny, 1994) (EPPO, 2005).

2.1. Biology

Tuta absoluta moths (Figure 2.1) are 5 - 7mm long with filiform antennae and a wing-span of 8 - 10 mm. The females are generally bigger and live longer than the males (Desneux *et al.,* 2010). Male as well as female moths mate multiple times during their lifespan (Desneux *et al*., 2010; Tropea Garzia *et al.,* 2012; USA-APHIS, 2011). Adult females lay eggs separately or in random groups (2 - 5 eggs) on both sides of the leaves, apical buds or green fruits. The leaves are the most preferred oviposition substrate and a single female can produce up to 260 eggs (Derbalah *et al.,* 2012; Sannino and Espinosa, 2010; Toševski *et al.,* 2011). *Tuta absoulta* eggs are oval and vary in colour from creamy-white to yellow (Figure 2.1), darken during its development and then turn to almost black before hatching (Imenes *et al.,* 1990; Sannino and Espinosa, 2010). Under optimal conditions, eggs hatch in 4 - 5 days, while the larval instars require 13 - 15 days to pupate (EPPO, 2005). There are four larval instars, with the first instar larvae approximately 0.9 mm long, and creamy in colour with dark heads which become greenish to light pink in the second to fourth instar (Figure 2.1) (EPPO, 2005). Immediately after hatching, neonate larvae tunnel into the plant parts and continue to feed inside (Cuthbertson *et al.,* 2013). Third instar larvae move to new fresh plant parts (Tropea Garzia *et al.,* 2012). Fully-grown fourth instar larvae drop to the soil or hide in curled leaves to pupate (Figure 2.1). The duration of the pupal stage is usually 9 - 11days (Desneux *et al.,* 2010; Tropea Garzia *et al.,* 2012). *Tuta absoluta* can complete 7 - 8 generations per year in Mediterranean conditions (Cocco *et al.,* 2015).

Figure 2.1: *Tuta absoluta* (A) eggs, (B) fourth instar larva, (C) pupa and (D) adult

2.2. Geographical distribution

Tuta absoluta was first described in Peru by Meyrick in 1917, but it has been recognized as a serious pest of tomato, *Solanum lycopersicum* L. (Solanales: Solanaceae) during the 1960’s in Peru, Equador, Chile, Colombia and Argentina (Guedes and Picanco, 2012; Lietti *et al.*, 2005; Siqueira *et al.*, 2001) and later in Bolivia, Uruguay, Venezuela and Brazil (Guedes and Picanco, 2012). In Europe, *T. absoluta* was included into the EPPO (A1) list in 2004 as a quarantine pest and consignments of tomato fruits from countries where the moth occurred should be free of the pest (EPPO, 2005). Despite these regulations, *T. absoluta* was accidently introduced into Spain by the end of 2006 (Urbaneja *et al.*, 2009). Within 10 years, the pest has spread to almost all countries in Europe, Africa, the Middle East and India (EPPO, 2017) (Figure 2.2).

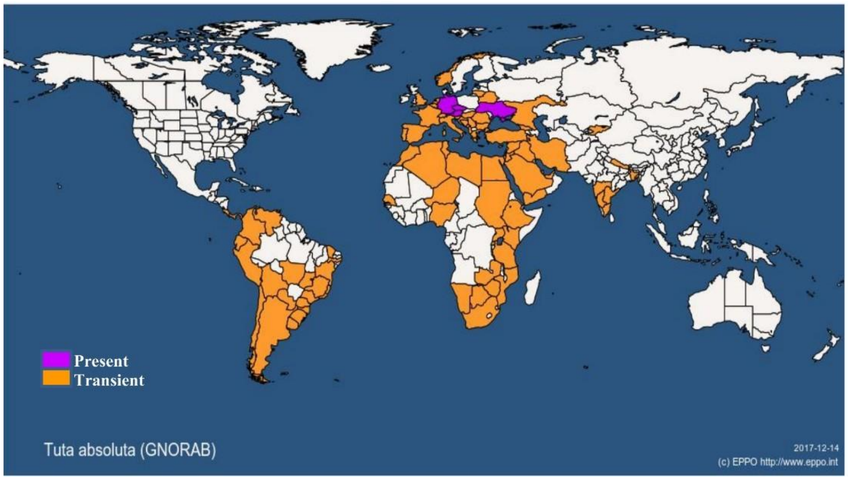

Figure 2.2: Geographic distribution of *Tuta absoluta* in South America, Europe, Africa and Asia. (EPPO, 2017).

2.3. Economic importance

Tuta absoluta is a major constraint to tomato production in both open fields and greenhouses in South America, Europe, the Middle East and Africa (Desneux *et al.*, 2010; Potting *et al.*, 2013). The pest also causes indirect loss through the loss of lucrative quarantine sensitive markets (Zolf and Suffert, 2012).

Crop protection costs also increase if *T. absoluta* is to be controlled (Guedes and Picanço, 2012; Harbi *et al.*, 2012). In the absence of effective control measures, up to 100% yield loss (Figure 2.3A and B) can be reached (Desneux *et al.*, 2010; Potting *et al.*, 2013). The invasion of *T. absoluta* into Tanzania caused a 50% decline in Tanzanian tomato production (Zekeya *et al.*, 2017). Damage by *T. absoluta* in protected tomato crops has been reported to be ranging from 11 to 43% during the first two years following its introduction into Tunisia (Abbes and Chermiti, 2014). Furthermore, 50% of tomato fields were infested by *T. absoluta* in 2013 in Senegal (Brévault *et al.*, 2014).

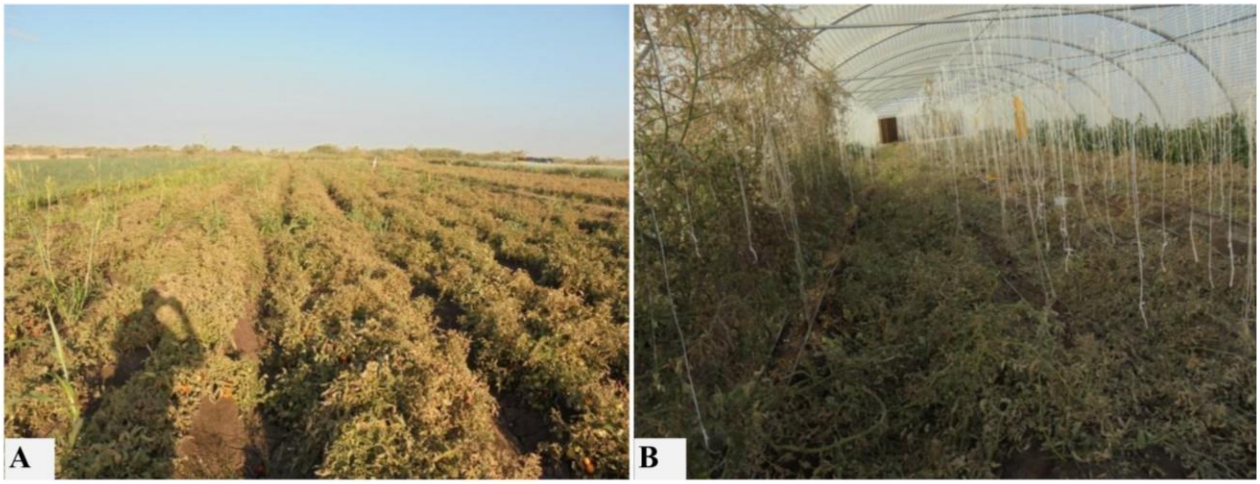

Figure 2.3: *Tuta absoluta* feeding can result in 100% loss of a tomato crops in (A) open fields and (B) greenhouses.

2.4. Host plants

Although tomato is the most preferred and suitable host plant for *T. absoluta* oviposition and development (Proffit *et al.*, 2011), the pest also attacks other Solanaceous vegetables such as eggplant (*Solanum melongena* L.), potato (*S. tuberosum* L.), and pepino (*S. muricatum* Aiton) (Arnó and Gabarra, 2011; Portakaldali *et al.*, 2013; Siqueira *et al.*, 2001; Zlof and Suffert, 2012). Wild Solanaceous plants such as *S. nigrum, S. elaeagnifolium, S. puberulum, Datura stramonium, D. ferox* and *Nicotiana glauca* were also reported as host plants of this pest (EPPO, 2005). Other wild host plants in the Convolvulaceae and Chenopodiaceae (Portakaldali *et al.*, 2013) as well as plants in the Asteraceae and Amaranthaceae families have also been reported (Mohamed *et al.,* 2015). *T. absoluta* also attacks Cape gooseberry (*Physalis peruviana* L.) (Solanaceae) (Tropea Garzia, 2009), French beans (*Phaseolus vulgaris* L.) (Fabaceae) (Speranza and Sannino, 2012; Mohamed *et al*., 2015), (*Citrullus lanatus*) (Cucurbitaceae), faba bean (*Vicia faba* L.) (Fabaceae) and alfalfa (*Medicago sativa* L.) (Fabaceae) (Mohamed *et al.,* 2015).

2.5. Damage caused by *Tuta absoluta*

Tuta absoluta infests all the above ground tomato plant parts (Braham *et al.*, 2012; Urbaneja *et al.*, 2012), as well as all the growth stages (Desneux *et al.*, 2010; Tropea Garzia *et al.*, 2012; Urbaneją *et al.*, 2012). Leaves are damaged by larvae tunnelling into the mesophyll (Figure 2.4A), thus reducing the plant's photosynthetic ability which results in lower tomato yields (Desneux *et al.*, 2010). Galleries in stems alter the general development of plants (Desneux *et al.*, 2010). Fruits are also attacked and secondary pathogen infections lead to fruit rot (Figure 2.4B) (USA-APHIS, 2011). *Tuta absoluta* infestation during the early plant stages cause crop damage and even kill the young plants (Tropea Garzia *et al.*, 2012). Direct feeding of the pest on the plants' growing tips can also halt plant development (Desneux *et al.*, 2010).

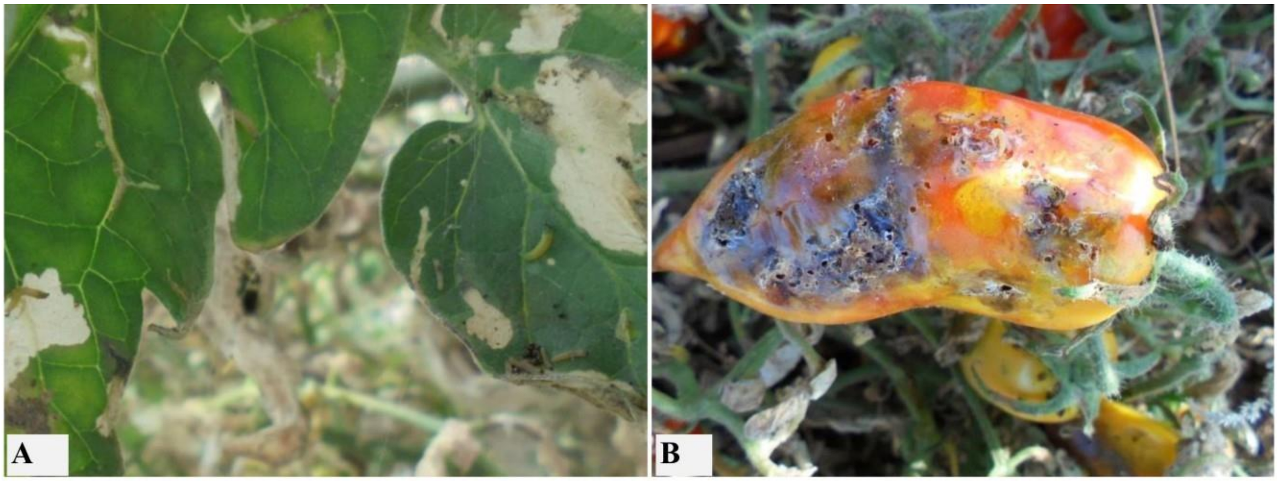

Figure 2.4: *Tuta absoluta* feeding (A) on leaves and (B) on fruits with secondary pathogen infection.

2.6. Control Measures

2.6.1. Monitoring

Sex pheromones have been developed for monitoring and mass trapping of *T. absoluta* in open fields and greenhouses (Caparros Megido *et al.,* 2013; Zlof and Suffert, 2012). Open shaped traps such as CICA-R and delta traps baited with 100µg of the synthetic sex pheromone (3E, 82, 11Z) -3, 8, 11- tetradecatriently acetate (TDTA) are used for *T. absoluta* adult males monitoring (Ferrara *et al.*, 2001). Traps should be placed in and around the borders of the open field or greenhouse (Al-Zaidi, 2009) at a level of 0.2 - 0.6 m according to the growth stage of the crop (Ferrara *et al.*, 2001). Treatments based on male captures is affected by various factors, such as population density and trap shape, position and pheromone types (Caparros Megido *et al.,* 2013). However, in Brazil a threshold of 45 ± 19.5 moths/trap/ day is recommend (Benvenga *et al.,* 2007), but in Tunisia a threshold of 30 - 50 moths/trap/week is recommended as a general threshold level (Abbes *et al.*, 2012a,b). *Tuta absoluta* can also be monitored by foliage examination for the presence of eggs and larvae. Sampling of expanded tomato leaves in the medium parts of the canopy are the best for estimation of larval population and mines (Gomide *et al.,* 2001). To estimate the number of eggs, sampling of the expanded tomato leaves from the apical plant parts is recommended (Gomide *et al.*, 2001). Cocco *et al.* (2015b) recommended sampling of 14 - 20 median leaves for a pest management decision to avoid fruit damage higher than 1% in greenhouse tomatoes.

2.6.2. Chemical control

In South America, *T. absoluta* has been primarily managed with insecticides (Moore, 1983; Lietti *et al.*, 2005). Organophosphates were commonly used, but were gradually replaced by pyrethroids in the 1970’s (Lietti *et al.*, 2005). During the 1980s’ Cartap hydrochloride (thiocarbamate), alternated with pyrethroids, and thiocyclam were widely used in *T. absoluta* control. These were again replaced with insecticides of various novel chemical classes such as abamectin, acylurea, insect growth regulators (IGRs), spinosad, tebufenozide and chlorfenapyr during the 1990’s (Desneux *et al.,* 2010; Lietti *et al.*, 2005). Nevertheless several cases of *T. absoluta* resistance to different classes of insecticides have been reported (Arnó and Gabarra, 2011; Lietti *et al.*, 2005). A contributing factor is the high reproduction rate of this pest resulting in a need for several treatments per growing season (Valchev *et al.,* 2013). *Tuta absoluta* has also already developed resistance to cypermethrin (Roditakis *et al.,* 2013) and diamides in Greece and Italy (Roditakis *et al.*, 2015), abamectin (Siqueira *et al.,* 2001), bifenthrin, triflumuron and teflubenzuro in Brazil (Gontijo *et al.*, 2013).

2.6.3. Biological control

A diversity of natural enemies are known to be associated with various stages of *T. absoluta*. These include predators, parasitoids, entomopathogenic nematodes and bacteria (Desneux *et al.,* 2010).

2.6.3.1. Parasitoids

Numerous hymenopteran parasitoids in the families Eulophidae, Braconidae and Trichogrammatidae were reported to attack *T. absoluta* in its area of origin (Desneux *et al.,* 2010; Luna *et al.,* 2010; Luna *et al.,* 2015) as well as in the new invaded areas of Europe and Asia (Doġanlar and Yiğit, 2011; Gabarra *et al.,* 2014; Urbaneja *et al.,* 2012; Zappalà *et al.,* 2012). A summary of parasitoids of *T. absoluta* reported worldwide is presented in table 2.1. Zappalà *et al.* (2013) reported 55 species of parasitoids associated with *T. absoluta* in the Mediterranean basin, and 49 parasitoid species in South America.

The most important parasitoids of *T. absoluta* in the Mediterranean basin are ectoparasitoid wasps of the family Eulophidae, including species from the genera *Necremnus, Stenomesius,* and *Neochrysocharis* (Gabarra *et al.,* 2014; Urbaneja *et al.,* 2012). Adult wasps prefer certain *T. absoluta* larval instars for oviposition and other instars for feeding, which results in high larval mortality rates (Calvo *et al.,* 2013; Ferracini *et al.,* 2012). *Bracon nigricans* Szépligeti (Hymenoptera: Braconidae) is an example of a larval ectoparasitoid which is widely distributed in European countries (Biondi *et al*., 2013; Urbaneja *et al.,* 2012) and the Middle East (Al-Jboory *et al.,* 2012). This parasitoid suppresses the larval population, particularly the third and fourth instar larvae through parasitism and host-feeding activity (Biondi *et al.,* 2013). Another braconid, *Pseudapantelea dingus* (Muesebeck) (Hymenoptera: Braconidae) studied by Luna *et al.* (2007) provided 30% parasitism of *T. absoluta* larvae after 24 hours exposure in a laboratory study.

Cabello *et al.* (2012) reported 85% reduction in *T. absoluta* infestation in greenhouse tomato with biweekly releases of *Trichogramma achaeae* (Nagaraja and Nagarkatti) (Hymenoptera: Trichogrammatidae) eight days after tomato transplanting at a rate of 50 adults/m^2, for four consecutive weeks. Under high infestation levels, only one inoculative release of 100 adults/m^2 is sufficient to achieve parasitism levels of up to 91% (Cabello *et al*., 2012). In contrast, a later study by Chailleux *et al.* (2013) reported low parasitism rates and poor control of *T. absoluta* by *T. achaeae*. Their efficacy did, however, increase when released in combination with the predator *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae).

2.6.3.2. Predators

In South America, approximately 50 species of *T. absoluta* predators belonging to different orders and families were reported by Desneux *et al.* (2010). In a study by Miranda *et al.*(1998), larval predators were reported to consume more than 79% of *T. absoluta* larvae. The mirid, *Dicyphus errans* (Wollf) (Hemiptera: Miridae) actively preys on *T. absoluta* eggs and first instar larvae. *Dicyphus errans* females consume a significant number of *T. absoluta* eggs and also prey on first instar *T. absoluta* larvae which are sufficient for completion of their development (Ingegno *et al.*, 2013).

The predators *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) and *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) are the most promising biological control agents of *T. absoluta* in the Mediterranean basin (Arnó and Gabarra, 2011; Desneux *et al.,* 2010). They actively prey on eggs and all larval stages of *T. absoluta,* but first-instar larvae are the most preferred (Urbaneja *et al.,* 2009).

Mollá *et al.* (2011) reported a combination of *B. thuringiensis* applications and *N. tenuis* releases to be highly effective in reducing the damage caused by *T. absoluta* with no fruit damage and a 97% reduction in leaf damage. In contrast to this finding, Calvo *et al.* (2012) reported that the effectiveness of *N. tenuis* was not increased by additional biological agents such as *T. achaeae* and *B. thuringiensis* which was reported to significantly reduce *T. absoluta* populations in greenhouse tomato. *Nesidiocoris tenuis* and *M. pygmaeus* did, however, fail to achieve acceptable *T. absoluta* control levels when released under field conditions at rate of 2 bugs/m^{2}per species (Nannini *et al.,* 2012).

2.6.3.3. Bacteria and nematode entomopathogens

Several entomopathogens have been documented to attack *T. absoluta*, with the bacterium, *B. thuringiensis* Berlinger var. *kurstaki* (Bt-formulated insecticide) showing high efficacy against all larval instars of the pest especially towards the first instar larvae (Gonzalez-Cabrera *et al.,* 2011). Hafsi *et al.* (2012) also reported *B. thuringiensis* var. *kurstaki* to be highly effective in controlling *T. absoluta* with up to 73% mortality rate. Integration of the Bt-based biopesticides with other biological control agents such as predators or parasitoids, have a synergistic effect. It results in higher mortality of *T. absoluta* eggs, thereby reducing the frequency of application of Bt-formulated biopesticides as well as synthetic insecticides (Gonzalez-Cabrera *et al.,* 2011).

The entomopathogenic nematodes, *Steinernema carpocapsae* (Steinernematidae), *S. feltiae* (Steinernematidae) and *Heterorhabditis bacteriophora* (Heterorhabditidae) are highly effective in controlling *T. absoluta* larvae and pupae (García-del-Pino *et al.,* 2013). Application of these nematode species resulted in parasitism of up to 95% of *T. absoluta* larvae and 10% of the pupae, in a greenhouse experiment (Batalla-Carrera *et al.*, 2010). Similar results were obtained in another greenhouse experiment with soil application of *S. carpocapsae* and *H. bacteriophora* (García-del-Pino *et al.*, 2013). Up to 100% mortality of the fourth instar larvae was achieved when these larvae dropped to and entering the soil to pupate. A soil application of *S. feltiae* resulted in 53% mortality of the final instar larvae on the soil (García-del-Pino *et al.*, 2013).

Table 2.1: *Tuta absoluta* parasitoids reported globally as well as the life stage targeted.

Family	Genus and species	Life stage targeted	Country	Reference
Braconidae				
	Agathis fuscipennis (Zetterstedt)	Larvae	Italy	Loni *et al.* (2011)
	Agathis sp.	Larvae	Argentina	Desneux *et al.* (2010)
	Apanteles dignus	Larvae	Colombia	Desneux *et al.* (2010)
	Apanteles gelechiidivoris	Larvae	Chile, Colombia and Peru	Desneux *et al.* (2010)
	Apanteles sp.	Larvae and pupae	Colombia and Spain	Desneux *et al.* (2010), Gabarra *et al.* (2014)
	Bracon (Habrobracon) didemie Beyarslan	Larvae (4th instar)	Turkey	Doğanlar and Yiğit (2011)
	Bracon (Habrobracon) hebetor Say	Larvae (4th instar)	Sudan, and Turkey	Doġanlar and Yiğit (2011), Mahmoud (2013)
	Bracon lucileae	Larvae	Argentina	Desneux *et al.* (2010)
	Bracon lulensis	Larvae	Argentina	Desneux *et al.* (2010)
	Bracon (*Habrobracon*) sp. nr. *nigricans* (Szépligeti, 1901)	Larvae (2nd, 3rd and 4th instar)	Italy, Jordan and Spain, Sudan	Al-Jboory *et al.* (2012), Biondi *et al.* (2013a), Gabarra *et al.* (2014), Mahmoud (2013), Zappalà *et al.* (2012)
	Bracon osculator	Larvae	Italy	Zappalà *et al.* (2012)
	Bracon sp.	Larvae (4th)	Algeria, Brazil, Colombia and Tunisia	Desneux *et al.* (2010), Boualem *et al.* (2012), Abbes *et al.* (2013)
	Bracon tutus	Larvae	Argentina	Desneux *et al.* (2010)
	Chelonus sp.	Larvae	Argentina, Brazil, and Spain	Desneux *et al.* (2010), Gabarra *et al.* (2014)
	Choeras semele (Nixon 1965)	Not specified	Spain	Gabarra *et al.* (2014)
	Cotesia sp.	Not specified	Spain	Gabarra *et al.* (2014)
	Diolcogaster sp.	Not specified	Spain	Gabarra *et al.* (2014)
	Earinus sp.	Larvae	Argentina and Brazil	Desneux *et al.* (2010)
	Goniozus nigrifemur	Larvae	Brazil	Desneux *et al.* (2010)
	Orgilus sp.	Larvae	Argentina	Desneux *et al.* (2010)
	Pseudapanteles dingus	Larvae (3rd instar)	Argentina and Chile	Desneux *et al.* (2010), Luna *et al.* (2010), Luna *et al.* (2015), Savino *et al.* (2016)
Chalcididae				

Table 2.1. Continued

Family	**Genus and species**	**Life stage targeted**	**Country**	**Reference**
	Brachymeria secundaria (Ruschka)	Larvae	Turkey	Doġanlar and Yiğit (2011)
	Conura sp. (syn Spilochalcis sp.)	Pupae	Argentina and Brazil	Desneux *et al.* (2010)
	Hockeria unicolor (Walker, 1834)	Larvae	Turkey and Spain	Doġanlar and Yiğit (2011), Gabarra *et al.* (2014)
	Invreia sp.	Pupae	Colombia	Desneux *et al.* (2010)
Echneumonidae				
	Hyposoter didymator	Not specified	Algeria	Boualem *et al.* (2012)
Elasmidae				
	Elasmus sp.	Larvae and pupae	Colombia and Italy	Desneux *et al.* (2010), Zappalà *et al.* (2012)
Encyrtidae				
	Arrhenophagus sp.	Egg	Brazil	Desneux *et al.* (2010)
	Copidosoma desantisi	Egg	Chile	Desneux *et al.* (2010)
	Copidosoma koehleri	Egg	Chile	Desneux *et al.* (2010)
	Copidosoma sp.	Egg	Argentina	Desneux *et al.* (2010)
Eulophidae				
	Baryscapus bruchophagi (Gahan)	Larvae	Turkey	Doġanlar and Yiğit (2011)
	Chrysocharis sp.	Larvae	Italy	Zappalà *et al.* (2012)
	Chrysonotomyia sp.	Larvae	Venezuela	Desneux *et al.* (2010)
	Closterocerus clarus (Szelenyi)	Larvae (1st instar)	Turkey	Doġanlar and Yiğit (2011)
	Clostrocerus formosus	Larvae	Argentina	Desneux *et al.* (2010)
	Dineulophus phthormiaeae	Larvae (3rd instar)	Argentina and Chile	Desneux *et al.* (2010); Luna *et al.* (2010); Luna *et al.* (2015), Savino *et al.* (2016)
	Diglyphus crassinervis	Not specified	Spain	Gabarra *et al.* (2014)
	Diglyphus isaea (Walker, 1838)	Not specified	Algeria and Spain	Boualem *et al.* (2012), Gabarra *et al.* (2014)
	Dineulophus phthorimaeae	Larvae (3rd instar)	Argentina	Luna *et al.* (2015)
	Elachertus sp.	Larvae	Italy	Zappalà *et al.* (2012)
	Elachertus inunctus	Larvae	Italy	Zappalà *et al.* (2012)

Table 2.1. Continued

Family	**Genus and species**	**Life stage targeted**	**Country**	**Reference**
	Elasmus phthorimaeae	Not specified	Spain	Gabarra *et al.* (2014)
	Horismenus sp.	Larvae and pupae	Brazil	Desneux *et al.* (2010)
	Necremnus cosmopterix (Ribeset Bernardo)	Larvae	Turkey	Bayram *et al.* (2016)
	Necremnus near *tidius* (Walker)	Larvae (1st and 2nd instar)	Italy	Ferracini *et al.* (2012); Zappalà *et al.* (2012)
	Necremnus sp.	Larvae	Italy and Spain	Gabarra *et al.* (2014), Zappalà *et al.* (2012)
			Italy	Zappalà *et al.* (2012)
	Necremnus sp. nr. artynes (Walker, 1839)	Larvae (1st, 2nd and 3rd instar)	Algeria, Italy, Spain and Tunisia	Boualem *et al.* (2012), Ferracini *et al.* (2012), Gabarra *et al.* (2014), Abbes *et al.* (2013), Zappalà *et al.* (2012)
	Neochrysocharis formosus (Westwood, 1833)	Larvae (1st, 2nd and 3rd instar)	Italy, Spain, Argentina and Turkey	Desneux *et al.* (2010), Zappalà *et al.* (2012), Gabarra *et al.* (2014), Sohrabi *et al.* (2014)
	Pnigalio cristatus	Larvae	Italy and Spain	Gabarra *et al.* (2014), Zappalà *et al.* (2012)
	Pnigalio incompletes	Larvae	Italy	Zappalà *et al.* (2012)
	Pnigalio soemius	Larvae	Italy and Spain	Gabarra *et al.* (2014), Zappalà *et al.* (2012)
	Ratzeburgiola christatus (Ratzeburg)	Larvae	Turkey	Doğanlar and Yiğit (2011)
	Ratzeburgiola incompleta Boucek	Larvae	Turkey	Doğanlar and Yiğit (2011)
	Retisympiesis phthorimaea	Larvae	Chile	Desneux *et al.* (2010)
	Sympiesis sp.	Larvae	Italy and Colombia	Zappalà *et al.* (2012)
	Tetrastichus sp.	Larvae	Colombia	Desneux *et al.* (2010)
	Zagrammosoma sp.	Larvae	Venezuela	Desneux *et al.* (2010)
Eupelmidae				
	Anastatus sp.	Egg	Colombia	Desneux *et al.* (2010)
Ichneumonidae				
	Campoplex haywardi	Larvae	Argentina	Desneux *et al.* (2010)
	Cryptinae gen. sp.	Larvae	Italy	Zappalà *et al.* (2012)
	Diadegma pulchripes	Larvae	Italy	Zappalà *et al.* (2012)
	Diadegma sp.	Larvae	Brazil, Colombia and Italy	Desneux *et al.* (2010), Zappalà *et al.* (2012)

Table 2.1. Continued

Family	Genus and species	Life stage targeted	Country	Reference
	Pristomerus sp.	Larvae	Colombia	Desneux *et al.* (2010)
	Temelucha anatolica (Sedivy)		Spain	Gabarra *et al.* (2014)
	Temelucha sp.	Larvae	Argentina and Colombia	Desneux *et al.* (2010)
	Zoophthorus macrops Bordera & Horstmann, 1995	Not soecified	Spain	Gabarra *et al.* (2014)
Pteromalidae				
	Halticoptera aenea	Larvae	Italy	Zappalà *et al.* (2012)
	Pteromalus intermedius (Walker, 1834)	Larvae	Turkey	Doġanlar and Yiğit (2011),
	Pteromalus semotus (Walker, 1834)	Not specified	Spain	Gabarra *et al.* (2014)
Tachinidae				
	Archytas sp.	Larvae	Brazil	Desneux *et al.* (2010)
	Elfia sp.	Larvae	Colombia	Desneux *et al.* (2010)
Trichogramatidae				
	Trichogramma achaeae (Nagaraja & Nagarkatti, 1969)	Egg	Spain and Canary islands	Cabello *et al.* (2012), Chailleux *et al.* (2013)
	T. bactrae	Egg	Chile	Desneux *et al.* (2010)
	T. dendrolimi	Egg	Chile	Desneux *et al.* (2010)
	T. exiguum	Egg	Colombia	Desneux *et al.* (2010)
	T. euproctidis	Egg	Switzerland and Egypt	Chailleux *et al.* (2013), El-Arnaouty *et al.* (2014)
	T. fasciatum	Egg	Argentina	Desneux *et al.* (2010)
	T. lopezandinensis	Egg	Peru	Desneux *et al.* (2010)
	T. minutum	Egg	Chile and Peru	Desneux *et al.* (2010)
	T. nerudai	Egg	Argentina and Chile	Desneux *et al.* (2010)
	T. pintoi	Egg	Peru	Desneux *et al.* (2010)
	T. pretiosum	Egg	Argentina, Brazil, Chile, Colombia, Paraguay, Venezuela, and France	Chailleux *et al.* (2013), Desneux *et al.* (2010), Faria *et al.* (2008)
	T. rojasi	Egg	Argentina	Desneux *et al.* (2010)
	T. telengai	Egg	Chile	Desneux *et al.* (2010)

2.6.4. Plant extracts

The triterpenoid (azadirachtin) extracted from Neem (*Azadirachta indica*) (Meliaceae) plants delays the development of *T. absoluta* causing larval mortality (Tomé *et al.,* 2012). Only 3.5% of the larvae were able to pupate after the triterpenoid application (Tomé *et al.*, 2012). Azadirachtin also affected larval walking (Tomé *et al.,* 2012) and can be used as a preventive or a curative measure when the *T. absoluta* populations are low.

Under greenhouse conditions, improved suppression of *T. absoluta* was obtained with *Artemisia cina* (Asteraceae) extracts compared to the synthetic insecticides imidacloprid and indoxacarb as well as their mixtures with the *A. cina* extract (Derbalah *et al.*, 2012). Hexane and ethanol extracts of *Acmella olevracea* (Asteraceae) can also be used for control of *T. absoluta* in both organic and conventional tomato crops (Moreno *et al.,* 2012).

2.6.5. *Cultural practices and resistant varieties*

Cultural practices such as destruction of alternative host plants have been used to prevent the build-up of T. *absoluta* populations (USA-APHIS, 2011). Removal of infested tomato plants, especially the upper parts, at least one month after seedling transplanting, could maintain the infestation rate of leaves at relatively low levels for the following three weeks (Abbes *et al.,* 2012a).

The use of adequate fertilizers and irrigation, crop rotation with non Solanaceous crop species and ploughing are cultural control methods which decrease *T. absoluta* populations (USA-APHIS, 2011). Wild *Lycopersicum* spp. (Solanaceae) are in general more resistant to arthropod pests than *L. esculentum*, due to the presence of glandular trichomes on the wild species which hamper oviposition and development of pests (Simmons and Gurr, 2005). In addition, the antixenotic 2-tridecanone (2-TD) released by the wild variety *L. hirsutum* f. *glabratum* negatively affects *T. absoluta* development (Leite *et al.,* 1999). The antixenosis resulting from the tricosane produced by the two tomato accessions HGB-674 and HGB-1497, also significantly reduced the infestations of *T. absoluta* on tomato leaves (Oliveria *et al.,* 2009). In Iran, among 11 tomato varieties tested for *T. absoluta* resistance, three varieties, *viz*. Atabay, Cluse and Servent were found to be resistant based on the number of damaged leaves, active mines and damaged terminal buds (Gharekhani and Salek-Ebrahimi, 2013).

2.6.6. Mass trapping and mating disruption

Mass trapping of *T. absoluta* males with sex pheromones can be an effective and economic management tool (Chermiti and Abbes, 2012; Junco and Herrero, 2008). In Tunisian open field tomatoes, adults and larvae of *T. absoluta* populations were significantly reduced by placing 32 sex pheromone water traps/ha^{-1} (Chermiti and Abbes, 2012). However, in greenhouse tomatoes, pest population has been significantly reduced by placing one trap/500 m^2 as a part of an integrated pest management program (Stoltman *et al.*, 2010). In Argentinean tomato crops, leaf infestation by *T. absoluta* was significantly reduced by using 48 homemade traps (translucent plastic cylinders, 9×11 cm, with a 4.5-×6.5-cm opening)/ha baited with sex pheromones, when compared to control with conventional insecticides (Lobos *et al.*, 2013). Delta traps and CICA-R trap types baited with 100µg *T. absoluta* sex pheromone are also successfully used for *T. absoluta* adult males mating disruption (Ferrara *et al.*, 2001). An effective mating disruption method for tomato greenhouses are reported by Vacas *et al.* (2011) and Cocco *et al.* (2013) with 30 – 60 g of pheromone used /ha to reduce the percentage of damaged fruits. Mass trapping programs must be used in an area wide approach for successful suppression of the pest (Chermiti and Abbes, 2012; Guedes and Picanço, 2012). Traps should also be deployed early in the plant growth cycle, when *T. absoluta* populations are present at low densities. The parthenogenetic reproduction of *T. absoluta* may negatively affect the mass trapping and mating disruption methods and should be investigated (Caparros Megido *et al.*, 2012; Abbes and Chermiti, 2014).

2.7. DNA Barcoding

Currently, taxonomists use DNA barcoding to identify, compare and examine the relationship within populations of the same species or groups and also use it to study the evolution of insects (Hebert *et al.*, 2003a,b). DNA barcoding is also used to create a standardized reference library for the DNA based identification of target species (Kerr *et al.*, 2007). The method relies on the short genetic sequence of mitochondrial DNA (mtDNA) which contains hereditary information involving evolutionary features of animals and exists in almost all eukaryote cells (Blaxter, 2004; Lunt *et al.*, 1996). In order to determine the Cytochrome Oxidase I (COI) sequence, the DNA is extracted from the cells using Proteinase K, which allows for the DNA to be extracted without destroying the exoskeleton of the specimen so it can also be used to make slides to observe the morphology (Morris and Mound, 2004). Following the extraction, the cytochrome oxidase I (COI) gene is amplified by Polymerase Chain Reaction (PCR) using universal primer pairs. The PCR product is analyzed on an agarose gel to confirm that amplification has occurred. If there is a band, the PCR product can be sent for DNA sequencing to determine the identity of the organism. The DNA sequence database of experimental species are edited and aligned and a phylogenetic tree is constructed to examine the relationships within species and groups using either Barcode of Life Data Systems (BOLD) or the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST).

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CHAPTER 3

Seasonal abundance, host plant range and damage level of *Tuta absoluta* and its indigenous natural enemies

3.1 Abstract

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) has been an economically destructive insect pest on tomato crops in Sudan since 2010. An extensive survey using sex pheromone-baited traps and field sampling of leaves and fruits of different cultivated and non-cultivated plant species was conducted to determine the host plant species of *T. absoluta* and its damage potential. The abundance of its indigenous natural enemies was also determined. The survey covered 43 sites in seven States of Sudan. The pest was found in all these States with the highest abundance in the winter and early summer seasons (December-April). However, population densities declined in the late summer season and remained very low during fall (July-October). The pest population density was higher in Sennar State in both early and late seasons compared to Gezira and White Nile States. Fifty-three plant species belonging to 16 families were sampled for the preference of *T. absoluta* but it was only recorded on four plant species in the Solanaceae family, namely *Solanum lycopersicum* L. (tomato), *S. melongena* L. (eggplant), *S. nigrum* L. (black nightshade) and *S. dibium* Fr. (locally known as Gubbain). The level of damage on open field tomato crops differed between the States and seasons, ranging from 46.5 - 56.5% and from 33.4 - 45.3 % on tomato leaves and fruit, respectively. In greenhouses,10.9% of fruit was damaged. Six hymenopteran parasitoid species belonging to four families were found in several surveyed sites on three Solanaceae plant species. These species are *Bracon nigricans* Szepligeti (Hymenoptera: Braconidae), *Bracon hebetor* (Say), *Dolichogenidea appellator* (Telenga) (Hymenoptera: Braconidae), *Eupelmus* sp. (Hymenoptera: Eupelmidae), Pteromalidae sp. (Hymenoptera: Pteromalidae) and Ichneumonid sp. (Hymenoptera: Ichneumonidae). The parasitism rate of all parasitoid species was low averaging 10% overall. Only *Nesidiocoris tenuis* (Hemiptera: Miridae) and *Chrysoperla* sp. (Neuoroptera: Chrysopidae) were reported as *T. absoluta* predators. These parasitoid and predator species could provide alternative control methods for *T. absoluta* which could minimize the extensive use of insecticides.

3.2. Introduction

The tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a devastating pest of tomato *Solanum lycopersicum* L. (Solanaceae) crops in South America since the 1960s (Guedes and Picanco 2012; Lietti *et al*., 2005). In 2006, the pest was reported in Spain, where it was considered to have arrived from Central Chile (Guillemaud *et al.,* 2015). The pest has widely spread in Afro-Eurasians countries within a few years and has become a real threat to tomato production in the newly invaded countries (Biondi *et al.*, 2018; Desneux *et al.*, 2010; 2011; EPPO 2017). In Sudan, *T. absoluta* was reported in 2010 in Khartoum State and subsequently spread to other tomato producing States (Mohamed *et al.*, 2012). The pest attacks all the aboveground plant parts and all developmental stages of tomato plants (Moreno *et al.*, 2012; Sánchez *et al.*, 2009; Urbaneja *et al.*, 2012; Zolf and Suffert, 2012). Larval feeding causes damage to leaves, flowers and fruits which significantly reduces the yield and quality (Cifuentes *et al.*, 2011; Desneux *et al.*, 2010). Except for the direct losses inflicted to fruit in terms of quality and a reduction in yield, other economic and environmental issues also arise due to the extensive and inappropriate use of chemical insecticides to control the pest (Campos *et al.*, 2017). Adding to the indirect costs caused by *T. absoluta* are the quarantine restrictions imposed by importing countries to prevent the entry and establishment of *T. absoluta* (USA-APHIS, 2011). Therefore, producers from countries where *T. absoluta* occur, have to apply additional measures before exporting the fruits or risk losing lucrative export markets for their produce.

Although *T. absoluta* is a primary pest of tomato, it is also an important pest of other Solanaceous crops such as eggplant (*Solanum melongena* L.) and potato (*Solanum tuberosum* L.) (Arnó and Gabarra, 2011; Desneux *et al.*, 2010; Tropea Garzia *et al.*, 2012). The pest has also been reported to attack non- solanaceous crops such as French bean (*Phaseolus vulgaris* L.) (Fabaceae) (Speranza and Sannino, 2012; Mohamed *et al.*, 2015), water melon (*Citrullus lanatus*) (Cucurbitaceae), faba bean (*Vicia faba* L.) (Fabaceae), and alfalfa (*Medicago sativa* L.) (Fabaceae) (Mohamed *et al.*, 2015). Other uncultivated plants have also been reported to serve as host plants for *T. absoluta* (Estay, 2000; Portakaldali *et al.*, 2013; Mohamed *et al.*, 2015).

The rapid development of the pest and its nature of damage and spread resulted in growers using various insecticides which resulted in development of resistance by the pest, both in native (Gontijo *et al.*, 2013; Lietti *et al.*, 2005; Siqueira *et al.*, 2001) and invaded areas (Europe) (Haddi *et al.*, 2012; Roditakis *et al.*, 2013). The intensive use of insecticides could result in other undesired effects to the natural enemies and pollinators (Campos *et al.*, 2017) as well as human health (Desneux *et al.*, 2010). These, together with the cryptic development of larvae (in mines) which is not easily exposed to insecticides, prompted the development of alternative, cost effective control methods. A pre-requisite for development of these control measures is a better understanding of the bio-ecology of the pest.

Therefore, the aims of this study were to (1) determine the damage level of *T. absoluta* as well its distribution and seasonality (2) catalogue the host plant range and (3) identify the indigenous natural enemies that formed new associations with the invasive moth.

3.3. Material and Methods

Study sites

A field survey was conducted in 43 sites in Sudan (Figure 3.1) from October 2014 to September 2016. Eighteen of these sites were in Gezira State, 12 in Sennar State and 7 sites in White Nile State. The remaining sites were in Khartoum, Northern, South and West Darfur States. The geo-referencing of these sites are provided in table 3.1. The sites include a diversity of agro-ecological zones (Desert, simi-desert, high rainfall and low rainfall savannahs). The desert in the Northern State receives less than 75 mm of annual rainfall and vegetation is absent except along the water courses. The semi-desert zone is from Khartoum in the north, for about 200 km southwards, with an average annual rainfall of 75 - 300 mm. The woodland savannah zone is south from the semi-desert and forms a belt stretching from the east to the west of the country. This zone is subdivided into the low and high rainfall savannahs with an annual rainfall of 300 - 500 mm and 500 - 1000mm, respectively. Most of the study sites (16 - 42) were located in the low rainfall savannah. The landscape of the study sites at Gezira, Sennar and White Nile States is mainly flat with dark cracking clay soils. Mainly vegetables, fodder crops and scattered orchards [citrus (*Citrus* sp.), guava (*Psidium guajava* L.) and mango (*Mangifera indica* L.)] are planted along the Blue and White Nile. To the west of the Blue Nile at Gezira State, the cropping system changes to mainly cereal crops, *viz*. sorghum (*Sorghum bicolor* L. Moench) and wheat (*Triticum aestivum* L.) but cotton (*Gossypium barbadense* L. and *G. hirsutum* L.), vegetables, and groundnut (*Arachis hypogaea* L.) are also cultivated. Tomato, faba bean and pigeon pea (*Cajanus cajan* L.) are planted in the Northern State (42 and 43) and *Acacia tortilis* shrubs also occur. Both sites at the western parts (40 and 41) were on the valley edges and vegetables tomato, eggplant, onions (*Allium cepa* L.) and leafy vegetables, alfalfa), and fruit trees (mango, guava, and citrus) are cultivated.

The sites in Gezira and Sennar States were sampled repeatedly every 21 - 30 days during the study period. The sites in the other States were sampled only two or three times (depending on the site) during the study period.

Tuta absoluta stock colony

Tomato leaves infested with *T. absoluta* larvae were collected from commercial fields at site 16 (Gezira State) (Figure 3.1). The collected samples were kept in aerated 2.5 L plastic containers, moistened and monitored daily until moths emerged. Once emerged, 20 male and 20 female moths were aspirated from the containers, released together in a ventilated Perspex cage (60 x 60 x 60 cm) and left for 48 hours to mate. Two potted tomato plants (cv. Strain B) were placed inside each cage for the moths to oviposit.

These plants were replaced after 48 hours and maintained in separate insect cages. Larvae emerging from the eggs were left on the plants to feed and develop. Additional plants were added to these cages as food when necessary. Two pieces of cotton wool were saturated with a 10% honey solution and secured with pins to two opposite sides of the cages to serve as food for the moths. The colony was maintained in a laboratory at 25 ± 1 °C and 16L: 8D photoperiod.

3.3.1. Monitoring *Tuta absoluta* larvae and mines in open field and greenhouse tomatoes

Tuta absoluta larvae and mines were regularly monitored on tomato plants from October 2014 to September 2015 in 36 open fields in Gezira State and 26 in Sennar State (Table 3.1). *Tuta absoluta* was monitored in 7 greenhouses in Gezira and 3 in Khartoum (Table 3.1). The temperature in all greenhouses was kept constant at 20 ± 2 °C. In each field and/or greenhouse, two expanded leaves from the median plant parts were sampled from 30 randomly selected plants on a transversal zigzag. These are referred to as sampling units per plant from here onwards. Collected leaf samples from each field at the respective sites were placed separately in paper bags, labelled and transported to the laboratory. The leaves were examined under a stereomicroscope (Leica WILD M3Z) and the numbers of larvae/leaf, mines/leaf, as well as the number of infested leaves were recorded. Predators found on the sampled leaves were counted and fixed in 95% alcohol for further identification. The infested leaf samples were placed either in ventilated insect cages (30 cm x 30 cm x 30 cm) or aerated plastic containers (2.5 L) (Figure 3.2). These leaf samples were moistened with water in a small spray bottle (0.5 L) and monitored daily. The *T. absoluta* moths or parasitoids that emerged, were recorded and parasitoids were fixed in 95% alcohol.

3.3.2. Tomato fruits damaged by *Tuta absoluta*

Tomato fruits (20/sampling date) were collected randomly from each of the respective fields and greenhouses. Fruits were examined under a stereomicroscope (Leica WILD M3Z) and the numbers of mines and larvae per fruit, as well as the number of infested and healthy fruits/sample were recorded. Infested fruits were incubated in aerated plastic containers (2.5 L) on heat-sterilized sand. Moths and parasitoids which emerged, were recorded.

3.3.3. Seasonal occurrence of *Tuta absoluta* moths

Seasonal occurrence of the *T. absoluta* moths was monitored by means of delta traps lined with sticky cards and baited with a *T. absoluta* sex pheromone lure (0.5 mg E3, Z8, Z11-Tetradecatrienyl acetate) which was suspended inside the trap. Traps were placed in both open tomato fields as well as in tomato greenhouses (Figure 3.3). A total of 127 pheromone-baited traps were installed in 61 open tomato fields (2 - 3 traps/ ha) at Gezira, Sennar and White Nile States.

The number of fields and greenhouses used for monitoring differed between the States (Table 3.2). Traps were placed at 0.6 - 1.0 m above soil level (depending on plant height) during the flowering stage. Traps were also placed in 10 greenhouses (2 traps/greenhouse) at four sites (2, 13, 14 and 15), at Gezira and Khartoum States (Figure 3.1). Each greenhouse was 350 m^2 in size. The sticky cards were replaced and the moths were counted weekly from October 2014 to July 2015. Pheromone lures were replaced monthly.

3.3.4. Survey of *Tuta absoluta* host plant species

3.3.4.1. Field sampling of leaves

Leaves of cultivated and weed species were collected from October 2014 to September 2016 from open fields at six of the States (Khartoum excluded) (Figure 3.1). These cultivated crops on which sampling was done, were adjacent to the selected tomato fields, while the weed species surrounded the tomato and other cultivated crop fields. Sampling of the leaves of cultivated crops was done as described above under section 3.3.1. Weeds were also inspected for *T. absoluta* damage and leaves with mines were sampled from the respective plants, placed separately in paper bags, labelled and transported to the laboratory. Infested leaves were sampled at random with a minimum of 30 leaves/sampling time. Leaf samples from both cultivated and weeds were examined using a stereomicroscope (Leica WILD M3Z) for the presence of *T. absoluta* larvae and then maintained similarly as described above (see 3.3.1) for emergence of moths and parasitoids. A total of 53 plant species (crops and weeds) were sampled at all sites (Table 3.3).

3.3.4.2. Field sampling of fruits and pods of potential crop hosts

Fruits and pods of eight different cultivated crop species were regularly inspected over a period of 21 - 30 days for the presence of *T. absoluta* larvae. Fruits were sampled from eggplant (*S. melongena L.*), bell pepper (*Capsicum annuum* L.) (Solanaceae), and okra, *Abelmoschus esculentus* L. (Malvaceae). Pods were sampled from the legumes crops, French bean (*P. vulgaris*), faba bean (*V. faba*), cowpea (*Vigna unguiculata*), Chickpea (*Cicer arietinum*) (Fabaceae) and pigeon pea (*Cajanus cajan* L.). Fruits and pods with mines were harvested and incubated in aerated plastic containers (2.5 L) on heat-sterilized sand or in ventilated insect cages (30 cm x 30 cm x 30 cm) in a laboratory at 25 ± 1 °C and 16L: 8D photoperiod until moths emerged.

3.3.5. Natural enemies survey

3.3.5.1. Field and greenhouse sampling

The *T. absoluta* infested leaves and fruits of the cultivated and weed species collected (see 3.3.1, 3.3.2, 3.3.4.1 and 3.3.4.2) were also examined for the presence of predators. These leaves and fruits were incubated until moths and/or parasitoids emerged as described above. Additionally, 22 open tomato fields and one greenhouse at Gezira and Sennar sites (Figure 3.1) were also sampled twice or three times during October 2014 to September 2016. The same sampling methods of leaves and fruits described under section 3.3.1 were followed. The samples collected were also incubated and the moths and/or parasitoids that emerged were recorded.

3.3.5.2. Collection of Tuta absoluta parasitoids using sentinel tomato plants

Tomato seeds (cv. Strain-B) were sown in seed trays (48 x 27.5 x 3.5 cm). Two seedlings/1 L pot, were transplanted four weeks after sowing. Plants were watered and fertilized regularly and kept in insect-proof cages until the 5 - 6 leaf stages (± 6 - 7 weeks old). Individual potted tomato plants were infested with 25 *T. absoluta* larvae each collected from the stock colony. Plants needed for collection of larval parasitoids were infested by placing larvae from the same instar (1st, 2nd, 3rd or 4thinstar)/plant. Plants for use in the collection of egg parasitoids were placed into oviposition cages and removed after 24 hours. A total of 50 eggs were left/plant and any additional eggs were carefully removed using a moistened brush prior to being placed in tomato fields. Potted plants infested with either *T. absoluta* eggs or larvae, were placed in selected tomato fields at sites 4, 11, 16 and 23 (Figure 3.1) from February to April 2016. To avoid exposure to insecticides, sentinel plants were placed in the border rows of the selected fields. The *T. absoluta* eggs and larvae were therefore not exposed to parasitism prior to exposure under field conditions. These plants were collected from the fields after 48 hours and kept in ventilated Perspex cages (50 x 50 x 50cm) or covered with cylinder shaped cages (Figure 3.4) in a laboratory. Parasitoids which emerged, were counted and fixed in 95% alcohol for further identification. A total of 181 infested plants in pots were distributed in four tomato fields at four sites over a period of eight weeks (Table 3.4).

3.3.5.3. Soil sampling for collection of Tuta absoluta pupal parasitoids

To collect pupal parasitoids of *T. absoluta*, random soil samples were taken from two infested tomato fields at site16, Gezira State. The soil of five 1 m^2 quadrates (3 - 4 cm deep) were sampled/field. This soil was soaked or washed in water, sieved with a fine mesh and the *T. absoluta* pupae which remained on the sieve, were collected. These pupae were placed in Petri dishes, moistened daily and kept at 25 ± 1 °C and 16L: 8D photoperiod until moths or parasitoids emerged.

3.3.6. Data analyses

The statistical analyses were performed using R version 3.3.1 (R Core Team, 2016). The adult males in trap catches as well as the number of mines and larvae were subjected initially to the Shapiro-Wilk normality test and the trap catches were log-transformed. The number of mines and larvae were log+1transformed prior to statistical analyses. The proportions of infested plants and fruits were arcsine- square-root transformed to normalize the data, but untransformed means are presented. Mixed model analysis was used to examine or correlate the effect of States and seasons on the number of mines and larvae as well as the male moth catches. Damage on tomato fruit and leaves and larval parasitism was expressed as percentages.

3.4. Results

3.4.1. Monitoring *T. absoluta* larvae and mines in open fields and greenhouse tomatoes

The number of larvae and mines/ sampling unit/ plant increased from November 2014 onwards and remained high in the early season crops and increased gradually from March to May in the late season crop at Gezira State (Figure 3.5). The percentage infestation which is a result of the number of larvae in mines, therefore, followed the same trend. The increase in the number of larvae and mines as well as % infestation also followed the same trend at Sennar State, expect for a decrease towards the end of the late season crop (May) (Figure 3.6). In both seasons, the mean number of larvae/sampling unit/ was significantly lower at Gezira State than at Sennar State, for the early ($\chi^2 = 276.57$; $df = 1$; $P < 0.001$) and late seasons ($\chi^2 = 14.864$; $df = 1$; $P < 0.001$), respectively (Figure 3.7A). Similarly, the mean number of mines per sampling unit per plant was also significantly lower at Gezira than Sennar State in the early season ($\chi^2 = 191.48$; $df = 1$; $P <= 0.001$) as well as late season ($\chi^2 = 65.02$; $df =1$; $P < 0.001$) (Figure 3.7B).

Monitoring of pest numbers and damage in the greenhouse at site 14 was done during November and December 2015. The number of larvae and mines/sampling unit/plant in greenhouses varied during the study period and locations ($\chi^2 = 25.587$; $df =1$; $P < 0.001$ and $\chi^2 = 14.401$; $df =1$; $P < 0.001$, for larvae and mines respectively) (Figure 3.8).

3.4.2. Tomato fruits damaged by *Tuta absoluta*

In the open fields, the damage level on fruit in Gezira State ranged between 42 - 45% and in Sennar State between 33% in the early season and 43% in the late season (Table 3.5). The greenhouse crops were destroyed by the owners due to heavy infestation by *T. absoluta*. Fruits were therefore sampled only once from three greenhouses at Wad Abbas, Gezira State (site 15). In these greenhouses, only 10.9% of fruit were damaged.

3.4.3. Seasonal occurrence of *Tuta absoluta* moths

Tuta absoluta moths were caught in pheromone traps throughout the early-, as well as the late seasons of the 2014/15 cropping season at Gezira, Sennar and White Nile States. The moth catches in early season crops were significantly higher than in the late season crops ($\chi^2 = 12.16$; $df = 1$; $P < 0.001$). There was also a statistically significant season by State interaction ($\chi^2 = 171.2$; $df = 64$; $P < 0.001$). Numbers fluctuated between 90 and 200 moths/trap/week in the early season crop in Gezira State. The mean number of moths/trap fluctuated more in the late compared to the early season. The lowest numbers of moths were caught towards the end of the late season crop in Gezira State (Figure 3.9). There was a marked reduction in moths caught in the pheromone traps (Figure 3.9) during June 2015. The mean number of *T. absoluta* moths caught in pheromone traps during the 2014/15 cropping season was generally higher at Sennar State (Figure 3.10) compared to Gezira State (Figure 3.9). The lowest number of moths was recorded early in the early season crop, but increased gradually and remained high until the end of the late season crop at Sennar State (Figure 3.10). The mean number of moths trapped at White Nile State was low during November and December 2014, but increased during January. The moth catches in this State remained generally high for the rest of the early season. It was also high early in the late season crop but declined later in the late season crop (Figure 3.11). The mean number of moths caught in pheromone traps in the White Nile State was significantly lower than in the other two States ($\chi^2 = 125.6$; $df = 1$; $P < 0.001$), in the early season crop. The mean moth catches in late season crops, were, however, significantly lower at Gezira State ($\chi^2 = 12.162$; $df = 1$; $P < 0.01$) compared to moth catches in the other two States in late season crops (Figure 3.12).

The number of males caught in pheromone-baited traps in the respective greenhouses at Gezira State varied with no moths caught in the greenhouses atsite13 while a mean of 235 males/trap/week was recorded at site 15.This *T. absoluta* population increased to a level so high that the farmer decided to destroy the plants. The number of moths caught per trap/week ranged between a minimum of 1 and a maximum of 184 moths/ trap/ week (Figure 3.13).

3.4.4. Survey of *T. absoluta* host plant species

3.4.4.1. Field sampling of leaves

During the field survey of *T. absoluta* host plants, a total of 133 *T. absoluta* larvae were collected from eggplant (*S. melongena*), nightshade (*S. nigrum*) and Gubbain (*S. dibium* Fr.) (Figure 3.14). The collected larvae were reared under laboratory conditions to adults on the plant species that they were collected from. A total of 10954 *T. absoluta* larvae were collected from its preferred host, tomato (*S. lycopersicum*).

The incidence of *T. absoluta* was on tomato (98.8%) followed by eggplant (0.7%), Gubbain (0.3%) and nightshade (0.2%).

3.4.4.2. Field sampling of fruits and pods of potential crop hosts

Eggplant fruits (*n* = 840), bell pepper (*n* = 660) and okra (*n* = 400) were collected and inspected for the presence of *T. absoluta* larvae. Pods of French bean were sampled 12 times, faba bean 15 times while 50, 20 and 3 samplings were done for pods of pigeon pea, chickpea and cowpea, respectively. No *T. absoluta* infestation occurred on either fruits or pods of any of these species.

3.4.5. Natural enemies survey

3.4.5.1. Field and greenhouse sampling

During the extensive survey of three years, sampling was done 299 times and a large number of leaves and fruit samples were collected from the cultivated and non-cultivated plant species. Six hymenopteran parasitoid species (Figure 3.15) belonging to four families were recovered from *T. absoluta* larvae. *Bracon nigricans (*Szépligeti) and *Bracon hebetor* (Say) (Hymenoptera: Braconidae) are larval ectoparasitoids and have also been reported as *T. absoluta* parasitoids in the Palaearctic region (Zappalà *et al.*, 2012). The solitary larval endoparasitoid, *Dolichogenidea appellator* (Telenga) (Hymenoptera: Braconidae) was reported during this study for the first time as a parasitoid of *T. absoluta*. An *Eupelmus* sp. (Hymenoptera: Eupelmidae) as well as parasitoids belonging to the families Pteromaidae and Ichneumonidae were also recorded. *Bracon nigricans* accounted for 74.8% of the parasitoids collected during the survey (*n* = 437), *D. appellator* for 18.8%, *B. hebetor*, 4.8% and the remaining three species 0.5%, each (Table 3.5). *Bracon nigricans* was the only parasitoid species found in open fields and greenhouses as well as at all States surveyed. *Bracon nigricans*, *Eupelmus* sp. and parasitoids from the Pteromaidae family were also recovered from tomato fruit samples (Table 3.6).

The parasitism rates were recorded at the sites in two of the States, namely Gezira and Sennar States, only. The parasitism levels of all parasitoid species were low with an overall mean of 10% (10.6% on tomato, 10.5% on eggplant and 9.5% on nightshade). The numbers of *B. nigricans* and *D. appellator* increased towards the end of the production cycle of the tomato crops when farmers did not apply insecticides (Personal observation).

Two predator species belonging to two orders were recorded from tomato and eggplant fields during the survey (Table 3.5). *Nesidiocoris tenuis* (Hemiptera: Miridae) and *Chrysoperla* sp. (Neuroptera: Chrysopidae) were observed preying on *T. absoluta* in all surveyed areas, with *N. tenuis* the most abundant. Larvae of *Chrysoperla* sp. fed on both larvae and adults of *T. absoluta* and *P. operculella* in the laboratory (Figure 3.16 A).

3.4.5.2. Collection of Tuta absoluta parasitoids using sentinel tomato plants

Only *B. nigricans* and *D. appellator* were recruited from the sentinel tomato plants distributed in four sites at Gezira and Sennar States (Table 3.7).

3.3.5.3. Soil sampling for pupal parasitoids

None of the *T. absoluta* pupae (*n* = 213) collected from the two tomato fields were parasitized and no pupal parasitoids were therefore found.

3.5. Discussion

Early monitoring of *T. absoluta* adults with pheromone-baited traps as well as that of the immature stages is important for successful application of an integrated pest management program. Low leaf infestation rates by *T. absoluta* were found early in the season but it increased with time from 2.7% to 66.9% leaf infestation per sampling units. The highest levels of fruit damage were also observed towards the end of the cropping cycle. In Tunisia, Chermiti *et al.* (2009) reported the fruit to be more vulnerable than the old leaves towards the end of the growing season.

The variability in infestation levels of *T. absoluta* between Gezira and Sennar States could be attributed to the weather conditions as well as differences in farming practices between Gezira and Sennar States. For example some of the sampling sites were located at the Al Gezira Scheme where insecticides were frequently applied aerially for control of cotton insect pests. This could also have indirectly resulted in the reduction of *T. absoluta* populations although it was not primarily being targeted. Differences in the level of the farmers’ knowledge about insecticides and its application could also have an effect. For example, farmers at Al Gezira State had received more training and extension services through the Farmers Field Schools (FFS) which were established by the Gezira Scheme in collaboration with the IPM Unit, Plant Protection Center, Agricultural Research Corporation in 1993 (Geberaldar *et al.,* 2014).

The greenhouses at two sites (13 and 15) were monitored for only five weeks because of crops that were destroyed by the owners due to either heavy infestation by *T. absoluta* or tomato leaf curl disease. Large variations in the numbers of *T. absoluta* larvae were, however, found among the greenhouses at the surveyed sites. The temperature in greenhouses was set at 20 °C and no parasitoids or predators were released (Personal observation). Although the infestation level was low during the first week of June in a greenhouse at site 15, a total crop loss occurred in the first week of July (100% damage) (Personal observation). The differences in infestation levels among the greenhouses might be due to differences in management practices employed to prevent infestations (i.e. use of insect-proof, exclusion nets on the opening, double doors, and insect free seedlings). These measures combined with sanitation procedures such as the removal of infested plant leaves and fruits were proved to reduce the infestation levels of *T. absoluta* in Tunisian greenhouses (Abbes *et al.*, 2012).

The peak in moth occurrence recorded towards the end of the season, coincided with maturation of the tomato crops. The establishment of *T. absoluta* can be explained by the similarity in climatic conditions of the Mediterranean region with some parts of South America from where the pest originates from, coupled with the availability of tomato crops year around either in the open fields or greenhouses (Desneux *et al.*, 2010). The number of generations of *T. absoluta* per year in the Mediterranean basin, is also similar to that in areas of its origin and it does also not enter into diapause (Sannino & Espinosa 2010). Desneux *et al.* (2010) reported 14°C as the minimum threshold temperature for development of *T. absoluta.* Rain has a profound effect on *T. absoluta* infestations on tomato under open field conditions. De Medeiros *et al.* (2011) as well as Chermiti & Abbes (2012) reported heavy rains to reduce *T. absoluta* populations. This tendency was also observed in the current study with fewer moths caught in the pheromone traps during the two weeks of rain in June at Al Gezira State.

A proper knowledge of the host plant range and its contribution to pest population build-up or growth are critical to develop effective control strategies (Norris and Kogan, 2005). An extensive survey was conducted in seven States of Sudan to determine the host plant range of *T. absoluta*. Fifty-three potential host plant species were sampled, of which only two cultivated plant species, namely tomato and eggplant as well as two wild plant species, namely black nightshade and Gubbain were found to host *T. absoluta*. All of these host plants belong to the family Solanaceae. This confirms the oligophagous nature of this

pest which has been vastly documented in its native area of South America (Cardozo *et al.*, 1994; Estay, 2000; Galarza, 1984). Although *T. absoluta* is known to be oligophagous in the areas where it is endemic, it also expands its host range in the newly invaded regions. Host plant species in addition to solanaceous crops, had been reported from countries where the pest had been recorded as an invader species, for example French bean (*P. vulgaris*) in Italy (Speranza and Sannino, 2012) as well as *Chenopodium album* (Chenopodiaceae) and *Convolvulus arvensis* (Convolvulaceae) in Turkey (Portakaldali *et al.*, 2013). Mohamed *et al.* (2015) reported even a wider host range for *T. absoluta* in Sudan, including water melon (*C. lanatus*) (Cucurbitaceae), faba bean (*V.*) (Fabaceae), alfalfa (*M. sativa*) (Fabaceae), ramtouk (local name) (*Xanthium brasilicum* Vell.) (Asteraceae), and spiny amaranth (*Amaranthus spinosus* L.) (Amaranthaceae). Although *V. faba, M. sativa, C. lanatus, P. vulgaris, X. brasilicum, A. spinosus* and jimson weed (*Datura stramonium* L.) (Solanaceae) were sampled adjacent to severely *T. absoluta* infested tomato crops during this study, no *T. absoluta* infestation was found on any of these plant species. These host plant species reported in Sudan by Mohamed *et al.* (2015) could therefore, not be confirmed in the present study. The pest did not move to and infest these crops which were in a better physiological condition than the tomato crops late in the season. Similarly in Turkey, Portakaldali *et al.* (2013) reported *C. album* and *C. arvensis* as host plants for *T. absoluta,* but Bawin *et al.* (2015) found that the pest did not lay eggs on these plant species under laboratory conditions. *T. absoluta* larvae inoculated onto leaves of *C. album* and . *arvensis* also died before tunneling into the leaves (Bawin *et al.*, 2015). Cardozo *et al.* (1994), Estay (2000) and Mohamed *et al.* (2015) all reported *D. stramonium* as a host plant of *T. absoluta,* but Abbes *et al.* (2016) reported the pest did not lay eggs on this plant species in a laboratory study. Although reported as hosts, these plants are therefore not preferred hosts of *T. absoluta* under natural conditions. Infestations found on some non-preferred plants could be considered as unusual attacks which might happen under certain conditions such as succession planting of tomato and bean in protected greenhouses (Speranza and Sannino, 2012).The fruit of eggplant, bell pepper, okra and tomato and the pods of French bean, faba bean, pigeon pea, chickpea and cowpea were sampled, no *T. absoluta* infestation on fruits or pods other than tomato, was found. Mohamed *et al.* (2012) reported that
T. absoluta attack eggplant fruit in Sudan, but this was not confirmed in the present study although infested eggplant fields were sampled several times during the season.

In the survey for parasitoids attacking *T. absoluta* in Sudan, six indigenous paraitoids were found. These were *B. nigricans*, *B. hebetor*, *D. appellator*, *Eupelmus* sp. Pteromaidae sp. and Ichneumonidae species. *Bracon nigricans* is a generalist and gregarious larval ectoparasitoids attacking host species mainly in the Lepidoptera and some coleopterans (Khalil *et al.*, 2016; Zappalà *et al.*, 2012). *Bracon nigricans* has been reported as a larval ectoparasite of *T. absoluta* in Europe and the Middle East (Al- Jboory *et al.*, 2012;

Gabarra *et al.,* 2014; Zappalà *et al.,* 2012) and Kassala State (Sudan) by Mahmoud (2013). This parasitoid infested *T. absoluta* larvae in tomato, eggplant and nightshade. This parasitoid occurred most frequently compared to the other parasitoid species and was also recorded in all surveyed States as well as in a greenhouse in Khartoum State. The parasitism rates of *T. absoluta* by *B. nigricans* did not exceed 10.5% in tomato and eggplant fields.

Bracon hebetor is a gregarious, idiobiont (they lay their eggs after killing the host or preventing the further development of their host), ectoparasitoid of a wide range of lepidopteran larvae (Faal- Mohammad-Ali and Shishehbor, 2013) and some coleopteran species (Khalil *et al.,* 2016). This parasitoid species was recovered from *T. absoluta* larvae from tomatoes sampled at Gezira and Sennar States only. Only 5% of the parasitoids collected from infected samples were *B. hebetor*. *Dolichogenidea appellator* (Braconidae) is a koinobiont (the host continues to develop and is only killed when the parasitoid reaches maturity), endoparasitoid and attacks lepidopteran species such as *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) (CABI, 2018) and *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) (Kazemzadeh *et al.,* 2014; Yu *et al.,* 2016). This parasitoid species was recovered from *T. absoluta* infested tomato and eggplant at Al Gezira and Sennar States as well as from sentinel tomato plants. It was also recovered from *P. operculella* infesting tomato and eggplants in both States.

Bracon nigricans was successfully reared on the larval instars of *T. absoluta* under laboratory conditions. However, *D. appellator* is an endoparasitoid of which the females lay eggs into the host larvae (Shaw *et al.,* 2009) without causing temporary paralysis to their hosts (Beckage and Gelman, 2004). *Dolichogenidea appellator* is also a generalist parasitoid that attacks lepidopteran species such as *P. operculella* (CABI, 2018), *P. xylostella* (Kazemzadeh *et al.,* 2014; Yu *et al.,* 2016) and *Etiellaz inckenella* (Treitschke) (Braconidae: Phycitidae) (Yu *et al.,* 2016).

A few years after *T. absoluta* invaded the Afro-Eurasian region, field surveys were conducted to determine the natural enemies which formed new associations with the pest (Desneux *et al.,* 2010). The highest number of parasitoid species was reported in Italy and Spain with more than 20 species in each country (Gabarra *et al.,* 2014; Zappalà *et al.,* 2012). Only one parasitoid species was reported in Jordan (Al- Jboory *et al.,* 2012), nine in Turkey (Doğanlar and Yiğit, 2011), six in Algeria (Boualem *et al.,* 2012) and two species in Tunisia (Abbes *et al.,* 2013). The variation in number of parasitoid species reported from the respective countries might be as a result of the difference in crop management practices such as insecticides use. For example, the composition of *T. absoluta* parasitoid species in the non-crop habitats and commercial crops in Spain, differed (Gabarra *et al.,* 2014). It is, however, known that the presence of weeds and evergreen crops or mixed crops play an important role in maintenance of biodiversity in agricultural landscapes (Fraser *et al.,* 2007; Gabarra *et al.,* 2014; Sääksjärvi *et al.,* 2006; Schmidt *et al.,* 2005; Zappalà *et al.,* 2012).

The guild of parasitoids species (55 species) of *T. absoluta* reported in the newly invaded areas (Western Palaearctic countries) is larger than that reported in its area of origin (49 species) (Zappalà *et al.,* 2013). The main parasitoids of *T. absoluta* in the Mediterranean basin are the ectoparasitoid wasps in the family Eulophidae, including species from the genera *Necremnus*, *Stenomesius*, and *Neochrysocharis* (Gabarra *et al.,* 2014; Urbaneja *et al.,* 2012). Since parasitoids prefer certain larval instars of *T. absoluta* for oviposition and others for host-feeding, the resultant larval mortality rate is high (Calvo *et al.,* 2013; Ferracini *et al.,* 2012). *Bracon nigricans* is widely distributed in European countries (Biondi *et al.,* 2013a; Urbaneja *et al.,* 2012; Zappalà *et al.,* 2013) and also in the Middle East (Al-Jboory *et al.,* 2012) and suppresses the larval population of *T. absoluta*, in particular the third and fourth larval instars through parasitism as well as host-stinging and host-feeding activity (Biondi *et al.,* 2013a).

In this survey, *B. nigricans* was found widely distributed over various geographical areas and represented the highest proportion of parasitoids recorded from *T. absoluta,* followed by *D. appellator* and *B. hebetor. Bracon nigricans* is reported to be the only species recovered from *T. absoluta* in Europe and the Middle East (Al-Jboory *et al.,* 2012; Gabarra *et al.,* 2014; Zappalà *et al.,* 2012), but *B. hebetor* is recovered from *T. absoluta* in Europe (Zappalà *et al.,* 2012). Other parasitoid species of *T. absoluta* reported from eastern Sudan (Kassala State), but not found in this study, are *Ecdamua cadenati* (Risbec) (Hymenoptera: Torymidae) and *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae) (Mahmoud, 2013). The sites samples at Al Gezira and Sennar States were richer in species compared to the sites in other States. It may be ascribed to the sampling periods as well as the extensive sampling conducted in the sites at Al Gezira and Sennar States.

The populations of *B. nigricans* and *D. appellator* declined during the fall season (July – September) which was also marked by higher rainfall. The lower infestation levels by these parasitoids could be ascribed to reduced areas of tomato crops compared to crops planted earlier in the season and low *T. absoluta* infestation of these crops during the rainy seasons. However, potato tuber moth, *P. operculella* present in eggplant fields also serve as a host for these parasitoid species and can therefore maintain the parasitoids from where they can later migrate to tomato crops. Their numbers did, however, increase towards the end of the production season. Insecticides are known to affect parasitoids negatively (Biondi *et al.,* 2013b). Farmers do not apply insecticides at the end of the season (Pers. obs.) which therefore indicates the negative effect of insecticides on these parasitoids. Parasitism rate was very low during the production season and did not exceed 10.5%, which was insufficient to reduce the populations of *T. absoluta* to sufficiently low limits to prevent yield losses. Abbes *et al.* (2015) also indicated that high temperatures may increase the negative effects of some insecticides on *B. nigricans* populations. *Bracon nigricans* and *D. appellator* were also recovered from the sentinel plants. No pupal parasitoids of *T. absoluta* were, however found and almost all of the collected pupae successfully eclosed to adults. There were also no pupal parasitoids found in the Mediterranean basin (Urbaneja *et al.*, 2012). Early larval instars of *T. absoluta* may cause significant damage because they usually feed on the newly-developed leaves (Cocco *et al.*, 2015; Taylor, 1987), therefore parasitization of the early larval instars could reduce the damage level. The second and third instar *T. absoluta* larvae are attacked by *D. appellator*, while *B. nigricans* attacks the third and fourth instars. When both these species occur together in high numbers, their synergistic parasitization could provide for good control of *T. absoluta*.

Predators are free-living insects or mites that depend on other insects for food during their lifecycle (Abdelrahman, 2014). During this survey, only *N. tenuis* and *Chrysoperla* sp. were collected as *T. absoluta* predators in all surveyed areas, with *N. tenuis* the most abundant. Both adults and nymphs of *N. tenuis* feed actively on eggs and all larval stages of *T. absoluta* (Urbaneja *et al.*, 2009). The generalist predators of *Chrysoperla* sp. prey on the larval stages of *T. absoluta* in South America (Desneux *et al.*, 2010). It was observed in this study that these species can prey on the adults of *T. absoluta* and *P. operculella* also. *Chrysoperla carnea* (Steph), *C. cannula* (Steph) and *C. vulgaris* (Sch) have also been identified as predators of *Bemisia tabaci* (Hemiptera: Aleyrodidae), *Helicoverpa armigera* (Lepidoptera: Noctuidae) and *Aphis gossypii* Glover (Hemiptera: Aphididae) in Sudan (Abdelrahman, 2014). Various species of *T. absoluta* predators occur naturally in South America and they can provide up to 79.4 % larval and 5% egg control respectively (Miranda *et al.*, 1998). In the newly invaded areas (Western Palaearctic countries) only 14 species belonging to seven families have been reported as *T. absoluta* predators (Zappalà *et al.*, 2013) compared to over 40 species in its area of origin (Desneux *et al.*, 2010). In the Mediterranean basin, the predators *N. tenuis* and *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) proved to be the most promising biological control agents of *T. absoluta* (Arnó and Gabarra, 2011; Desneux *et al.*, 2010). To achieve acceptable control of *T. absoluta* under field conditions, both *N. tenuis* and *M. pygmaeus* should occur at a density of more than two insects/ m^2 (Nannini *et al.*, 2012). The efficacy of biocontrol of *T. absoluta* could be improved when *N. tenuis* is used in combination with *B. thuringiensis*. Mollá *et al.* (2011) reported damage to tomato leaves to be reduced with up 97% as a result of this combination being applied.

3.6. Conclusion

Tuta absoluta was recorded in seven States of Sudan surveyed in this study. The number of moths caught in pheromone traps was the lowest in October and increased gradually, with a peak in occurrence during January and February. The pest did, however, decrease during the rainy months (June and July). The number of leaf mines and larvae as well as the proportion of infested leaves followed the same trend as the moth catches. During the host plant survey, *T. absoluta* was found infesting only four plant species in the Solanaceae family. These were *S. lycopersicum, S. melongena*, *S. nigrum* and *S. dibium*.

Six hymenopteran parasitoid species belonging to four families (Braconidae, Eupelmidae, Pteromaidae and Ichneumonidae) were recovered from *T. absoluta*. Of these, *B. nigricans* is widely distributed across the country while *D. appellator* and *B. hebetor* were found in the central region only. *Bracon nigricans* and *B. hebetor* are ectophagous parasitoid species of lepidopteran larvae. However, *D. appellator* is generalist and an endoparasitoid species recorded for the first time as a *T. absoluta* larval parasitoid.

Nesidiocoris tenuis and *Chrysoperla* sp. were recorded as *T. absoluta* predators. These species are widely distributed across the country.

3.7. References

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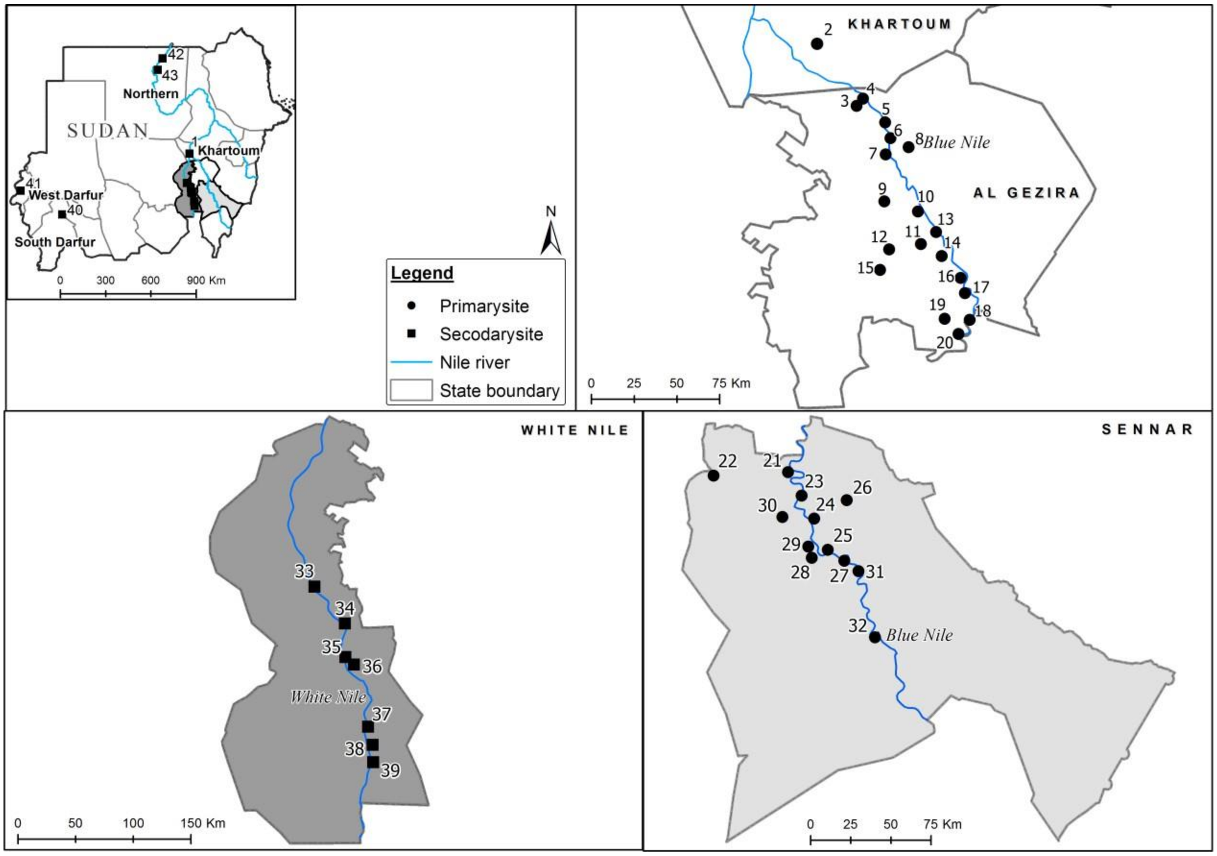

Figure 0.1: Maps of the States of Sudan indicating the sites used for sampling of *Tuta absoluta* larvae, potential host plants and natural enemies, as well as monitoring of moths with pheromone traps.

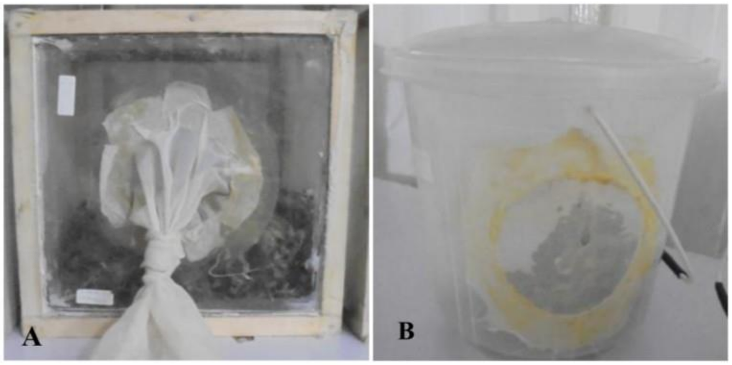

Figure 0.2: *Tuta absoluta* infested leaf samples were incubated in (A) insect cages (30 x 30 x 30 cm) and (B) in aerated plastic containers (2.5 L) to record the emergence of moths and parasitoids.

Figure 3.3: Delta traps baited with *Tuta absoluta* sex pheromone lures were placed in (A) open tomato fields and (B) in tomato greenhouses to monitor the flight activity of the adults by counting the male moths captured on (C) the sticky card of these delta traps.

Figure 3.3: A sentinel tomato plant (A) placed in a tomato field and (B) covered with a cylindrical cage in the laboratory after it was exposed to parasitoids in a field.

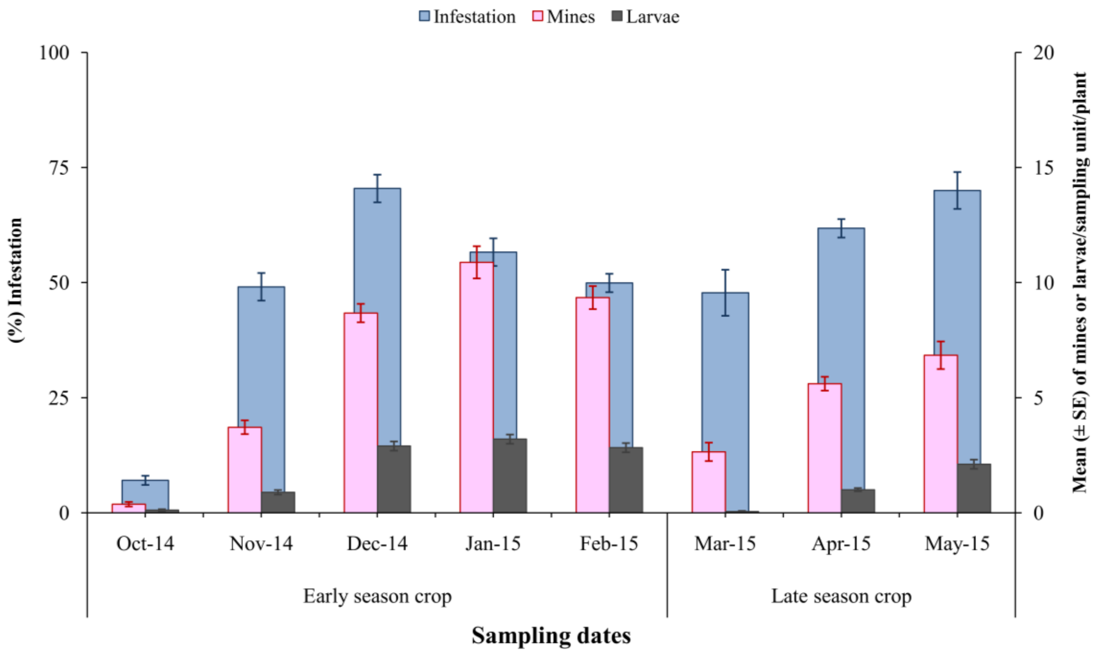

Figure 3.4: Monthly mean number (± SE) of *Tuta absoluta* mines and larvae per sampling unit per plant and the percentage of infested plants in open tomato fields in Gezira State.

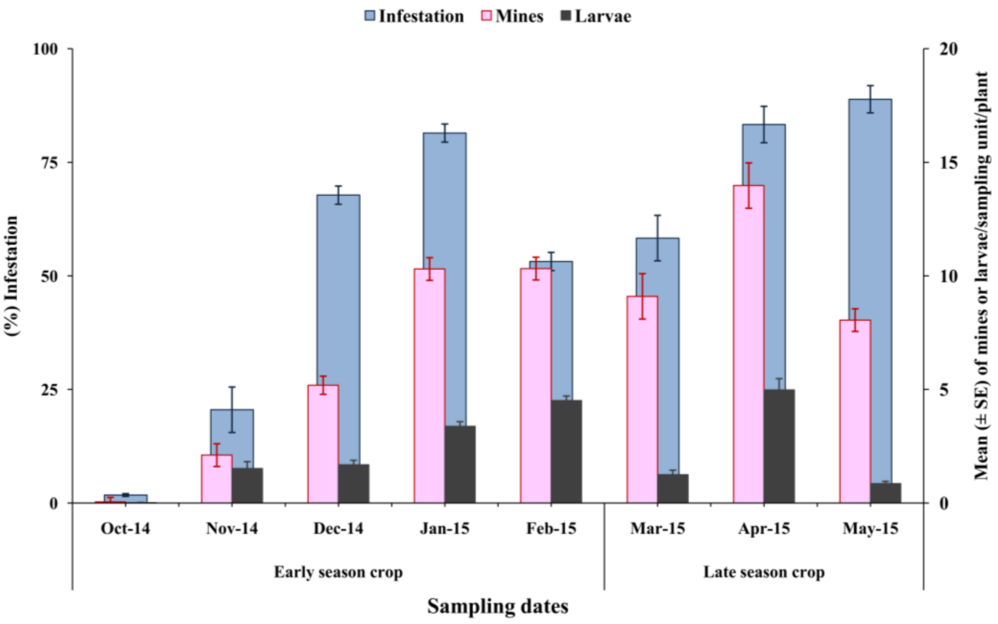

Figure 3.5: Monthly mean number (± SE) of *Tuta absoluta* mines and larvae per sampling unit per plant and the percentage of infested plants in open tomato fields in Sennar State.

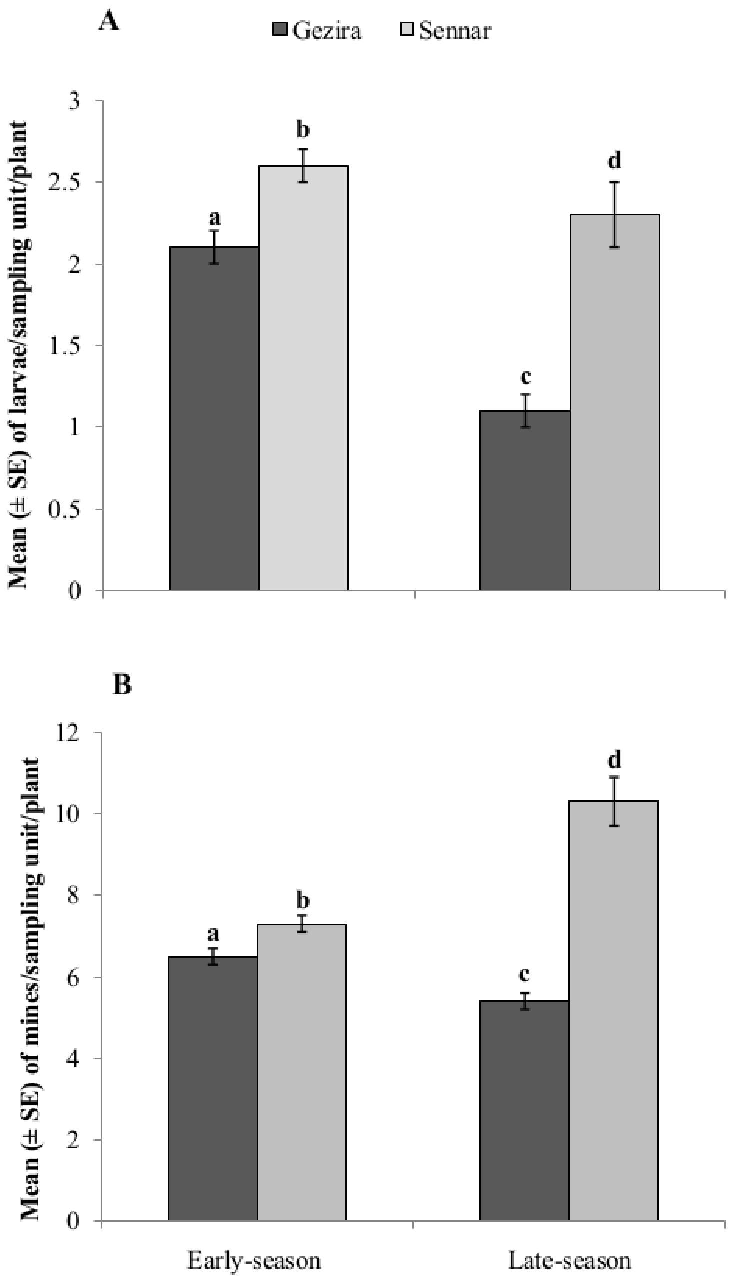

Figure 3.6: (A) Mean number of *Tuta absoluta* mines per sampling unit plant per season (B) mean number of *T. absoluta* larvae per sampling unit plant per season in the Gezira and Sennar States. Bars capped with different letters are significantly different from the corresponding season (Chi-square test at α= 0.05)

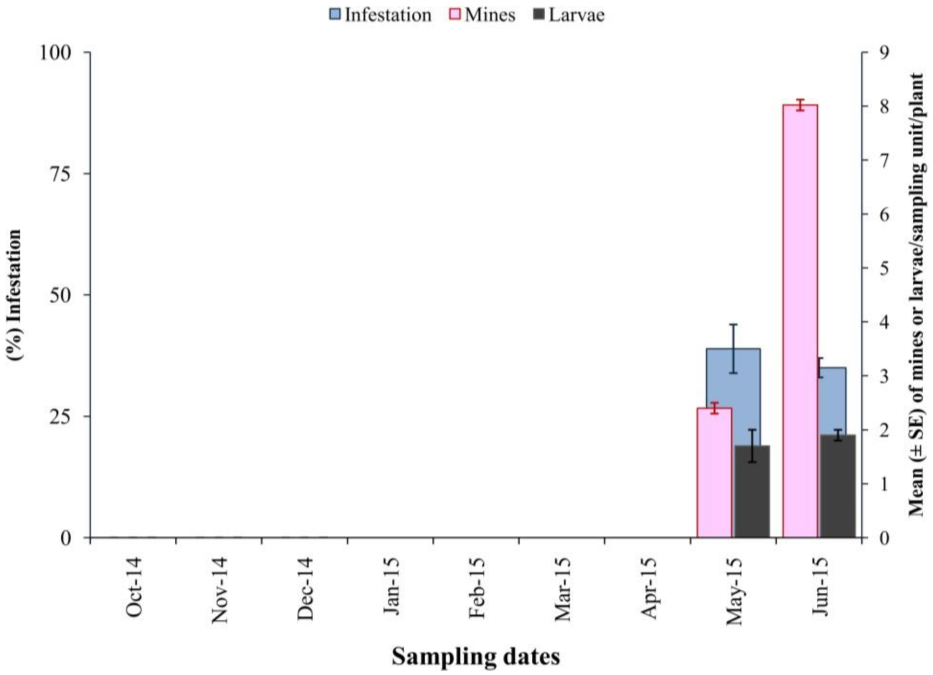

Figure 3.7: Monthly mean number (± SE) of *Tuta absoluta* mines and larvae per plant and the percentage of infested plants in greenhouse tomatoes in Gezira State.

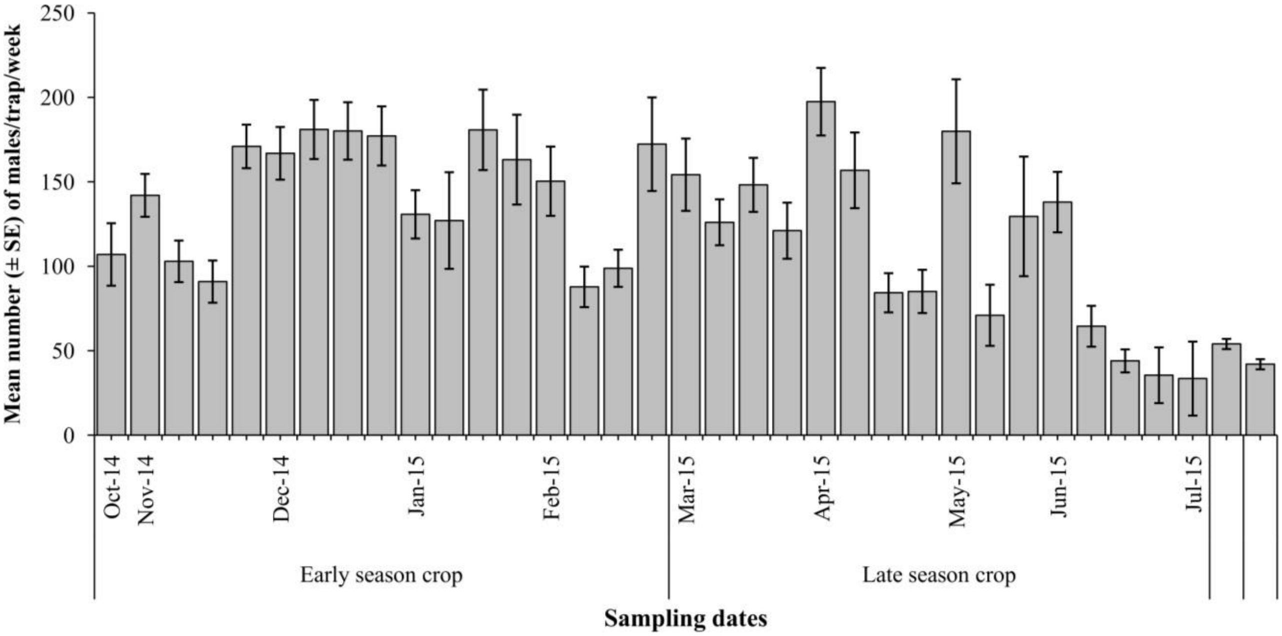

Figure 3.8: Weekly mean number (± SE) of *Tuta absoluta* males trapped in open tomato fields at Gezira State.

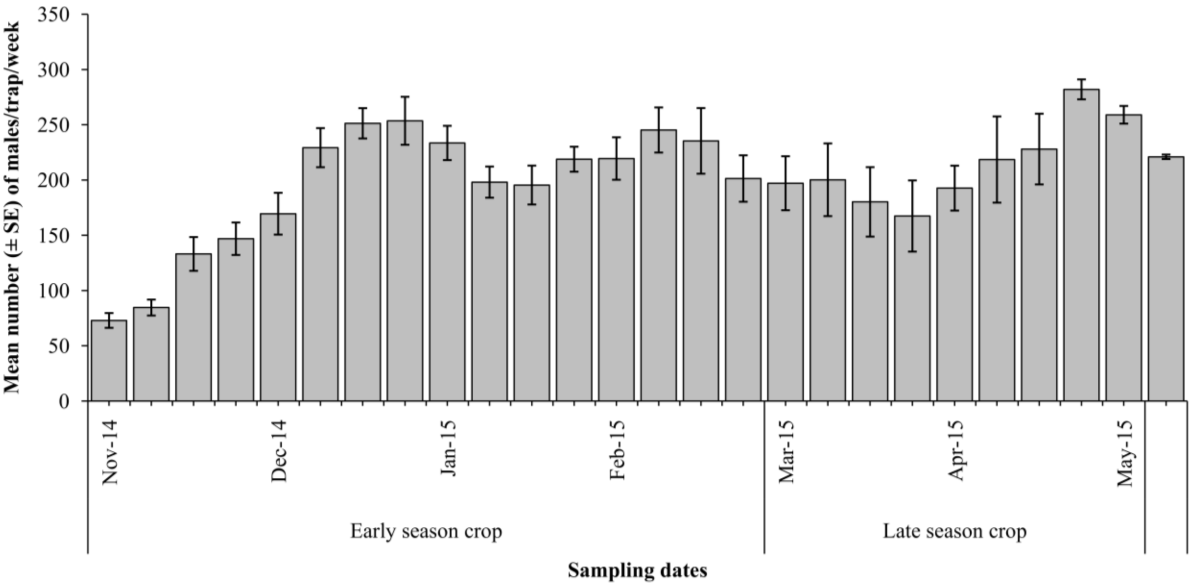

Figure 3.9: Weekly mean number (± SE) of *Tuta absoluta* males trapped in open tomato fields at Sennar State.

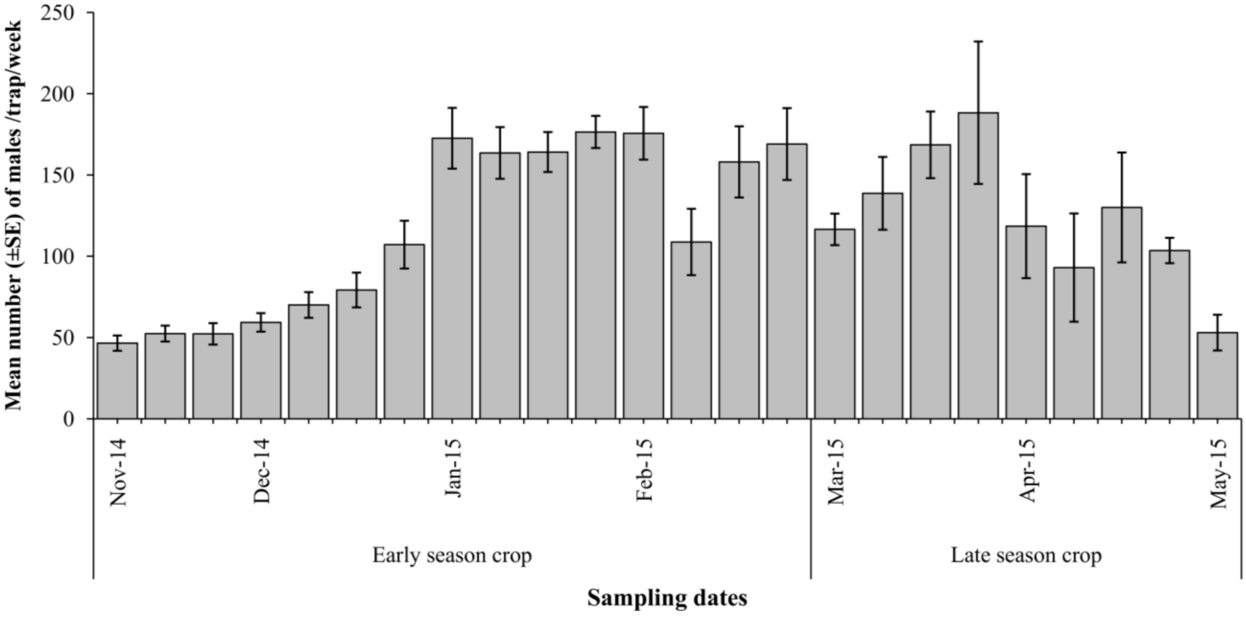

Figure 3.10: Weekly mean number (± SE) of *Tuta absoluta* males trapped in open tomato fields at White Nile State.

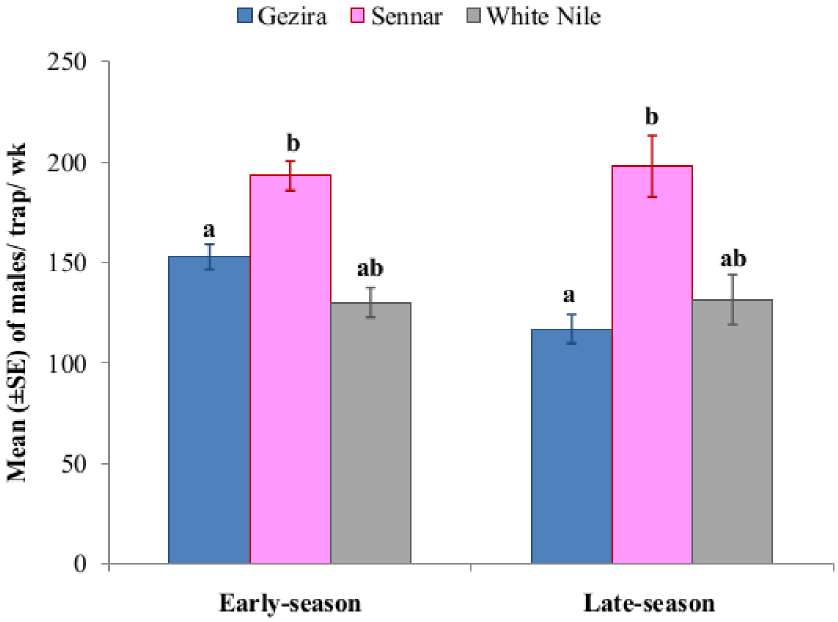

Figure 3.11: Mean number of *Tuta absolute* male moths in traps in early (October- February) and late season (March- July) in the three States. Bars capped with different letters are significantly different (Tukey's test, α = 0.05).

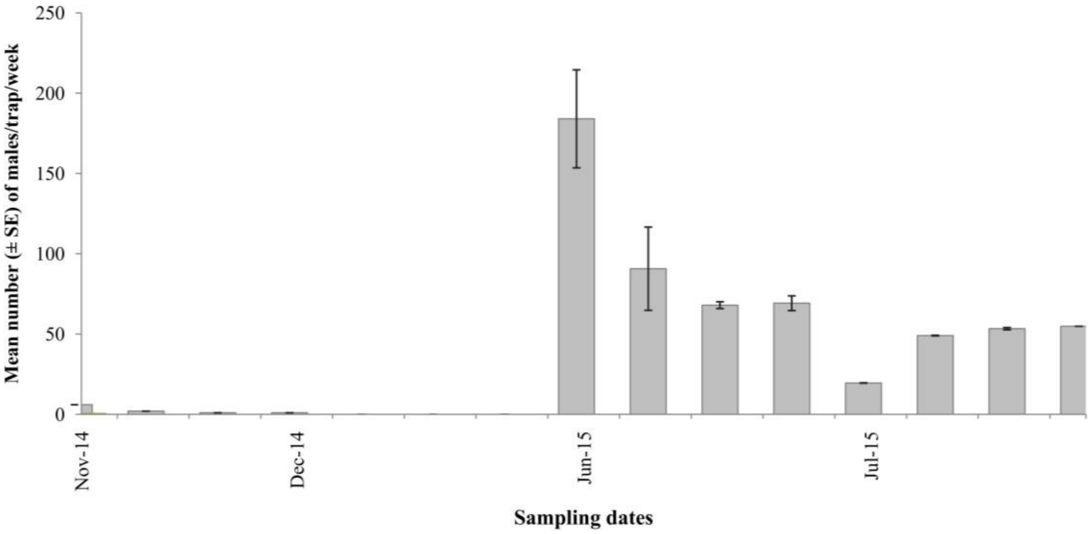

Figure 3. 12: Weekly mean number (± se) of *Tuta absoluta* males trapped in three tomato greenhouses in Gezira State.

Figure 3.13: (A) Mines in leaves caused by *Tuta absoluta* larvae in *S. melongena* (B) *S. nigrum* and (C) *S. dibium*

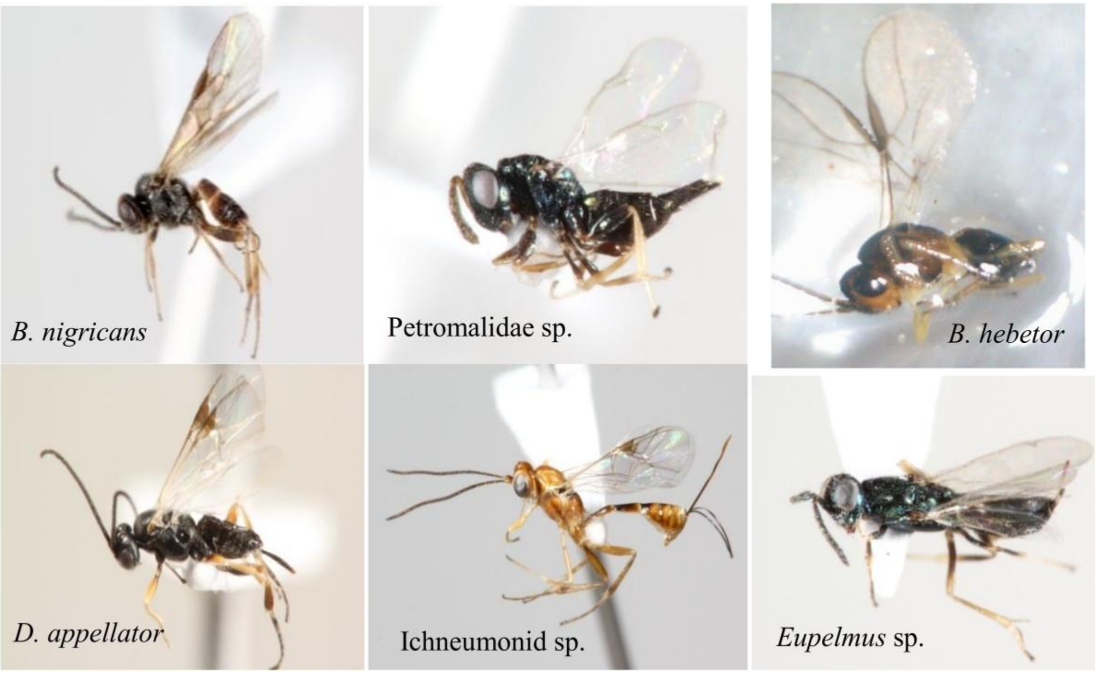

Figure 3.14: Parasitoid species collected during the survey

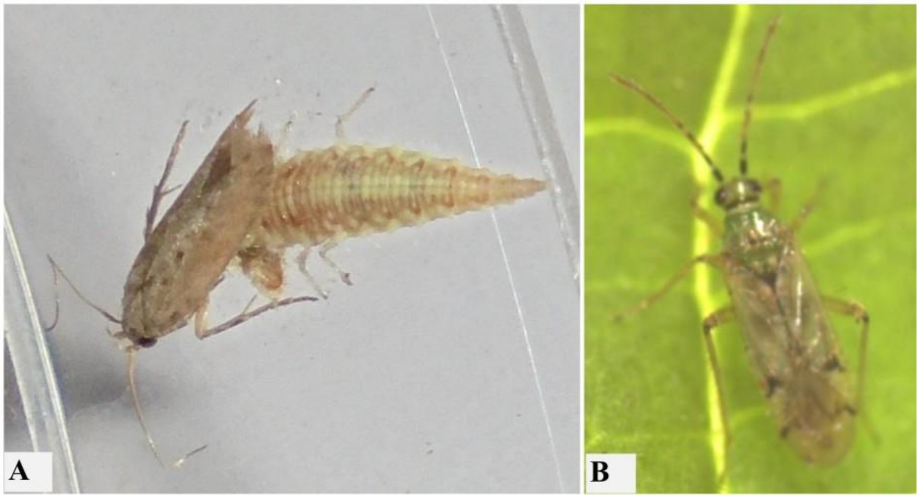

Figure 3.15: (A) A larva of *Chrysoperla* sp. feeding on a *Tuta absoluta* adult and (B) an adult of *Nesidiocoris tenuis*

Table 3.1: Locations of *Tuta absoluta* larvae and adult monitoring, and natural enemies and host plants survey sites during 2014-2016.

State	Site	Location	No. of tomato fields	Sampling methods
Khartoum	1*	15° 38' 51. 4" N 32° 31' 03.43' E	1	Natural enemies survey
	2*	15° 19' 50. 4" N 32° 50' 09.3' E	3	Leaves and fruit sampling and males monitoring
Gezira	3	15° 10' 22. 4" N 33° 02' 37.5" E	3	Larvae and adult monitoring, natural enemies and host plants survey
	4	15° 12' 38. 5" N 33° 04' 38.8" E	2	Larvae and adult monitoring, natural enemies and host plants survey
	5	15° 05' 07. 3" N 33° 11' 35.9" E	2	Larvae and adult monitoring, natural enemies and host plants survey
	6	15° 00' 08. 6" N 33° 13' 18.2" E	1	Larvae and adult monitoring, natural enemies and host plants survey
	7	14° 55' 02. 2" N 33° 11' 48.8" E	2	Larvae and adult monitoring, natural enemies and host plants survey
	8	14° 57' 17. 7" N 33° 18' 59.5" E	1	Larvae and adult monitoring, natural enemies and host plants survey
	9	14° 40' 10. 7" N 33° 11' 23.1" E	2	Larvae and adult monitoring, natural enemies and host plants survey
	10	14° 37' 02. 4" N 33° 21' 57.9" E	2	Larvae and adult monitoring, natural enemies and host plants survey
	11	14° 26' 43. 48" N 33° 22' 51. 81" E	3	Larvae and adult monitoring, natural enemies and host plants survey
	12	14° 25' 01. 5" N 33° 12' 51.8" E	4	Larvae and adult monitoring, natural enemies and host plants survey
	13*	14° 30' 31. 5" N 33° 27' 39.3" E	3	Larvae and adult monitoring and natural enemies survey
	14*	14° 22' 59. 0" N 33° 29' 27.7" E	1	Larvae and adult monitoring and natural enemies survey
	15*	14° 18' 33. 4" N 33° 34' 19.1" E	3	Larvae and adult monitoring, and natural enemies survey
	16	14° 16' 03. 2" N 33° 35' 27.9" E	4	Larvae and adult monitoring, natural enemies and host plants survey
	17	14° 11' 18. 4" N 33° 36' 47.3" E	4	Larvae and adult monitoring, natural enemies and host plants survey
	18	14° 02' 52. 6" N 33° 38' 15.2" E	4	Larvae and adult monitoring, natural enemies and host plants survey

Table 3.1: Continued

State	Site	Location	No. of tomato fields	Sampling methods
	19	13° 51' 13. 6" N 33° 34' 31.7" E	1	Larvae and adult monitoring, natural enemies and host plants survey
	20	13° 58' 21. 5" N 33° 34' 46.7" E	1	Larvae and adult monitoring, natural enemies and host plants survey
Sennar	21	13° 47' 16. 6" N 33° 32' 04.1" E	1	Larvae and adult monitoring, natural enemies and host plants survey
	22	13° 46' 10. 0" N 33° 32' 46.1" E	1	Larvae and adult monitoring, natural enemies and host plants survey
	23	13° 39' 23. 7" N 33° 36' 39.7" E	5	Larvae and adult monitoring, natural enemies and host plants survey
	24	13° 31' 42. 5" N 33° 40' 53.2" E	1	Larvae and adult monitoring, natural enemies and host plants survey
	25	13° 21' 13. 5" N 33° 45' 23.5" E	4	Larvae and adult monitoring, natural enemies and host plants survey
	26	13° 37' 57. 0" N 33° 51' 49.9" E	5	Larvae and adult monitoring, natural enemies and host plants survey
	27	13° 17' 33. 0" N 33° 50' 56.9" E	2	Larvae and adult monitoring, natural enemies and host plants survey
	28	13° 18' 32. 0" N 33° 40' 02.1" E	2	Larvae and adult monitoring, natural enemies and host plants survey
	29	13° 22' 18. 2" N 33° 38' 56.1" E	1	Larvae and adult monitoring, natural enemies and host plants survey
	30	13° 32' 13. 9" N 33° 30' 14.7" E	1	Larvae and adult monitoring, natural enemies and host plants survey
	31	12° 51' 53. 2" N 34° 01' 16.5" E	1	Larvae and adult monitoring, natural enemies and host plants survey
	32	13° 14' 06. 7" N 33° 55' 43.4" E	2	Larvae and adult monitoring, natural enemies and host plants survey
White Nile	33	13° 54' 22. 9" N 32° 21' 56.0" E	1	Adult monitoring, natural enemies and host plants survey
	34	13° 37' 04. 8" N 32° 36' 13.7" E	5	Adult monitoring, natural enemies and host plants survey
	35	13° 21' 25. 0" N 32° 36' 26.9" E	2	Adult monitoring, natural enemies and host plants survey
	36	13° 17' 51. 8" N 32° 40' 26.5" E	1	Adult monitoring, natural enemies and host plants survey

Table 3.1: Continued

State	Site	Location	No. of tomato fields	Sampling methods
	37	12° 48' 50. 6" N 32° 47' 01.7" E	2	Adult monitoring, natural enemies and host plants survey
	38	12° 40' 22. 0" N 32° 49' 07.3" E	1	Adult monitoring, natural enemies and host plants survey
	39	12° 32' 10. 0" N 32° 49' 29.4" E	2	Adult monitoring, natural enemies and host plants survey
South Darfur	40	12° 01’55.4” N 24° 55' 48. 9" E	2	Natural enemies and host plants survey
West Darfur	41	13° 26' 46. 6" N 22° 27' 49.1" E	1	Natural enemies and host plants survey
Northern	42	21° 19' 14. 6" N 30° 55' 04.4" E	3	Natural enemies and host plants survey
	43	20° 38' 50. 18" N 30° 38' 05.44" E	1	Natural enemies and host plants survey

*Greenhouses

Table 3.2: Number of pheromone traps installed in open fields and tomato greenhouses during the survey from October 2014 to July 2015 at the respective sites.

State	**No. of traps**
Gezira	53
Gezira*	14
Khartoum*	8
Sennar	44
White Nile	27
Total	146

*Greenhouses

Table 3.3: Plant species sampled from October 2014 to September 2016 to determine the host plant range of *Tuta absoluta.*

Plants species sampled	No. of fields	No. of sampling occasions
Amaranthaceae		
Amaranthus spinosus.(Spiny amaranth)*	6	18
Amaranthus viridis (Slender or Green amaranth)*	3	9
Amaranthus viridis (Amaranth)*	3	9
Apiaceae		
Coriandrum sativum (Coriander)	2	8
Aristolochiaceae		
Aristolochia bracteolata (Birthwort, pipevine or Dutchman's pipe)*	-	21
Asteraceae		
Helianthus annuus (Sunflower)	3	10
Sonchus corntus (Sow thistles)*	3	15
Xanthium stramonium (Rough cocklebur)*	25	50
Caesalpiniacae		
Cassia senna (Alexandrian senna)*	3	9
Capparisaceae		
Gynandropsisgynandra (African spider flower)*	8	24
Convolvulaceae		
Ipomoea batatas (Sweet potato)	1	3
*Ipomoea cordofana**	3	15
Cucurbitaceae		
Cucumis melo var. agrestis (Wild melon)*	3	9
Cucumis melo var. cantalupensis (Cucumber)	1	3
Cucumis melo var. flexuosus (Snake cucumber)	10	30
Cucurbitaceae		
Cucumis prophetarum	5	15
Cucurbita pepo (Pumpkin)	1	3
Euphorbiaceae		
Acalypha indica (Indian nettle)*	2	6
Euphorbia aegyptiac (Milkweed)*	5	15
Euphorbia heterophylla (Milkweed)*	5	15
Phyllanthus maderaspatensis (Soreb kabir)*	3	9

Table 3.3. Continued

Plants species sampled	**No. of fields**	**No. of Sampling occasions**
Phyllanthus niruri (Gale of the wind)*	2	6
Euphorbiaceae		
Ricinus communis (Castor oil plant)*	3	9
Fabaceae		
Arachis hypogaea (Groundnuts)	3	9
Cajanus cajan (Pigeon pea)	25	125
Cicer arietinum (Chickpea)	10	48
Indigofera hochstetteri	1	3
Medicago sativa (Alfalfa)	5	18
Phaseolus vulgaris (French bean)	4	12
*Sesabania arabica**	3	9
Vigna unguiculata (Cowpea)	1	3
Vicia faba (Faba bean)	5	15
Lamiaceae		
Leucas urticifolia *	2	6
Ocimum basilicum L.(Sweet basil)*	2	6
Malvaceae		
Abelmoschus esculentus (Wild okra)*	4	12
*Abutilon figarianum**	12	36
Gossypium barbadense (Cotton)	7	39
Abelmoschus esculentus (Okra)	10	57
Hibiscus sabdariffa (Red sorrel)*	4	15
Nyctaginaceae		
Boerhavia erectal L.(Erect spiderling)*	3	9
*Boerhavia repens**	3	9
Papaveraceae		
Argemone mexicana (Mexican poppy)*	4	12
Corchorus olitorius (Jewa Mallow)	7	21
Sorghum bicolor (Sorghum)	7	21
Portulacaceae		
Portulaca oleracea (Purslane)	4	12
Solanaceae		

Table 3.3. Continued

Plants species sampled	**No. of fields**	**No. of Sampling occasions**
Capsicum annuum (Pepper and Bell pepper)	11	76
Datura stramonium (Jimsonweed)*	15	45
Nicotiana tabacum L.(Tobacco)	1	3
S. dibium Fr. (Gubbain)*	10	30
S. lycopersicum L. (Tomato)	84	241
*S. melongena*L. (Eggplant)	14	48
*S. nigrum*L. (Nightshade)*	7	15

Weed *

Table 3.4: Number of sentinel tomato plants, *Tuta absoluta* eggs and larvae from the respective instars that were placed in tomato fields during February and March 2016 in four sites at Al Gezira Sate, Sudan.

Site	**Location**	**No. of exposed larvae of different immature stages**				
		Eggs	Instars			
			1st	2nd	3rd	4th
4	15° 12' 38. 5" N 033° 04' 38.8" E	600 (12)	300 (12)	300 (12)	150 (6)	75 (3)
10	14° 37' 02. 4" N 033° 21' 57.9" E	300 (6)	100 (4)	100 (4)	100 (4)	100 (4)
17	14° 11' 18. 4" N 033° 36' 47.3" E	800 (16)	300 (12)	300 (12)	225 (9)	200(8)
23	13° 39' 23. 7" N 033° 36' 39.7" E	800 (16)	300 (12)	300 (12)	225 (9)	200 (8)

Numbers in brackets indicate the number of potted plants

Table 3.5: Infestation level of tomato fruit at Gezira and Sennar States during 2014‒2015.

State	**Season**	**Mean no. of mines/fruit**	**Mean no. of larvae/fruit**	**% of infested fruit**
Gezira	Early	1.7	0.4	45.3
	Late	1.6	0.2	42.1
Gezira*		0.6	0.0	10.9
Sennar	Early	1.9	0.4	33.4
	Late	2.0	0.1	42.5

*Greenhouses

Table 0.6: Number. of parasitoid and predator species recovered from *Tuta absoluta* on Solanaceae plants during a survey (2014-2016).

State	**Plant species**	**Parasitoid species**						**Predator species**	
		Bracon nigricans	*Dolichogenidea appellator*	*Bracon hebetor*	*Eupelmus* sp.	Pteromaidae sp.	Ichneumonid sp.	*Chrysoperla* sp.	*Nesidiocoris tenuis*
Khartoum	*S. lycopersicum**	100% (21)	-	-	-	-	-	-	75
Gezira	*S. lycopersicum*	67.3% (259)	23.9% (92)	7.1% (27)	0.5% (2)	0.5% (2)	0.8% (3)	139	488
	*S. lycopersicum**	-	-	-	-	-	-	-	46
	S. melongena	66.7% (4)	33.3% (2)	-	-	-	-	72	6
	S. nigrum	100% (2)	-	-	-	-	-	-	-
	S. dibium	-	-	-	-	-	-	-	-
Sennar	*S. lycopersicum*	72.7% (48)	21.2% (14)	3% (2)	1.5% (1)	1.5% (1)	-	82	99
	S. melongena	50% (2)	50% (2)					29	-
	S. nigrum	-	-	-	-	-	-	-	-
	S. dibium	-	-	-	-	-	-	-	-
White Nile	*S. lycopersicum*	100% (22)	-	-	-	-	-	15	27
	S. dibium	-	-	-	-	-	-	-	-
South Darfur	*S. lycopersicum*	100% (43)		-	-	-	-	7	19
	S. melongena	-	-	-	-	-	-	3	-
West Darfur	*S. lycopersicum*	100% (15)	-	-	-	-	-	-	7
Northern	*S. lycopersicum*	100% (21)	-	-	-	-	-	13	39
	S. melongena	-	-	-	-	-	-	-	-
Total		74.8 % (437)	18.8 % (110)	4.8 % (28)	0.5 % (3)	0.5 % (3)	0.5 % (3)	30.9% (360)	69.1 % (806)

Table 0.7: Number of parasitoid species recovered from *Tuta absoluta* larvae and their parasitism rate on fruit samples.

Plant species	**No. of fruit collected**	**No. of larvae**	**No. of *T. absoluta***	**No. of parasitoid species**		
				B. nigricans	*Eupelmus* sp.	*Pteromaidae* sp.
S. lycopersicum	2629	371	203	7 (1.9%)	3 (0.8%)	1 (0.2)
*S. lycopersicum**	280	67	32	4 (1.4%)	-	-
S. melongena	162	-	-	-	-	-

*Greenhouses

Table 3.8: Number of parasitoid species recovered from sentential tomato plants and their parasitism rate.

Host stage	**No. of exposed host**	**No. of *T. absoluta* moths**	**No. of parasitoids recovered**	
			B. nigricans	*D. appelator*
Egg	2500	746 (29.8%)	-	-
1st instar	1000	278 (27.8%)	-	-
2nd instar	1000	320 (32%)	-	5 (0.5%)
3rd instar	700	212 (30.3%)	12 (1.7%)	7 (1%)
4th instar	575	58 (10%)	25 (4.3%)	-

CHAPTER 4

Population incidence of *Tuta absoluta* (Meyrick) on tomato and eggplant

4.1. Abstract

The population dynamics and damage levels of the invasive pest *T. absoluta* were investigated on tomato (*Solanum lycopersicum* L.) and eggplant (*Solanum melongena* L.) crops under open-field conditions at two sites in Gezira State, Sudan. The sites were at Wad Medani (14° 22' 59. 0"N; 033° 29' 27.7" E) and at Al Kamleen (15° 05' 07. 3"N /033° 11' 35.9" E). *Tuta absoluta* larvae were monitored by sampling the leaves and fruits of the two crops in the 2014/15 and 2015/16 cropping seasons. The numbers of larvae and mines were determined at weekly intervals during the cropping season. The larvae of the first generation *T. absoluta* appeared approximately two months after tomato and eggplant were planted. Pest density in tomato crops was significantly higher (0.3 larvae and 0.9 mines/sampling unit, respectively) than in eggplant (0.1 larvae and 0.3 mines/sampling unit, respectively).

Only *Nesidiocoris tenuis*, a *T. absoluta* predator species was recorded and from tomato fields only . No parasitoids were recorded from this trial during the study period.

4.2. Introduction

Tuta absoluta is a non-diapausing insect pest (Cocco *et al.,* 2015a). Females mate multiple times during their life time, are highly fecund and oviposit over a long period depending on the environmental conditions and diets (Desneux *et al.*, 2010; Lee *et al.,* 2014; Sannino and Espinosa, 2010; Tropea Garzia *et al.,* 2012). A single female can produce up to 260 eggs during her lifespan (Sannino and Espinosa, 2010). More than 10 overlapping generations/yr occur in South America where *T. absoluta* originates from, and 8-12 generations/yr occur in the Mediterranean region (EPPO, 2005; Cocco *et al.,* 2015a ; Sannino and Espinosa, 2010).

Tomato, *Solanum lycopersicum* L. (Solanaceae), is the main host plant of *T. absoluta* (Cocco *et al.,* 2015a; Sannino and Espinosa, 2010; Proffit *et al.*, 2011). The pest has the ability to utilize other Solanaceae plant species such as potato (*S. tuberosum* L.), eggplant (*S. melongena* L.) and nightshade (*S. nigrum* L.) (Abbes *et al.,* 2016; Arnó and Gabarra, 2011; Portakaldali *et al.,* 2013; Siqueira *et al.*, 2001; Zlof and Suffert, 2012). The abundance and distribution patterns of these crops and weeds are important factors in the population dynamics of *T. absoluta* (Abbes *et al.,* 2016; Cocco *et al.,* 2015a). However, other factors such as plant growth, physiological factors, and nutrient availability can restrict *T. absoluta* populations (Leite *et al.,* 1999; Torres *et al.,* 2001). The mean number of infested leaves (leaves with one mine or more) is considered as the infestation rate (Assaf *et al.,* 2013), while the number of mines (with live larvae)/leaf is considered as an indicator of larval density (Cocco *et al.,* 2015a,b). For fruit damage, the percentage of fruits with galleries is considered as yield loss (Balzan and Moonen, 2012).

The aim of this study was to determine the incidence of *T. absoluta* in tomato and eggplant fields.

4.3. Material and Methods

Tomato and eggplant field establishment

The study was conducted at the Agricultural Research Corporation (ARC) Farm in Wad Medani (14° 22' 59. 0"N; 033° 29' 27.7" E, 406 m above the sea level) and at Al Kamleen (15° 05' 07. 3"N /033° 11' 35.9" E, 387 above the sea level), Gezira State, Sudan, during the 2014/15 and 2015/16 production seasons. At both locations, two separate plots (60 x 70 m) were prepared with ridges at 1.4 m for tomato and at 0.8 m for eggplant. At Wad Medani, six week old seedlings of tomato (cv. Strain-B) and eggplant (cv. Black Beauty) were transplanted with an intra-ridge spacing of 0.3 m on 29 November 2014. At Al Kamleen, the two crops were directly sown into the respective plots on 18 December 2014. The seedlings were thinned four weeks after planting to obtain an intra-row spacing of 0.3 m. In the 2015/16 season, crops were directly sown into the field on 17 November 2015 at Wad Medani and 30 October 2015 at Al Kamleen. In all plots, mono-ammonium phosphate was broadcasted manually at a rate of 120 kg ha^{-1} and mixed into the soil before planting. A similar rate of nitrogen (120 kg ha^{-1} urea) was additionally applied three and six weeks after planting, while the same quantity was applied to the plants approximately four and seven weeks after sowing. Compound fertilizer NPK (20-20-20) + TE was applied as a foliar spray at weekly intervals from the fourth week after planting until the flowering stage. No insecticides was applied in both the seasons. A fungicide mixture, pyraclostrobin 40g/L + dimethomorph 72g/L was applied to all plots at a rate of 6.0 g a.i/ha pyraclostrobin and 10.8 g a.i/ha dimethomorph in a spray volume of 300 L water/ha. Applications were done four times during the season for disease control. Hand-weeding of plots was done regularly to keep the fields free of weeds.

4.3.1. *Tuta absoluta* larvae and mines on tomato and eggplant

Two leaflets from the upper and median parts of each of 25 randomly selected plants in each plot were collected weekly until the onset of senescence. The sampling period was from 24 January until 19 April 2015, and from 23 January until 30 March 2016. Leaf samples were transported in plastic bags to the laboratory and examined under a stereomicroscope (Leica WILD M3Z) to determine the number of the larvae/leaflet, mines/leaflet and the total number of *T. absoluta* infested leaves/week. The number of *Nesidiocoris tenuis* (Hemiptera: Miridae) (predator) found on leaves was also recorded. One fruit from 20 randomly selected plants from each experimental plot were also sampled weekly. Fruit damage was recorded on a grid of six sampling quadrats (1 m^2) during the second season. The number of larvae/fruit, number of mines/fruit and the proportion of infested fruits were determined. The leaves and fruit containing *T. absoluta* larvae were maintained in aerated plastic containers (2.5 L) until emergence of *T. absoluta* moths or parasitoids which were then recorded.

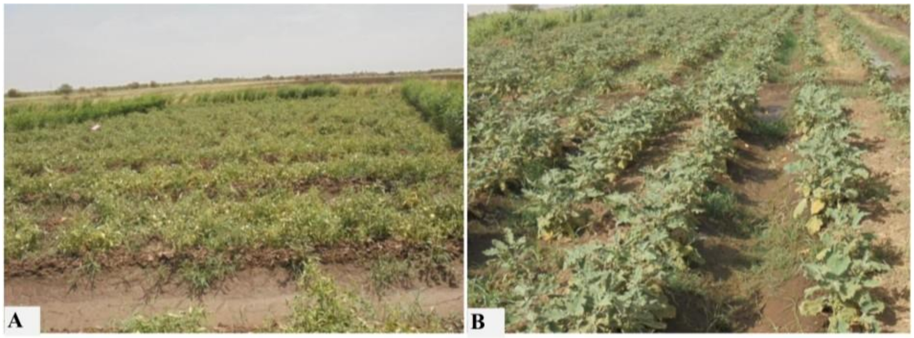

Figure 4.1: Tomato field (A) and eggplant field (B) at Al Kamleen site during 2014/15 season.

4.3.3. Data analysis

All statistical analyses were performed using R version 3.3.1 (R Core Team, 2015). The numbers of living *T. absoluta* larvae and mines/leaf were subjected to the Shapiro-Wilk normality test. To normalize the data, counts were transformed to $\log_{10}$ (counts + 1) before performing analyses. Mixed model analyses were used to evaluate the effect of the crops (tomato and eggplant) on *T. absoluta* populations levels (number of larvae) and damage symptoms (mines/leaf). The effect of seasons and sites on the number of *T. absoluta* larvae was also subjected to a mixed model analyses. Proportions of infested leaves and fruits were transformed to arcsine-square-root before analyses, but the actual percentages are presented in the figures. Percentage leaves and fruits damaged were also calculated.

4.4. Results

4.4.1. *Tuta absoluta* larvae and mines on tomato and eggplant

4.4.1.1. Season 2014/15: Tomato

Monitoring of *T. absoluta* larvae on tomato commenced late in January 2015 (2014/15 season) when the mines were noticed on leaves. At the Wad Medani site, the initial mean number of larvae was only 0.1 larvae/leaf, and the numbers gradually increased during February. The number of larvae increased rapidly in early March reaching a mean number of 0.7 larvae/leaf. Thereafter, the larval numbers decreased and remained very low until the end of the cropping season (Figure 4.2).

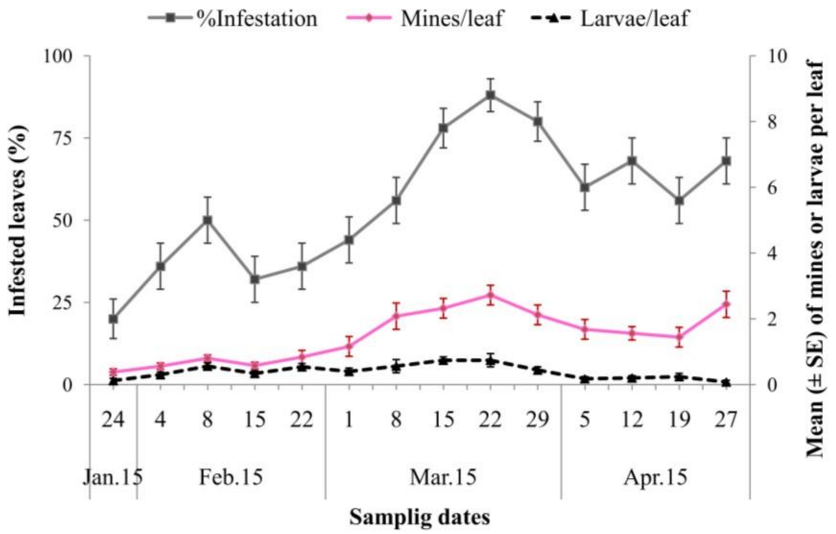

Figure 0.2: Mean number (± SE) of live *Tuta. absoluta* larvae and mines per leaf (right y-axis) and the percentage leaves infested by larvae (left y-axis) on tomato at Wad Medani in 2014/15.

At the Al Kamleen site, first notice of larvae (first generation) on tomato was in the last week of February. Numbers increased slightly from early March and reached the highest infestation level during the third week of March, but declined to zero only one week later (Figure 4.3). The mean number of mines/leaf and larval infestation rate followed almost the same pattern. The overall number of larvae and mines/leaf were significantly lower in the field at Al Kamleen than at Wad Medani (larvae: $t = 7.256$; $P <$ 0.001 and mines: $t = 12.068$; $P < 0.001$, respectively).

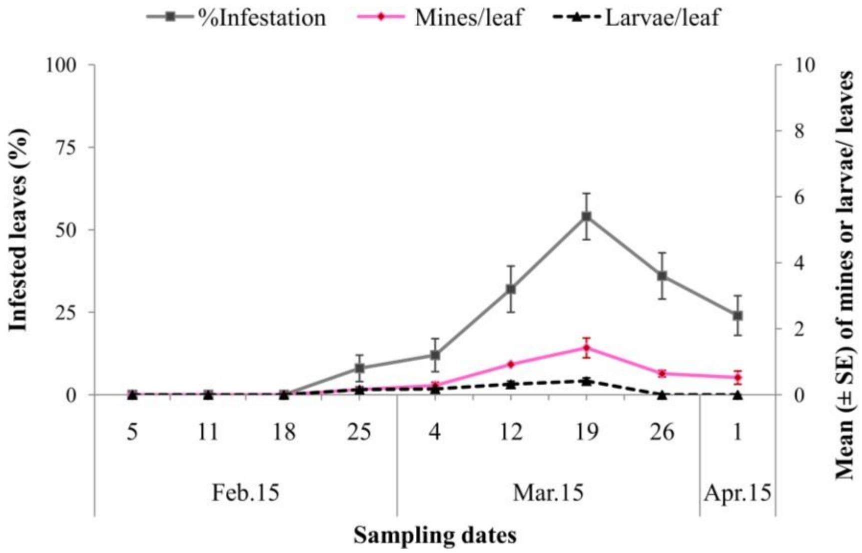

Figure 0.3: Mean number (± SE) of live *Tuta absoluta* larvae and mines per leaf (right y-axis) and the percentage leaves infested by larvae (left y-axis) on tomato at Al Kamleen in 2014/15.

4.4.1.1. Season 2014/15: Eggplant

The number of *T. absoluta* larvae on eggplant followed generally the same trend as recorded on tomato over the same period, but with lower numbers (Figure 4.4). The overall mean number of larvae/leaf was significantly lower than on tomato (t = 2.696; P = 0.007). The mean number of larvae per leaf at Wad Medani was initially very low, starting from 0.04 larvae/leaf during the second week of February and increased slowly to 0.2 larvae/leaf during the third week of March (Figure 4.4). This number then decreased to 0.04 larvae/leaf during the following week (29 March). The number of larvae remained this low until the end of the cropping season, with no significant differences among the observation dates. The highest mean number of mines/leaf (0.5 mines per leaf) was recorded on 22 March with a 25% infestation rate.

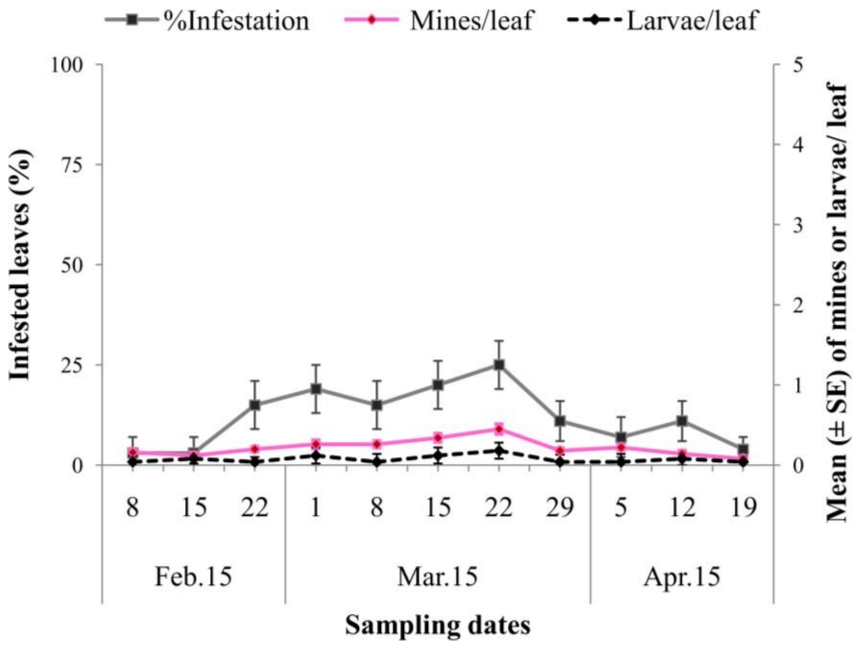

Figure 0.4: Mean number (± SE) of live *Tuta absoluta* larvae and mines per leaf (right y-axis) and the percentage leaves infested by (left y-axis) on eggplant at Wad Medani in 2014/15.

At Al Kamleen, larvae from the first generation on eggplant appeared five weeks after observations commenced. There was a mean of 0.1 larva/leaf recorded in the second week of March, and gradually increased over time (Figure 4.5). The mean number of larvae and mines did not exceed 0.2 larvae or mines/leaf during the entire observation period. Leaf infestation rate was relatively low during the season and did not exceed 19%.

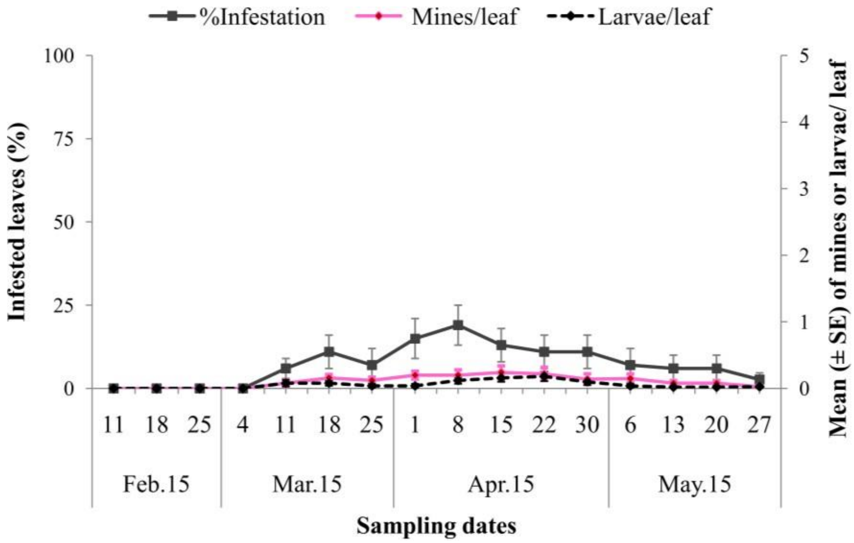

Figure 0.5: Mean number (± SE) of live *Tuta absoluta* larvae and mines per leaf (right y-axis) and the percentage leaves infested by larvae (left y-axis) on eggplant at Al Kamleen in 2014/15.

4.4.1.2. *Season 2015/16: Tomato*

The numbers of *T. absoluta* larvae at the Wad Medani site, differed significantly from that recorded in the previous year (t = 3.274; $P < 0.01$), but the 2015/16 season was also a shorter season than the 2014/15 season (Figure 4.6). Starting from the second week of February, the mean numbers of larvae/leaf increased gradually reaching its maximum level (0.4) one week before the end of the trial. Similar to the previous season, the period of peak population growth occurred in March. The maximum number of mines/leaf recorded was 1.4 and an infestation rate of 34% only.

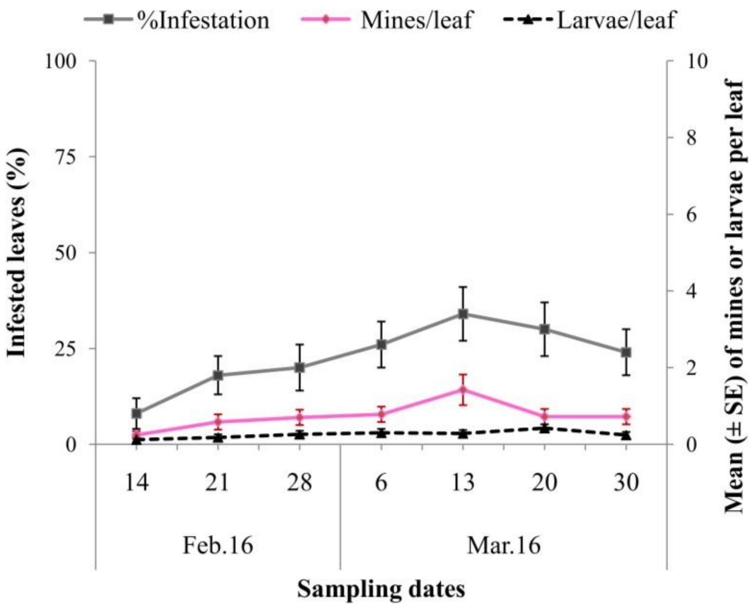

Figure 0.6: Mean number (± SE) of live *Tuta absoluta* larvae and mines per leaf (right y-axis) and the percentage leaves infested by larvae (left y-axis) on tomato at Wad Medani in 2015/16.

The number of *T. absoluta* larvae recorded on tomato followed the same trend at the Al Kamleen site as was recorded at Wad Medani. The number of larvae in the tomato crop was significantly higher ($t = 5.509$; $P < 0.001$) than in the previous season with two population peaks (Figure 4.7). There was a longer period of infestation and concurrently a longer observation time in the 2015/16 season compared to the 2014/15 cropping season. The infestation rate was also higher.

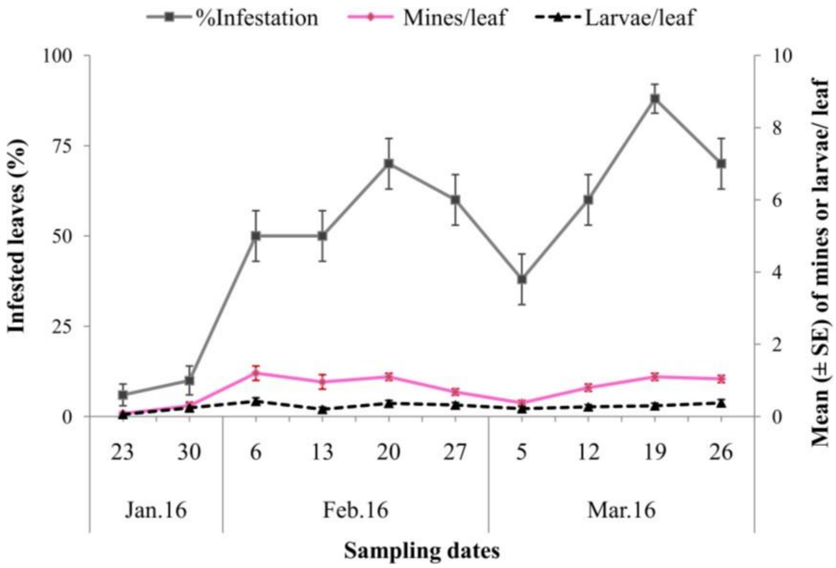

Figure 0.7: Mean number (± SE) of live *Tuta absoluta* larvae and mines per leaf (right y-axis) and the percentage leaves infested by larvae (left y-axis) on tomato at Al Kamleen in 2015/16.

4.4.1.2. Season 2015/16: Eggplant

The mean number of larvae/leaf was very low throughout the production season at Wad Medani site (Figure 4.8).

At the Al Kamleen site, larval numbers were initially at a mean of 0.02 larvae/leaf, and increased gradually throughout the remaining weeks of evaluation. Counts reached an average of 0.2 larvae/ leaf during the second week of February, but decreased later in March (Figure 4.9).

The only *T. absoluta* natural enemy that was recorded in tomato fields at both sites and seasons, was the predator, *N. tenuis*. The mean numbers of *N. Tenuis*/leaf were 0.2 ± 0.02 and 0.2 ± 0.04 in the first and second season respectively at the Wad Medani site and 0.2 ± 0.02 and 0.1± 0.01 in first and second season at Al Kamleen.

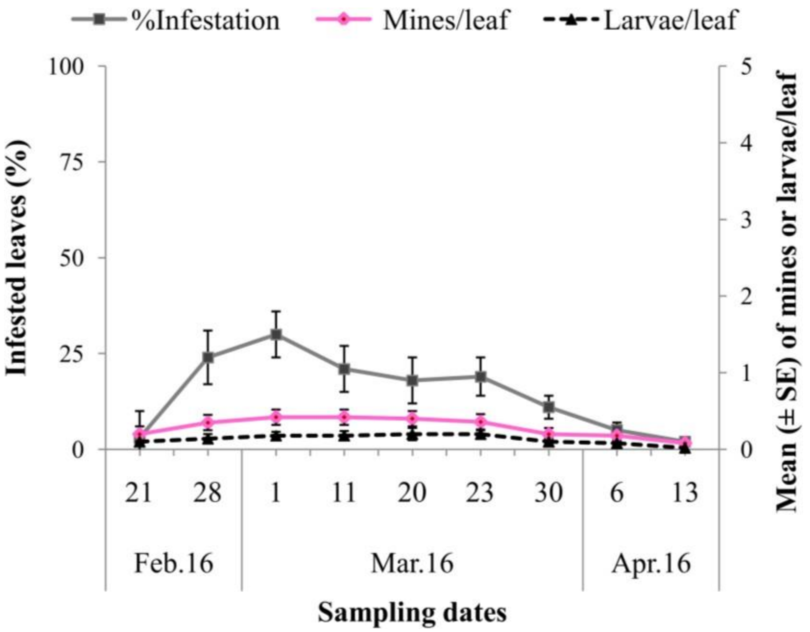

Figure 4.8: Mean number (± SE) of live *Tuta absoluta* larvae and mines per leaf (right y-axis) and the percentage leaves infested by larvae (left y-axis) on eggplant at Wad Medani in 2015/16.

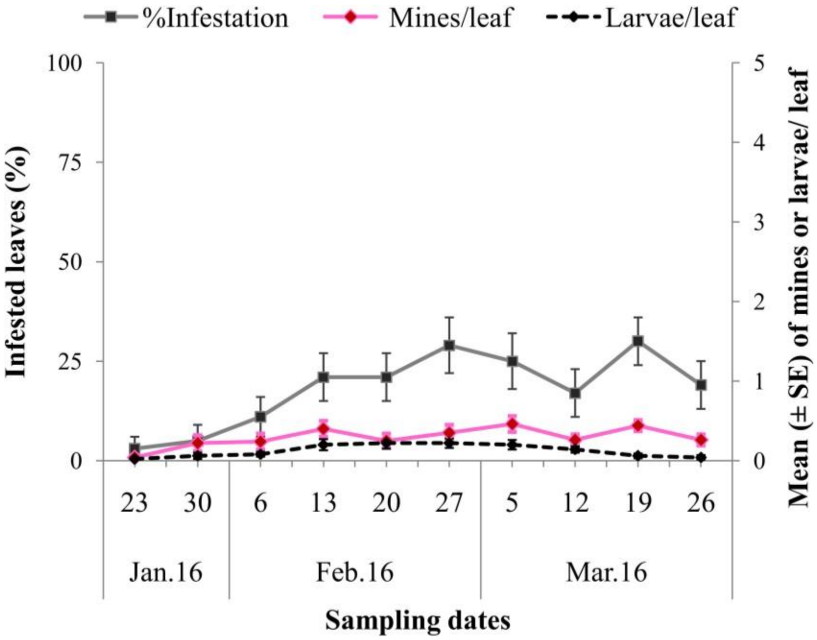

Figure 4.9: Mean number (± SE) of live *Tuta absoluta* larvae and mines per leaf (right y - axis) and the percentage leaves infested by larvae (left y - axis) on eggplant at Al Kamleen in 2015/16.

4.5. Discussion

In Sudan, tomato seed is sown directly or seedlings are transplanted in October and November as a winter season crop. The developmental duration and size of *T. absoluta* populations in both open fields and greenhouses in several countries have been shown to be positively correlated with the environmental factors such as temperature and relative humidity or rainfall (Cocco *et al.,* 2015a; Medeiros *et al.,* 2011; Sannino and Espinosa, 2010). Additionally, in open fields mated females can move from other host plants surrounding tomato fields which may extend the oviposition period and cause overlapping generations (Cocco *et al.,* 2015a).

Tuta absoluta larval numbers, were low during the entire sampling period. These numbers were much lower than the population density recorded in commercial fields in Sudan (see chapter 3) as well as to the numbers reported in a similar study carried out in Tunisia (Abbes *et al.,* 2012). Population density of *T. absoluta* on both tomato and eggplant, was low after planting, but increased gradually towards the end of both seasons. Other studies also showed low *T. absoluta* population levels during the early stages of tomato crops that gradually increased with time (Abd El-Ghany *et al.,* 2016; Balzan & Moonen 2012; Mahmoud *et al.,* 2015). The larvae of the first generation on the crops were recorded approximately two and three months after the transplanting or sowing date in the first and second seasons respectively. This is in accordance with the findings of Allache & Demnati (2012) who reported the first phenological stages of tomato plants to be free from *T. absoluta* infestation.

Larvae of the first generation developed on leaves and infested tomato fruits during the reproductive phase as a result of an increase in population density as well as the old leaves which became less favoured (Chermiti *et al.,* 2009). The number of larvae was significantly higher on the lower leaves of the plant than on the upper leaves but populations move progressively to the upper leaves (Harizanova *et al.,* 2009; Trottin-Caudal *et al.,* 2012).

Although the aim of this study was not to determine the number of generations/cropping season, on the two crops, the distinct peaks in larval numbers recorded during the sampling period could indicate the number of generations per season (Cocco *et al.,* 2015; Asma and Kaouthar, 2017).

The secondary host plant status of eggplant (Sannino and Espinosa 2010; Shehata *et al.,* 2016; Uzun *et al.,* 2015) was also confirmed in this study since very low larval densities and no infestation of fruits were recorded. Additionally, the infestation rate on eggplant leaves did not exceed 30% compared to the infestation rates on its favoured host plant tomato, which was 88%. Results from this study did not confirm the findings of Mohamed *et al.* (2012) that *T. absoluta* could attack eggplant fruit.

The percentage of infested plants or infestation based on the presence or absence of live larvae in mines on sampled leaves are useful indices (Cocco *et al.,* 2015b). However, according to Urbaneja *et al.* (2013) the percentage infestation could be an over estimation in the current study, since we counted the number of infested leaves with mines in general without any consideration if the larvae were live or dead.

Natural enemies, including hymenopteran parasitoids, predators and parasitic nematodes are reported to attack *T. absoluta* under natural conditions (Batalla-Carrera *et al.,* 2010; Desneux *et al.,* 2010; Gabarra *et al.,* 2014). Natural enemies can be used successfully to manage *T. absoluta* in greenhouses (Batalla- Carrera *et al.,* 2010; Cabello *et al.,* 2012; García-del-Pino *et al.,* 2013; Urbaneja *et al.,* 2009). However, the importance of natural enemies in suppression of *T. absoluta* populations in fields is controversial (Nannini *et al.,* 2012). Although six species of hymenopteran parasitoids of *T. absoluta* were found during an extensive survey (Chapter 3), none of these parasitoids were found in the experimental plots of this study in either the 2014/15 or 2015/16 production seasons.

4.6. Conclusion

Results from the current study showed that in winter season (November and December) *T. absoluta* infestation initiated on tomato and eggplant fields two months after planting. Eggplant proved to be a less favoured host plant and larvae were only observed to feed and develop on the leaves. This study was done during the dry season, but results from the rainy season might differ and should be investigated also.

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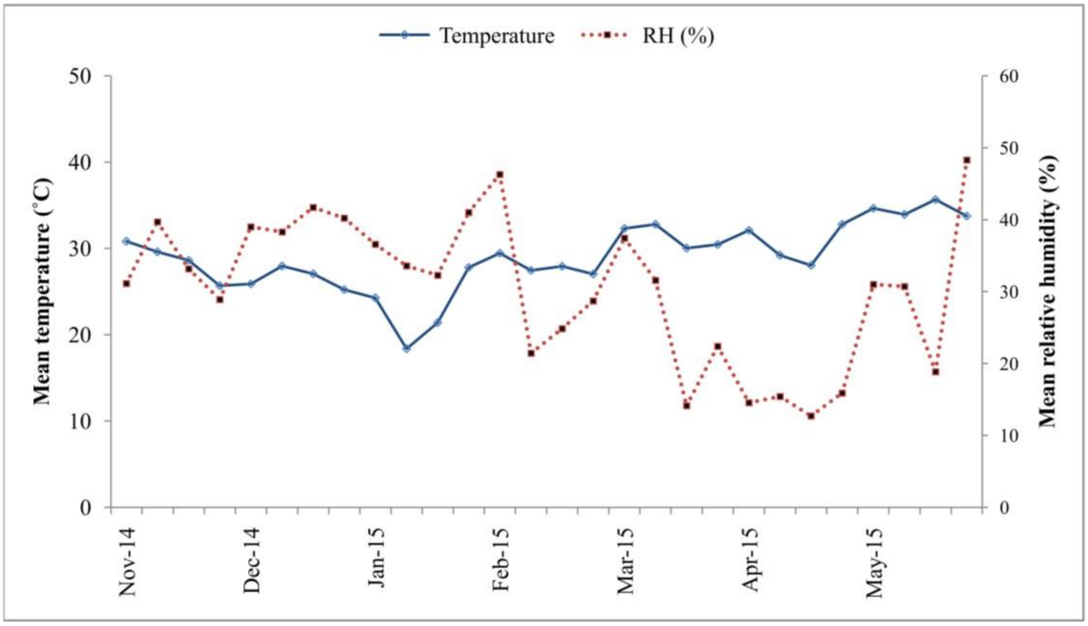

Figure 4.10: Mean weekly temperature and relative humidity in Wad Medani site season 2014/15.

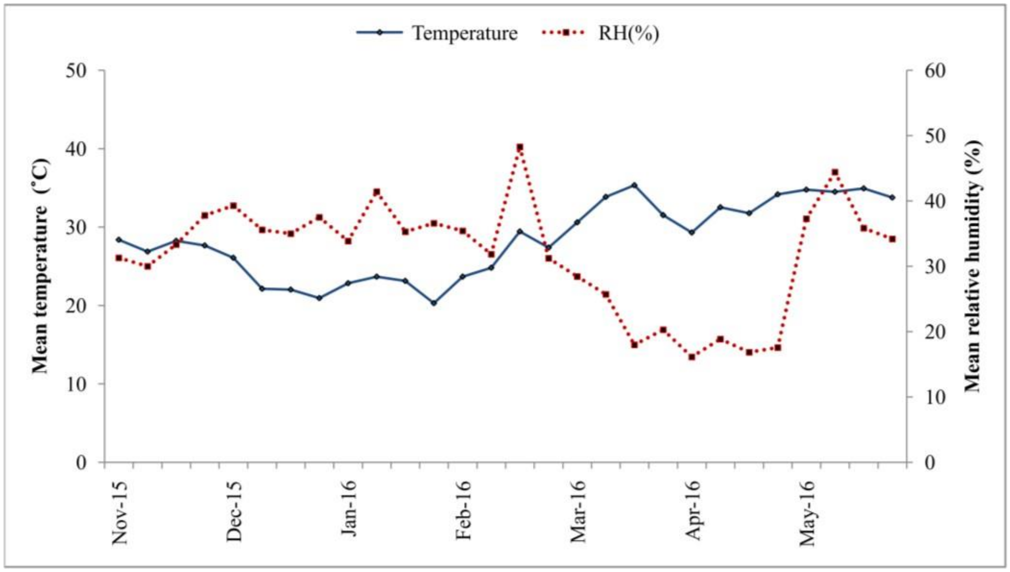

Figure 4.11: Mean weekly temperature and relative humidity in Wad Medani site season 2015/16.

CHAPTER 5

Effects of plant species on preference and performance of *Tuta absoluta*

5.1. Abstract

Since *Tuta absoluta* is a new invasive pest in Sudan, information on its bio-ecology including host plant range and the effect of these host plants on fitness traits, are virtually lacking. The effect of host plant species on the oviposition preference of moths and performance of larvae on selected host plants were evaluated in laboratory experiments. These plants were, tomato (*Solanum lycopersicum* L.), nightshade (*Solanum nigrum* L.), French bean (*Phaseolus vulgaris* L.) and peppers (*Capsicum annuum* L.). In choice and no-choice tests, tomato was the most preferred host plant for *T. absoluta* oviposition, followed by nightshade, French bean and pepper. The developmental time of *T. absoluta* was significantly affected by plant species. The larvae developed quickest on tomato followed by nightshade and French bean. The percentage survival the adult stage was significantly higher on tomato (88.5%), compared to survival on nightshade (68.5%) and French bean (3%). The male and female pupal mass and length as well as adult hind tibia length and wingspan did not differ in two consecutive generations when reared on tomato and nightshade. Fecundity and longevity as well as oviposition preference were not affected by the rearing on different host plants. The finding confirms that *T. absoluta* is an oligophagous pest with a preference for tomato. Black nightshade could be a potential source of *T. absoluta* build-up during the tomato off-season and since fecundity and longevity are not affected, it will not have a negative effect on population growth. Black nightshade is one of the major African indigenous vegetables used both as a cash and food crop. Use of this crop by *T. absoluta* as a host plant, resulted in economic losses to farmers.

5.2. Introduction

Although tomato, *Solanum lycopersicum* L. (Solanaceae) is the most preferred host plant for *T. absoluta* oviposition and also suitable for larval development, the pest feeds and survives on various other cultivated and wild solanaceous plants (Arnó and Gabarra, 2011; EPPO, 2005; Siqueira *et al.*, 2001; Zlof and Suffert, 2012). In newly invaded areas (Eoupe and Africa), it also attacks plants other than Solanaceae such as French bean, *Phaseolus vulgaris* L. (Fabaceae) (Speranza and Sannino, 2012), *Chenopodium album* L. (Chenopodiaceae) and *Convolvulus arvensis* L. (Convolvulaceae) (Portakaldali *et al.*, 2013), water melon, *Citrullus lanatus* (Cucurbitaceae), faba bean, *Vicia faba* L. (Fabaceae), alfalfa, *Medicago sativa* L. (Fabaceae), and Spiny amaranth, *Amaranthus spinosus* L. (Amaranthaceae)

(Mohamed *et al.*, 2015). The wild host plants may act as hosts for this pest during the off season from where they can later migrate to adjacent cultivated crops (Abbes *et al.*, 2016; Mohamed *et al.*, 2015). However, attractive and susceptible host plants may function as trap crops which can divert the pest away from the cultivated crops (Hokkanen, 1991). A better understanding of the oviposition preference and larval performance of *T. absoluta* on different plant species could contribute to the development of sound control strategies for this pest.

5.3. Material and Methods

Plant cultures

The seed of tomato (cv. Strain-B), nightshade, pepper and bell pepper (cv. California Wonder) were sown in separate trays (48 x 27.5 x 3.5 cm) and seedlings were later transferred into pots (1.0 L). French bean and faba bean seeds were planted directly in the pots (1.0 L). The potted plants were maintained at the same conditions as described in section 3.3.2.

Tuta absoluta stock colony

Tuta absoluta infested plants were collected from commercial fields at Gezira State (14° 02' 52. 6" N; 033° 38' 15.2" E) and reared under laboratory conditions as described in chapter 3 (see 3.3), F1 moths were used for oviposition preference tests.

5.3.1. Oviposition preference

5.3.1.1. Choice test

The oviposition preferences of *T. absoluta* towards six plant species, namely tomato, black nightshade, French bean, pepper, bell pepper and faba bean were evaluated under laboratory conditions at a constant temperature of 25 ± 1 °C and 16L:8D photoperiod. Male and female *T. absoluta* moths were kept together in rearing cages for two days after eclosion to allow for mating. Twenty male-female pairs were transferred to each of six ventilated Perspex cages (50 x 50 x 50 cm). A 10% honey solution was provided on two pieces of cotton wool/ cage. One potted plant of each of the six plant species was placed/ cage, 24 hours after the moths were released into the cages. The plants were removed after 24 hr and the number of eggs laid/plant was counted under a stereomicroscope (Leica WILD M3Z). Each plant species was kept in a separate cage (50 x 50 x 50 cm) and larvae that hatched were provided with additional plants of the same species as food until adults emerged. The number of moths and time to moth eclosion were also recorded.

5.3.1.2. No-choice test

The six plant species evaluated in the choice test for oviposition preference, were also evaluated in a no-choice test. The same procedure was followed, with the exception that only one plant of a single species was placed in the experimental cage which contained five male-female pairs of *T. absoluta* moths. There were six replicates/plant species

5.3.2. Survival and development time

Survival and development time of larvae which hatched on the respective host plant species evaluated in the choice and no-choice tests were recorded. The effect of the six plant species as well as green French bean pods on the larval performance of *T. absoluta* was also evaluated. Neonate *T. absoluta* larvae (<1 hour old) reared on tomato, were obtained from the stock colony. Neonate larvae were inoculated onto eight potted plants (25 larvae/plant) of each plant species as well as onto 40 green French bean pods. Infested plants were maintained in separate Perspex cages (50 x 50 x 50 cm).

Larval survival and was noted daily until they reached the third instar (L3). The leaves of each plant species containing larvae were cut and placed in separate transparent plastic containers (120 mm diameter x 60 mm height) and covered with fine netting material for ventilation. The larvae were provided with fresh leaves of the same plant species daily, until pupation. The number of days until pupation was recorded. Two days after pupation, a random sample of pupae were obtained from each plant species and sexed according to the sex-specific morphological differences described by Sannino and Espinosa (2010). The sexed pupae (30♂:30♀) were weighed, measured and placed into separate Petri dishes for each sex and plant species until moth emergence. Hind tibia length and wingspan of these females (*n* = 30/host plant species) were also measured.

5.3.3. Effect of plant species on fecundity and longevity of *T. absoluta* offspring (F1)

The first generation (F1) *T. absoluta* moths which emerged from pupae obtained from tomato, nightshade and French bean were used for this experiment. A single pair (♀: ♂) reared on the same host plant species was released/insect cage (6 x 12 x 12 cm) and provided with a 10% honey solution as described above. Twelve pairs originated from tomato and black nightshade were used while only 11 pairs reared on French bean were used. One compound tomato leaf (2-3 leaflets) was cut and the petiolule was placed into a small vial (8 ml) filled with tap water and covered with cotton wool around the petiolule. One vial containing a leaf was placed in each cage as an oviposition substrate. The vials were removed and replaced after 24 hours. The number of eggs laid/female was recorded daily for the entire lifespan of the female, using a stereomicroscope (Leica WILD M3Z). Males from pairs that died before the females were replaced with males which originated from the same F1 colony as their respective females. To determine adult longevity, mortality of moths was recorded daily, and dead moths were removed from the cages.

5.3.4. Effect of plant species on larval performance of F 2 populations

The development time of the F2 generation from eggs laid on tomato by the F1 females (see 5.3.5) was recorded. F2 larvae that emerged from these eggs were transferred to leaves of their initial host plant species used for F1 larval rearing. These larvae therefore had no previous feeding experience on tomato. Survival and development time of these larvae were recorded daily until adult emergence. The experiment was conducted at 25 ± 1 °C and 45 ± 2% relative humidity. The moths from these pupae were used to produce offspring (F2) for an experiment on oviposition preference (see 5.3.7).

5.3.5. Effect of larval host plant on oviposition preference of subsequent adults

The oviposition preference of first generation (F1) *T. absoluta* moths were evaluated. Naive moths (2-3 days old) from larvae reared on either tomato, black nightshade or French bean, were released inside experimental cages (15 x 15 x 20 cm). Each cage contained vials with leaves of the three host plants *viz.* tomato, black nightshade and French bean. These vials were similar to those described under section 5.3.5. Five pairs of moths (5♀: 5♂) originating from larvae reared on tomato and night shade and only one pair of moths (1♀: 1♂) from larvae reared on French bean (due to few F1 offspring) were released/cage. The numbers of eggs laid on the leaves of each host plant were counted under a stereomicroscope (Leica WILD M3Z) and recorded. There were five replicates/F1 rearing host.

The oviposition preference of *T. absoluta* F2 and F3 offspring reared on black nightshade were also evaluated using the same methodology as described for the F1 offspring. Only tomato and black nightshade plants were provided as oviposition choice in these experiments which was each replicated 10 times.

5.4. Data analysis

Prior to analyses, data were tested for normality using the Shapiro-Wilk normality test. The Generalized Linear Models (GLMs) with poisson models (link=log) was used to analyse the number of eggs laid by *T. absoluta* on different host plant species in the ovipositional preference and developmental time of immature stages. Host plants with no *T. absoluta* eggs were excluded from analyses in both ovipositional preference tests (choice and no-choice). Host plants effects on female fecundity and size (hind tibia and wingspan) of *T. absoluta* were analyzed using (GLMs) with negative binomial and quasi-poisson, respectively. The effects of host plants on *T. absoluta* survival and both pupal mass and length were analyzed using Chi-square tests of independence and T-test, respectively. Means separations were carried out using Tukey's honest significant difference (HSD) test. All data were analyzed using R (version 3.3.1; R Core Team, 2016).

5.5. Results

5.5.1. Oviposition preference

5.5.1.1. Choice test

Low numbers of eggs were laid by females in the choice test. The number of eggs laid varied significantly with host plant species ($\chi^2 = 315.81$, $df = 5$, $P < 0.001$). Eggs were laid on tomato and nightshade only, with significantly more eggs being laid on tomato. No eggs were laid on French bean, pepper, bell pepper and faba bean (Table 5.1).

Table 5.1: Effects of larval host plant species on the oviposition preference (choice test) and survival of *Tuta absoluta.*

Host plant species	**Eggs/20 females**	**%Survival (Egg to adult)**
Solanum lycopersicum	41.2 ± 6.2a	37.9 a
Solanum nigrum	17.0 ± 4.4 b	24.7 a
Phaseolus vulgaris	0.0	-
Capsicum annuum	0.0	-
Capsicum annuum)	0.0	-
Vicia faba	0.0	-

Means within a column followed by different letters are significantly different (Tukey's HSD test α = 0.05).

5.5.1.2. No-choice test

Tuta absoluta moths laid eggs on only four of the six plant species (Table 5.2), *viz*. tomato, nightshade, French bean and pepper. The number of eggs laid on the respective host plant species varied significantly ($\chi^2 = 94.15$, $df = 5$, $P < 0.001$). Significantly more eggs were laid on tomato, followed by black nightshade, French bean, and pepper. No eggs were laid on bell pepper and faba bean (Table 5.2).

Table 5.2: Effects of larval host plant species on the oviposition preference (no-choice test) and survival of *Tuta absoluta.*

Host plant species	Eggs/5females	%Survival (Egg to adult)
Solanum Lycopersicum	122.7 ± 38.7 a	52.0 a
Solanum Nigrum	35.2 ± 9.4 b	33.5 b
Phaseolus vulgaris	3.5 ± 2.9 c	0.0 c
Capsicum annuum	1.8 ± 1.8 c	0.0 c
Capsicum annuum	0.0	-
Vicia Faba	0.0	-

Means within a column followed by different letters are significantly different (Tukey's HSD test, α = 0.05).

5.5.2. Survival and development time

From the four host plant species on which *T. absoluta* laid eggs, it was only able to complete its development on tomato and black nightshade (Table 5.3), with respective percentage survival of 37.9% and 24.7%, and 52% and 33.5% in choice and no-choice test, respectively. Larvae that hatched from eggs laid on French bean died after mining into leaves as either second or third instars, while those that hatched on pepper died as neonates before tunneling into the leaves. On the two host plant species on which *T. absoluta* were successfully survived to adult, the developmental duration of larvae on black nightshade was significantly longer ($\chi^2 = 4.7$, $df = 1$, $P < 0.05$) than that on tomato (Table 5.3).

Table 5.3. Effects of host plant species on the developmental time of *Tuta absoluta* (choice test).

Host plant species	No. of initial eggs	Developmental time (days ± S. E)			
		Egg	Larva	Pupa	Adult
Solanum lycopersicum	192	6.8 ± 0.1a	10.9 ± 0.2a	10.8 ± 0.2a	27.9 ± 0.3a
Solanum nigrum	85	6.6 ± 0.1a	12.3 ± 0.8b	10.7 ± 1.0a	29.0 ± 1.2b

Means within a column followed by different letters are significantly different (GLM, α = 0.05).

All the *T. absoluta* neonates placed on tomato and black nightshade tunneled into their respective host, while only 21% (*n* = 42) were able to tunnel into French bean leaves. No larvae tunneled into the leaves of pepper, bell pepper, and faba bean as well as French bean pods.

Among the plant species into which *T. absoluta* were able to tunnel, the percentage larval survival was significantly higher (χ^2 = 364.13, *df* = 2, *P* < 0.001) on tomato and the lowest on French bean leaves (Table 5.4). The percentage pupal survival also varied with the host plant (χ^2 = 14.16, *df* = 2, *P* < 0.001), again being the highest on tomato, although there was no significant difference between tomato and black nightshade (Table 5.4). The overall percentage survival from larval stage to adult was significantly higher (χ^2 = 321.44, *df* =2, *P* < 0.001) on tomato compared to that recorded on black nightshade and French beans (Table 5.4).

Table 5.4. Effects of host plant species on the survival (%) of *Tuta absoluta* larvae on selected host plant species.

Life stages	***S. lycopersicum***	***S. nigrum***	***P. vulgaris* leaves**	**Statistical test**	***P-value***
Larvae	94.5 a	75.0 b	5.0 c	χ^2= 364.13	<0.001
Pupae	93.7 a	91.3 a	60.0 b	χ^2= 14.16	<0.001
Larvae to adult	88.5 a	68.5 b	3.0 c	χ^2= 321.44	<0.001

Percentages within a row followed by different letters are significantly different.

The mean mass and length of both female and male pupae reared on tomato and black nightshade did not differ significantly for both F1 and F2 offspring (Table 5.5). There were also no significant difference in the hind tibia length and wingspan of both the F1 and F2 *T. absoluta* offspring reared on tomato, nightshade and French bean (Table 5.6). However, development time varied with host plant species , for the larvae (χ^2 = 12.05, *df* = 2, *P* = 0.002) and pupae (χ^2 = 12.05, *df* = 2, *P* = 0.008), respectively. Larval duration was significantly shorter on tomato than on nightshade and French bean. The longest larval development time was recorded on French bean pods. The pupal duration was similar for larvae reared on tomato and nightshade, but significantly longer for pupae from larvae reared on French bean. The development time from neonate larvae to adults was also significantly affected by host plant species (χ^2 = 8.60, *df* = 2, *P* = 0.0001). The duration from neonate larvae to adults was significantly shorter on tomato than on nightshade and French bean. The longest larvae-to-adult development time was recorded on French bean (Table 5.7).

Table 5.5. Effects of host plant species on mean pupal mass and pupal length of *Tuta absoluta* offspring.

Sex	**Parameters**	**First generation**			**Second generation**		
		S. lycopersicum	***S. nigrum***	**Statistics**	***S. lycopersicum***	***S. nigrum***	**Statistics**
Females	Mass (mg)	3.01 ± 0.2	3.0 ± 01	$t = 0.09$ $P = 0.9$	2.8 ± 0.1	2.8 ± 0.1	$t = 0.37$ $P = 0.7$
Males		2.6 ± 0.1	2.4 ± 0.1	$t = 1.83$ $P = 0.07$	2.4 ± 0.04	2.5 ± 0.1	$t = 0.66$ $P = 0.5$
Females	Length (mm)	3.3 ± 0.1	3.3 ± 0.1	$t = 0.12$ $P = 0.9$	2.5 ± 0.04	2.6 ± 0.04	$t = 1.66$ $P = 0.1$
Males		3.8 ± 0.1	3.7 ± 0.03	$t = 1.34$ $P = 0.2$	2.4 ± 0.02	2.5 ± 0.04	$t = 1.29$ $P = 0.2$

Table 5.6. Effects of host plant species on the hind tibia length and wingspan of *Tuta absoluta* offspring.

Parameters	**First generation**				**Second generation**		
	Solanum lycopersicum	***Solanum nigrum***	***Phaseolus vulgaris***	**Statistics**	***Solanum lycopersicum***	***Solanum nigrum***	**Statistics**
Hind tibia (mm)	1.6 ± 0.03a	1.7 ± 0.03a	1.7 ± 0.1a	$\chi^2 = 0.09$; $P = 0.07$	1.7 ± 0.02a	1.7 ± 0.02a	$\chi^2 = 0.01$; $P = 0.2$
Wingspan (mm)	4.0 ± 0.06a	4.0 ± 0.06a	3.9 ± 0.2a	$\chi^2 = 0.04$; $P = 0.5$	3.8 ± 0.2a	4.0 ± 0.1a	$\chi^2 = 0.1$; $P = 0.5$

Table 0.7. Effects of host plant species on the developmental time of *Tuta absoluta* F2 offspring.

Plant species	**Mean no. of days ± S. E**		
	Larvae	**Pupae**	**Larvae – Adults**
S. lycopersicum	13.7 ± 0.4a	9.6 ± 0.2a	24.2 ± 0.3a
S. nigrum	15.2 ± 0.2b	9.5 ± 0.3a	25.9 ± 0.3b
P. vulgaris	17.9 ± 0.2c	12.9 ± 1.6b	27.40 ± 1.1c

Means within a column followed by different letters are significantly different (Tukey's HSD test, α = 0.05).

5.5.3. Effect of plant species on fecundity and longevity of *T. absoluta* offspring (F1)

The pre-oviposition period of *T. absolut*a females is one day. There was no significant difference in the number of eggs laid by F1 moths that originated from larvae reared on tomato, nightshade and French bean (χ^2 = 3.08, *df* = 2, *P* = 0.2). The highest egg production of *T. absoluta* moths originated from larvae that were reared on tomato was earlier (day 4), than those that originated from black nightshade (day 6) and from French bean (day 9) (Figure 5.1). In general, more than 65% of the eggs were laid during the first 10 days of a female's life span for *T. absoluta* originating from all host plants (Figure 5.1).

Longevity of females was not affected by the rearing host (χ^2 = 0.2, *df* =2, *P* = 0.9) while males lived significantly longer when larvae were reared on tomato (χ^2 = 7.48, *df* = 2, *P* = 0.02). On average male longevity were 27.8 ± 1.6, 22.8 ± 1.6 and 22.0 ± 0.9 days, and female longevity, 26.0 ± 2.2, 24.9 ± 2.2 and 25.1 ± 1.4 days , for moths originating from larvae reared on tomato, black nightshade and French bean respectively. The survival of *T. absoluta* females originated from larvae reared on the different host plant species followed a type I survival curve (Figure 5.2).

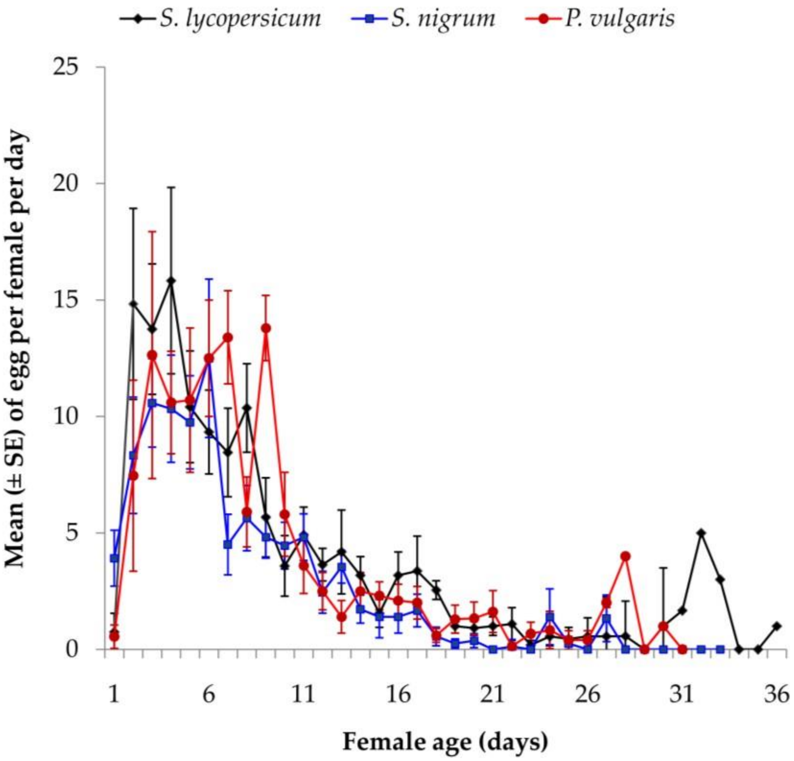

Figure 0.1: Effects of rearing host plant species (tomato, nightshade and French bean) on mean number of eggs laid daily and in a life time by *Tuta absoluta*.

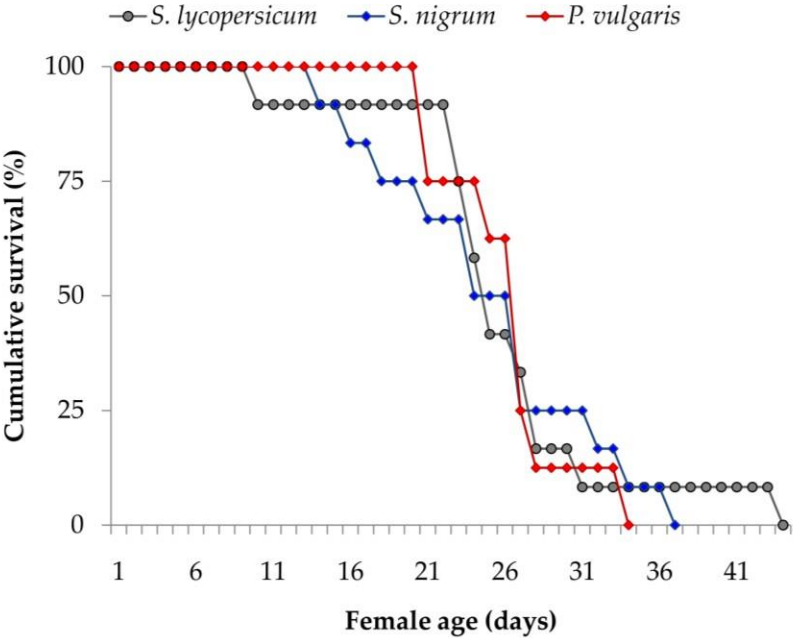

Figure 0.2: Survival curves of *Tuta absoluta* females moths reared on different host plant species at 25 ± 0.2 °C and 50 ± 2% RH.

5.5.4. Effect of plant species on larval performance of F 2 populations

There was no significant difference in the viability (χ^2 = 0.32, *df* = 2, *P* = 0.8) as well as the incubation period (χ^2 = 0.08, *df* = 2, *P* = 0.9) of the eggs laid by F1 offspring reared on the different host plant species (Figure 5.3). However, survival from larval to adult stage (χ^2 = 377.86, *df* = 2, *P* = 0.0001) (Figure 5.3), and the overall developmental duration (χ^2 = 13.9, *df* =2, *P* = 0.0001) for F2 offspring varied with the rearing host plants (Figure 5.4). Development time was significantly shorter on tomato than on nightshade and French bean, but development time was significantly longer on French bean than on night shade.

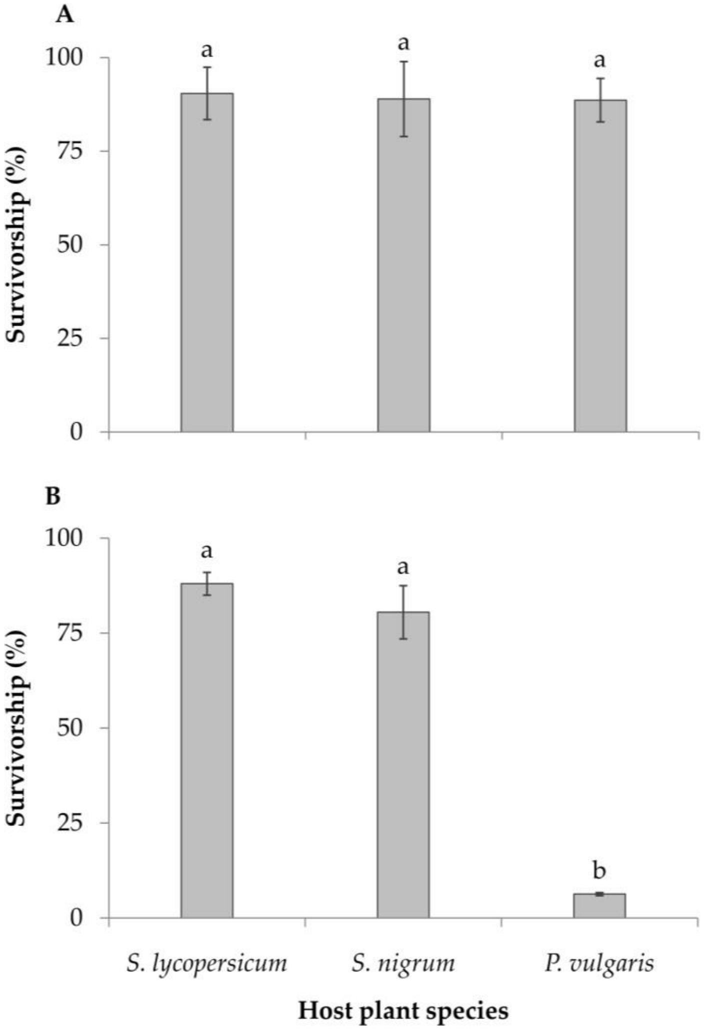

Figure 0.3: Effects of rearing host plant species (tomato, nightshade and French bean) on the offspring (F2) performance: percentage survival of eggs (A) and egg-adult (B) stage fed on different host plants. Bars caped with different lower case letters are significantly different (Tukey's test, α= 0.05).

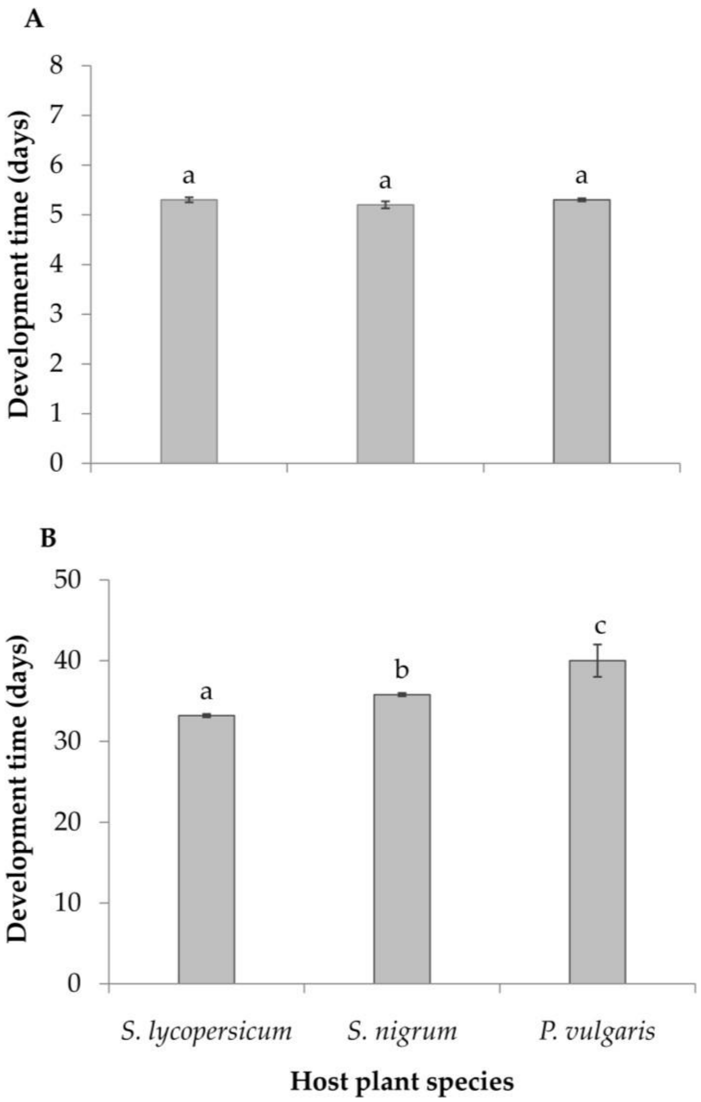

Figure 0.4: Effects of rearing host plant species (tomato, nightshade and French bean) on the development time of eggs (A) and the egg-adult stage (B) of the F2 offspring. Bars caped with different lower case letters are significantly different (Tukey's test, α= 0.05).

5.5.5. Effect of larval host plant on oviposition preference of subsequent adults

When given a choice between tomato, black nightshade and French bean, *T. absoluta* F1 offspring reared on tomato (χ^2 = 9.6, *df* = 2, *P* = 0.0001) and French bean (χ^2 = 12.77, *df* = 2, *P* = 0.002) laid significantly more eggs on tomato. However, *T. absoluta* F1 moths originating from the previous generation that was reared on black nightshade showed no significant oviposition preference for any of the three host plant species (χ^2 = 6.86, *df* = 2, *P* = 0.03) (Figure 5.5). When *T. absoluta* F2 and F3 originated from the previous generation being reared on black nightshade were provided with tomato and black nightshade for oviposition, both F2 (χ^2= 0.04, *df* = 1, *P* = 0.8) and F3 (χ^2 = 2.38, *df* = 1, *P* = 0.1) offspring did not show any significant oviposition preferences towards either host plant species (Figure 5.5).

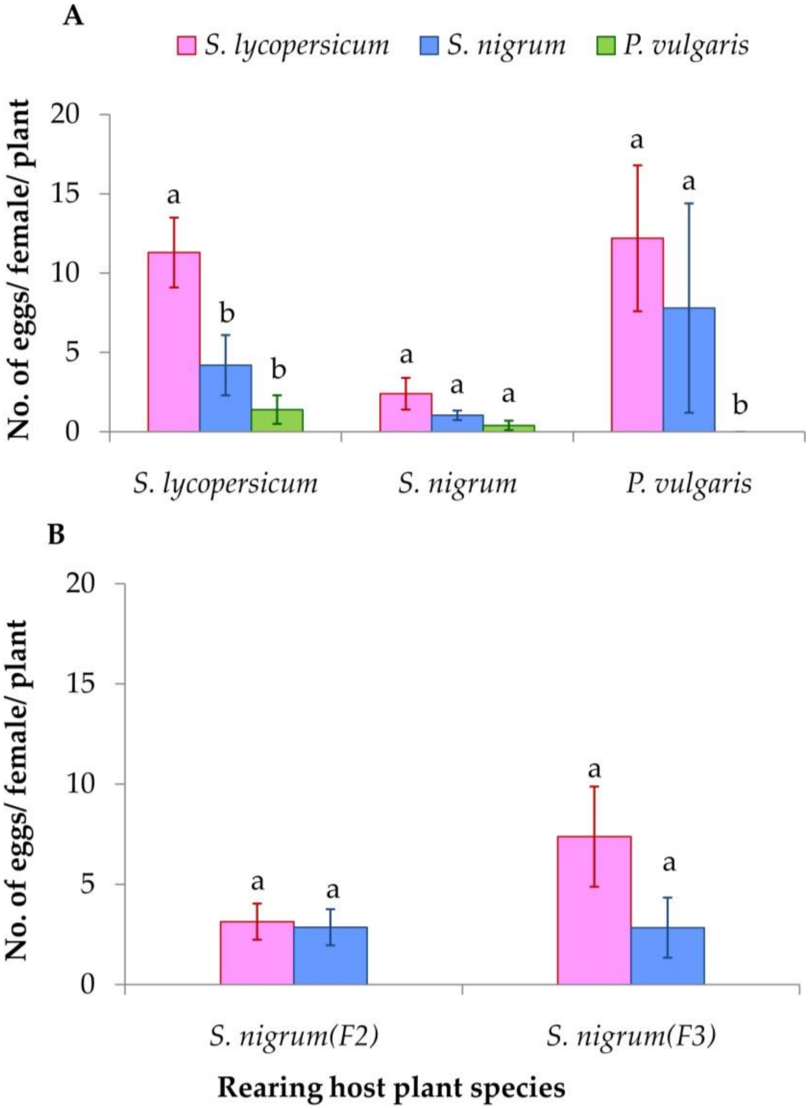

Figure 5.5: Offspring preference: (A) oviposition preference of *T. absoluta* (F1) reared on *S. lycopersicum, S. nigrum* and *P. vulgaris* for the same three host plant species (B) the oviposition preference of (F2) and (F3) reared on *S. nigrum* for *S. lycopersicum* and *S. nigrum*. Paired means indicated by the same letter do not differ significantly (Tukey's HSD test, α= 0.05).

5.6. Discussion

In the choice test, *T. absoluta* laid eggs on tomato and nightshade only, but preferred tomato. However, in no-choice tests, *T. absoluta* also oviposited on French bean and pepper, but to a lesser extent, compared to tomato and black nightshade. No eggs were laid on bell pepper and faba bean in both choice and no- choice tests. It therefore confirms the field observations of no *T. absoluta* infestations on faba bean and bell pepper. Faba beans has, however, been reported as a host for this pest by Mohamed *et al.* (2015). The oviposition preference determined in this study confirms the findings reported by Uzun *et al.* (2015), that tomato is the most preferred host for oviposition compared to eggplant and pepper in choice tests. Bawin *et al.* (2015a) also reported plants in the Solanaceae family to be the most preferred host plant species.

The oviposition preference of insect herbivores has shown to be directly linked to host plant suitability, which impacts on the survival and development of their offspring (Via, 1986; Pereyra and Sánchez, 2006; Gripenberg *et al.*, 2010; Jiang *et al.*, 2015). In the current study *T. absoluta* was able to complete its development on tomato and black nightshade only. Although eggs hatched on French bean and pepper, all larvae died on these plant species. The effect of host plants on survival of *T. absoluta* has been reported by Bawin *et al.* (2015a,b) who demonstrated that survival of *T. absoluta* larvae was high on Solanaceous plant species. The failure of *T. absoluta* to complete its development on non-Solanaceous plant species in this study is also in accordance with the findings of Bawin *et al.* (2015a). Only a small number of eggs were able to hatch on faba bean and *Malva sylvestris* L. (Malvaceae) and died as neonates before tunneling into plants leaves (Bawin *et al.*, 2015a).

In assessing larval performance on different plant species, all neonates placed on tomato and black nightshade successfully mined into their respective hosts, while none was able to mine into leaves of pepper, bell pepper, and faba bean as well as French bean pods. Similarly, Bawin *et al.* (2015a) reported that *T. absoluta* neonates inoculated onto *Beta vulgaris vulgaris* L. (Amaranthaceae), *C. album*, *C. arvensis*, *Calystegia sepium* L. Brown (Convolvulaceae) and *Nicandra physalodes* L. Gaertner (Solanaceae), were unable to tunnel into these plants. These plants were also rejected for oviposition by the moths in oviposition tests.

Tuta absoluta larvae that hatched from eggs laid on French bean were not able to complete their development on this plant species. However, a very small proportion (3%) of larvae inoculated onto French bean was able to complete their development to the adult stage. The high larval mortality on this plant could be explained by physical properties of the leaves as most of the dead larvae were found physically impaled/entrapped by trichomes present on French bean. A similar observation was made by Szyndler *et al.* (2013), who reported that French bean trichomes negatively affected the movement of

Cimex lectularius L. (Heteroptera: Cimicidae). Studies using wild tomato accessions (*S. habrochaites, S. pennellii* and *S. galapagense*) with trichomes type IV, have been shown to impair the suitability of wild tomato for different insect herbivores including *T. absoluta* (Simmons and Gurr, 2005; Maluf *et al.*, 2010; McDowell *et al.,* 2011; Mulusew, 2013). Abbes *et al.* (2016) reported that different solanaceous host plants have no effect on the development duration of *T. absoluta*. Results from this study are therefore in contrast to the findings of Abbes *et al.* (2016), but in agreement with the results reported by Pereyra and Sánchez (2006), as well as Bawin *et al.* (2015a,b). Different varieties of tomato (Gharekhani and Salek- Ebrahimi, 2014) and potato (Megido *et al.*, 2013) were also reported to significantly affect the developmental time of *T. absoluta*. Other *T. absoluta* fitness traits such as pupal mass, pupal and adult size, female fecundity and longevity were comparable among the three host plants evaluated in this study. Adult longevity and fecundity did also not differ for *T. absoluta* offspring which originated from potato, black nightshade and belladonna *Atropa belladonna* L. (Solanaceae) (Bawin *et al.*, 2015b). Pereyra and Sánchez (2006), however, reported offspring to attain a higher pupal mass and high fecundity when reared on its preferred host plants, tomato. Longevity and fecundity was found to vary with host plants (Bawin *et al.,* 2015a; Abbes *et al.*, 2016).

Previous experience or breeding host of insect herbivores affects the oviposition preference of the subsequent offspring, in what is commonly known as the Hopkins-host selection principle (HHSP) (Barron, 2001). In the current study, the ovipositional preference of *T. absoluta* offspring did not follow the HHSP principle. Most evident was those reared on French bean which showed a similar oviposition preference to their parental generation and strongly maintained tomato as the most preferred host. *Tuta absoluta* offspring (F1, F2, and F3) maintained on black nightshade showed weak associative learning as they exhibited comparable ovipositional preference to black nightshade and tomato (the pest's natural host). The lack of associative learning in *T. absoluta* has also been documented in other studies. For example, *T absoluta* females reared on tomato showed a strong preference for tomato, while those reared on potato were equally attracted to both plant species. Females did, however, oviposit a significantly higher number of eggs on tomato plants regardless of the rearing host plant (Caparros Megido *et al.*, 2014). Rearing of *T. absoluta* on a resistant tomato genotype (Aromata) did not affect the oviposition- preference of the subsequent generation (F1), and females consistently preferred the susceptible variety (Santa Clara) over the resistant variety in a choice test (Proffit *et al.*, 2011). The oviposition preference of

***T.** absoluta* reared for 15 consecutive generations on a resistant tomato genotype, was reported to be towards the susceptible genotype in choice tests (Curtinhas, 2011). Similar results of innate oviposition preferences have also been reported for other lepidopteran species, such as *Heliconius erato phyllis* Fabricius (Nymphalidae) (Kerpel and Moreira, 2005), *Papilio polyxenes* Fabricius (Papilionidae) (Heinz and Feeny, 2005) and *Polygonia c-album* L. (Nymphalidae) (Janz *et al.,* 2009). This study confirmed that *T. absoluta* is oligophagous as only four Solanaceae host plants were found to be attacked by this pest.

5.6. Conclusion

This study confirmed tomato as the preferred host plant of *T. absoluta*, but nightshade is also preferred and suitable for *T. absoluta* oviposition and larval development. French bean was found not to be suitable and it might be related to the physical properties of the plant leaves (trichomes) which significantly affected larval movement, causing high mortality. The preference and suitability of black nightshade has far reaching consequences for food and nutritional security in sub-Saharan Africa. This vegetable is one of the major African indigenous vegetables used both as a cash and food crop providing the much needed essential micronutrients for the impoverished communities. Therefore, this vegetable should be considered when developing and implementing any management strategies targeting *T. absoluta*.

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CHAPTER 6

Biology and performance of two indigenous larval parasitoids on *Tuta absoluta* (Lepidoptera: Gelechiidae) in Sudan

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RESEARCH ARTICLE

Biology and performance of two indigenous larval parasitoids on *Tuta absoluta* (Lepidoptera: Gelechiidae) in Sudan

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ABSTRACT

Tuta absoluta is an alien invasive pest in Sudan. Since it was detected in the country, the pest continues to cause major tomato losses. Alarmed by its devastating nature and the speed of spread, tomato growers resorted to the indiscriminate use of broad-spectrum insecticides. Promising indigenous parasitoid(s) may contribute to suppression of this pest. The biology and performance of the native parasitoids, *Bracon nigricans* Szépligeti (Braconidae) and *Dolichogenidea appellator* (Telenga) (Braconidae), was therefore evaluated against different immature stages of *T. absoluta* under laboratory conditions. A significantly higher number of fourth instar larvae was accepted for oviposition by *B. nigricans*. Similarly, fourth instar larvae yielded a considerably higher number of parasitoid offspring compared to third instar larvae. The male ratio of *B. nigricans* offspring which emerged from both larval instars was high, 0.96 and 0.66 for third and fourth instar larvae of *T. absoluta*, respectively. There was, however, no significant difference in the number of males and females that emerged from fourth instar larvae. Conversely, the number of hosts killed by stinging behaviour or host feeding was significantly higher on third instar larvae. *B. nigricans* potential fecundity varied with the age of the wasp with the lowest fecundity on the day of eclosion. The performance of *D. appellator* in terms of the total number of offspring produced and female progeny was similar for second and third instar larvae of *T. absoluta*. The preimaginal developmental time for both parasitoid species did not vary with either host stage or sex of the parasitoid. *B. nigricans* adult longevity was similar for both sexes, while that of *D. appellator* significantly varied with females living longer than males. Prospects for conservative biological control of *T. absoluta* are discussed in the light of the results of this study.

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1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most consumed and widely grown vegetables in the world, second to potato (Desneux, Luna, Guillemaud, & Urbaneja, 2011; Nelson, 2008). The world production is about 177 M tonnes fresh fruit produced on 4.8 million hectares (Food and Agriculture Organization [FAO], 2016). Tomato cultivation generates high economic returns for farmers, national economics, as well as creating

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employment opportunities for rural populations and other communities along the tomato value chain. In addition to its economic value, tomato plays an important role in improving human health by providing vital vitamins and minerals (Bhowmik, Kumar, Paswan, & Srivastava, 2012). In Sudan, tomato is the second most important vegetable crop next to onion, and covers about 28% of the total area under vegetable production (Ahmed, 1994, pp. 12–15). A recent FAO report estimated for Sudan the area under tomato production, annual production and average productivity to be at 46,746 ha, 617,400 tonnes and 13.2 tonnes/ha, respectively (FAO, 2016).

Among the various insect pests that hamper tomato production, the South American tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is the major pest in both native and invaded regions. Following the transatlantic invasion by *T. absoluta* into Europe in 2006 (Urbaneja, Montón, & Mollá, 2009) and subsequent spread and establishment in Africa and Asia (Sankarganesh, Firake, Sharma, Verma, & Behere, 2017; Sylla et al., 2017), tomato production on these continents has been negatively affected. Infestation of tomato by *T. absoluta* very often results in considerable reduction in yield (up to 100%) and quality of the fruit (Desneux et al., 2010). Damage by this pest occurs throughout the tomato growth cycle, from seedling to maturity. It also attacks all aerial parts of the plant including the apical buds, leaves, stems, flowers and fruits. Damage is normally caused by the larvae mining in the leaves and producing large galleries (Biondi, Guedes, Wan, & Desneux, 2018). The photosynthetic capacity of these plants is therefore reduced and the leaves eventually dry up and die. Galleries on the stems alter the development of the plant and lead to necrosis. Fruits can be attacked soon after formation and the galleries left by feeding larvae provide opportunities for the development of secondary pathogens resulting in fruit rot (Campos, Biondi, Adiga, Guedes, & Desneux, 2017). Beside the direct losses on fruit quality and quantity, indirect losses are associated with quarantine restrictions imposed by importing countries to prevent the entry and establishment of *T. absoluta*. For example, the United States Department of Agriculture–Animal and Plant Health Inspection Services (USDA–APHIS) (2011) has issued a federal order prohibiting the import of any plant material that is a host or potential host of *T. absoluta* from the countries where the pest has been recorded. *T. absoluta* has also been reported to attack other vegetable crops of economic importance such as potato and eggplant (Mohamed, Mahmoud, Elhaj, Mohamed, & Ekesi, 2015; Portakaldali, Öztemiz, & Kütük, 2013).

Management of *T. absoluta* in South America has relied heavily on application of synthetic insecticides which has resulted in the development of resistance by the pest, both in the native (Gontijo et al., 2013; Lietti, Botto, & Alzogaray, 2005; Siqueira, Guedes, Fragoso, & Magalhaes, 2001) and in the newly invaded areas (Haddi et al., 2012; Roditakis, Skarmoutsou, & Staurakaki, 2013). Moreover, several insecticides have been shown to cause significant lethal and sublethal effects to *T. absoluta* predators (Biondi, Desneux, Siscaro, & Zappalà, 2012) and parasitoids (Biondi, Zappalà, Stark, & Desneux, 2013). This scenario is likely to repeat itself in Sudan and Africa, if nothing is done to minimise the over-dependence on synthetic insecticides. Shortly after the pest invaded the Mediterranean basin, surveys for natural enemies were carried in the region. Numerous native parasitoids were identified that are able to parasitise *T. absoluta* with varying degrees of success (Gabarra et al., 2014; Zappalà et al., 2012) as well as predators which are being considered as potential biological control agents (Jaworski, Bompard, Genies, Amiens-Desneux, & Desneux, 2013; Salehi, Yarahmadi, Rasekh, & Sohani, 2016; Zappalà et al., 2013). Some of the identified parasitoids have been further evaluated under laboratory and greenhouse conditions with promising results in reducing *T. absoluta* populations (Biondi, Desneux, Amiens-Desneux, Siscaro, & Zappalà, 2013; Chailleux et al., 2012; Ferracini et al., 2012).

In Sudan, similar surveys were undertaken during which several parasitoids and predator species associated with *T. absoluta* have been reported (Mahmoud, 2013). Among these, the ectoparasitoid *Bracon nigricans* Szépligeti (Hymenoptera: Braconidae) and the endoparasitoid *Dolichogenidea appellator* (Telenga) (Hymenoptera: Braconidae) are widely distributed across the country, accounted for 94% of total species collected during a field survey, with ≈10% parasitism rate (G. E. A. Idriss, unpub.). However, information regarding their biology and potential as biological control agents of *T. absoluta* is scarce. This study examined the host-stage preference, suitability and adult longevity of both *B. nigricans* and *D. appellator* parasitising *T. absoluta* under laboratory conditions. Additionally, both the life history and female fecundity of *B. nigricans* were studied.

2. Material and methods

2.1. Tomato plants culture

Tomato plants (cv. Castlerock) were grown in seed trays filled with gardener's soil standard type containing 1.2 kg/m^3 NPK fertiliser (18:10:20) for four weeks. Plants were later transferred into pots (1.0 L) at a density of two plants per pot. Plants were kept in screen cage under natural weather conditions, watered and fertilised regularly. The plants were kept free of insecticides, whereas the fungicide Car Bio Due 112 EC (pyraclostrobin 40 g/L dimethomorph 72 g/L) was used to protect the plants from fungal diseases (5 mL in 10 L of water) until the 5–6 leaf stage (± 6–7 weeks old). These plants were used for the experiments.

2.2. T. absoluta *rearing*

The colony was initiated with an initial cohort (≈ 120♂:120♀) which was collected from Wadi Halfa (21° 19′ 14.6″N; 030° 55′ 04.4″E), (Northern Sudan), by sampling *T. absoluta*-infested tomato leaves. The collected samples were kept inside aerated 2.5 L plastics containers until adults emerge. These moths were paired (20♂:20♀) and released in Perspex cages (60 × 60 × 60 cm) containing three potted tomato plants and maintained in the laboratory maintained at 25 ± 0.5°C and a 16L:8D photoperiod.

This rearing method provided a consistent supply of *T. absoluta* of all larval stages for the experiments.

2.3. Parasitoids rearing

Laboratory colonies of *B. nigricans* and *D. appellator* were initiated with individuals (≈ 100♂:100♀ for each species) obtained from *T. absoluta*-infested tomato leaves that were collected from Wad Medani (14° 16′ 03.2″N; 033° 35′ 27.9″ E). Infested samples were placed inside Perspex cages (30 × 30 × 30 cm) until parasitoid wasps and *T. absoluta* emerged. Identification of *B. nigricans* was confirmed by Dr Konstantin Samartive (Laboratory of Experimental Entomology and Biocontrol Theory, Zoological Institute of the Russian Academy of Sciences) while *D. appellator* was identified by Dr José L. Fernández-Triana (Canadian National Collection of Insects, Arachnids, and Nematodes) and the later was further confirmed using DNA barcoding. Each parasitoid species was transferred from the incubation cages to separate Perspex rearing cages (50 × 50 × 50 cm) containing tomato plants infested with second, third and fourth instar *T. absoluta* larvae and kept in a laboratory under the conditions described above. Adult wasps were provided with a 20% diluted honey solution on a cotton wick on a daily basis. Parasitoid colonies were regularly rejuvenated by adding field-collected adult wasps.

2.4. Bioassays

2.4.1. Host-stage acceptability

The acceptability of the different larval instars of *T. absoluta* to *B. nigricans* and *D. appellator* was evaluated under the same laboratory conditions as described for insect and parasitoid rearing. Four potted tomato plants each infested with 20 individuals of either first, second, third or fourth instar *T. absoluta* larvae were placed together in an experimental cage (50 × 50 × 50 cm). Morphological differences as specified by Sannino and Espinosa (2010) were used to distinguish among the four larval instars. Twenty naïve male–female pairs of either *B. nigricans* (7–10 days old) or *D. appellator* (3–4 days old) wasps were released into a cage and allowed to forage on the larvae of the infested plants. After 24 hours exposure, the parasitoids were removed. To determine the preference and acceptability of each of the host larval stages by the parasitoids for oviposition, all exposed individuals were dissected under a stereomicroscope (Leica WILD M3Z) and the numbers of parasitised host stages as well as the number of parasitoid eggs per host stage were recorded. The experiment was replicated three times for each parasitoid species.

2.4.2. Host-stage suitability

To determine the physiological suitability of *T. absoluta* for the immature development of *B. nigricans* and *D. appellator*, various larval stages of the host were exposed to the two parasitoid species in a similar set-up as described above (Section 2.4.1). In this experiment, however, 40 naïve male–female pairs of either *B. nigricans* or *D. appellator* were exposed to four potted tomato plants each infested with 40 individuals of either first, second, third or fourth instar *T. absoluta* larvae for 24 hours. After the exposure time, these plants containing *T. absoluta* larvae were kept individually in separate cages and monitored daily until host pupation or parasitoid cocoon formation. The number of adult moths and parasitoids that emerged were sexed and recorded. The developmental times of the parasitoid offspring as well as the host larvae were recorded.

B. nigricans parasitised *T. absoluta* larvae which dropped from the tomato plants were examined under a stereomicroscope for wounds inflicted by the parasitoid ovipositor. If no parasitoid eggs were found, the larvae were regarded as dead due to stinging behaviour or feeding by the parasitoid. The experiment was replicated three times for each parasitoid species.

2.5. Adult longevity

Adult longevity of *B. nigricans* and *D. appellator* was assessed in a laboratory under the same conditions as described above. Newly emerged wasps of *B. nigricans* (25 pairs) and *D. appellator* (15 pairs) were placed in separate Perspex cages (30 × 30 × 30 cm). The wasps were provided with a 20% diluted honey solution. Wasp mortality was recorded and dead wasps were removed daily.

2.6. Potential fecundity test of female B. nigricans

Four groups of 30 (1:1 ♀:♂) newly emerged *B. nigricans* were kept in separate Petri dishes and provided with diluted honey (20%) on a small cotton wick and maintained in a laboratory under conditions described under Section 2.2. Females were dissected in a phosphate buffer solution under a stereomicroscope and the number of mature eggs per female were counted and recorded. Dissection of the first group was done approximately 8 hours after emergence and the remaining groups on the third, sixth, and ninth day post-emergence.

2.7. Statistical analyses

Data on host acceptability in terms of a number of parasitised host stages and number of parasitoid eggs/host stage were analysed using the Generalized Linear Model (GLM) with binomial regression model (link = 'logit') and Poisson regression model (link = log), respectively. The total numbers of emerged parasitoid offspring as well as the number of males and females in the host suitability tests were analysed using chi-square (χ^2) goodness-of-fit test for the hypothesis that the parasitoids choose between the respective instars on a 1:1 ratio. The GLMs with poisson regression model was applied to examine the effect of host stages on the total developmental time and the number of eggs laid per female *B. nigricans* at different days after emergence (potential fecundity of females). The adult longevity of both parasitoid species was analysed by means of the negative-binomial model to avoid data overdispersion. All statistical analyses were performed using R version 3.3.1 (R Core Team, 2016).

3. Results

3.1. Host-stage acceptability

B. nigricans did not parasitise the first and second instar larvae of *T. absoluta* (Figure 1). The fourth instar larvae were significantly more preferred for parasitism compared to the third instar larvae ($\chi^2 = 162.13$; $df = 3$; $P = 0.0001$) (Figure 1). Similarly, the number of eggs laid per larvae were significantly higher on fourth instar larvae than that on third instar larvae ($\chi^2 = 88.93$; $df = 3$; $P = 0.0001$) (Figure 1).

Results of host acceptability could not be recorded for *D. appellator* as eggs of this parasitoid could not be seen when dissecting the exposed larvae, even though adult wasps emerged from the exposed second and third instar larvae of *T. absoluta* after incubation.

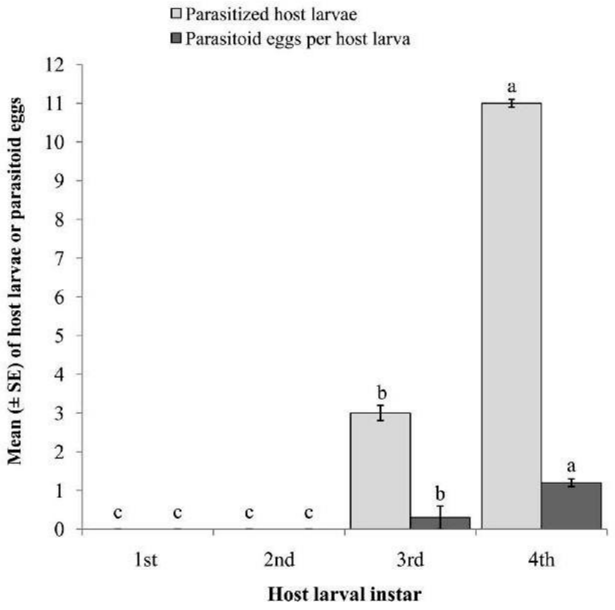

Figure 1. *T. absoluta* stages acceptance for oviposition by *B. nigricans*. Bars caped with different letters for the same category are significantly different ($\alpha = 0.05$, Tukey's test).

3.2. Host-stage suitability

Fourth instar *T. absoluta* larvae yielded a considerably higher number of *B. nigricans* offspring compared to the third instar ($\chi^2 = 31.646$; $df = 1$; $P = 0.0001$). Significantly, more female offspring was also produced when the parasitoid was reared on fourth instar compared to third instar *T. absoluta* larvae ($\chi^2 = 23.31$; $df = 1$; $P < 0.0001$) (Table 1). The sex ratio was male biased when the parasitoid was reared on third instar (0.96) but there was no significant difference in sex ratio when was reared on fourth instar (0.66) of *T. absoluta* larvae (Table 1). The number of host larvae killed by female *B. nigricans* through stinging behaviour/paralysis or host feeding was significantly higher on third instar than fourth instar ($\chi^2 = 14.3$; $df = 1$; $P = 0.0001$) (Figure 2). Mean development time of male and female *B. nigricans* was similar when reared on the third as well as the fourth instar *T. absoluta* larvae for males ($\chi^2 = 0.000$; $df = 1$; $P = 1.0$) and females ($\chi^2 = 0.025$; $df = 1$; $P = 0.9$), respectively. Developmental time of both male and female *B. nigricans* did not differ significantly when reared on third instar *T. absoluta* larvae ($\chi^2 = 0.77$; $df = 1$; $P = 0.4$) as well as on fourth instar larvae ($\chi^2 = 0.001$; $df = 1$; $P = 1.0$) (Table 2).

Table 1. Host suitability of *T. absoluta* larval instars for development of parasitoid species (*B. nigricans* and *D. appellator*).

Parasitoid species	Larval instar	Mean no. of offspring	Mean no. of females	Mean no. of males
B. nigricans	Third	4.7 ± 2.2a	0.3 ± 0.3aA	4.3 ± 1.9aB
	Fourth	21.7 ± 2.3b	9.3 ± 0.7bA	12.3 ± 2.3bA
D. appellator	Second	24 ± 1.26a	14 ± 0.6a	10 ± 0.6a
	Third	20 ± 2.1a	12 ± 1.0a	8 ± 1.2a

Note: Means in a column followed by the same lower-case letters are not significantly different ($\alpha = 0.05$, Chi-square test). Means in a row followed by the same upper-case letters are not significantly different ($\alpha = 0.05$, Chi-square test).

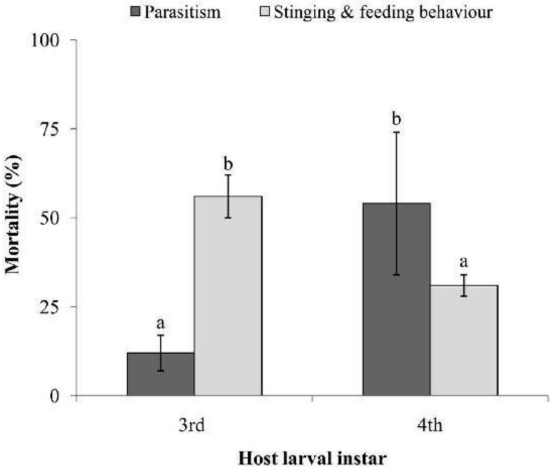

Figure 2. Percentage mortality of third and fourth instars *T. absoluta* larvae caused by parasitism and stinging and feeding behaviour of *B. nigricans*. Bars caped with different letters for the same category are significantly different ($\alpha = 0.05$, Chi-square test).

D. appellator was able to develop on second and third instars of *T. absoluta* only. The performance of the parasitoid in terms of the total number of offspring produced was similar between the two larval instars ($\chi^2 = 0.92$; $df = 1$; $P = 0.3$). There was also no significant difference in parasitoid female progeny reared from the two larval instars ($\chi^2 = 0.32$; $df = 1$; $P = 0.6$). In contrast to *B. nigricans*, both larval instars yielded a female-biased sex ratio of 0.58 (second instar) and 0.6 (third instar) (Table 1). There was also no significant difference in the developmental time of both sexes when reared on either host instar ($\chi^2 = 0.001$; $df = 1$; $P = 1.0$ and $\chi^2 = 0.012$; $df = 1$; $P = 0.9$, for second and third larval instars, respectively) (Table 2). Developmental time of the same sex did not vary with the rearing host instar ($\chi^2 = 1.48$; $df = 1$; $P = 0.2$ and $\chi^2 = 1.86$; $df = 1$; $P = 0.2$, for male and female, respectively).

3.3. Adult longevity

Longevity of adult *B. nigricans* was similar for both male (14.4 ± 1.4 days) and female (12 ± 1.3) ($\chi^2 = 3.37$; $df = 1$; $P = 0.07$). However, the mean lifespan of adult *D. appellator* varied with sex with significantly longer female longevity (8.7 ± 0.8 days) than for male (6.1 ± 0.7

Table 2. Developmental time (days ± SE) of *B. nigricans* and *D. appellator* on different larval instars of *T. absoluta*.

		Means (days ± SE)	
Parasitoid species	Larval instar	Female	Male
B. nigricans	Third	12.0 ± 0.0aA	12.6 ± 0.3aA
	Fourth	12.6 ± 0.1aA	12.6 ± 0.2aA
D. appellator	Second	19.9 ± 0.3aA	19.8 ± 0.4aA
	Third	18.5 ± 0.2aA	18.4 ± 0.3aA

Note: Means in a column followed by the same lower-case letters are not significantly different ($\alpha = 0.05$, GLM). Means in a row followed by the same upper-case letters are not significantly different ($\alpha = 0.05$, GLM).

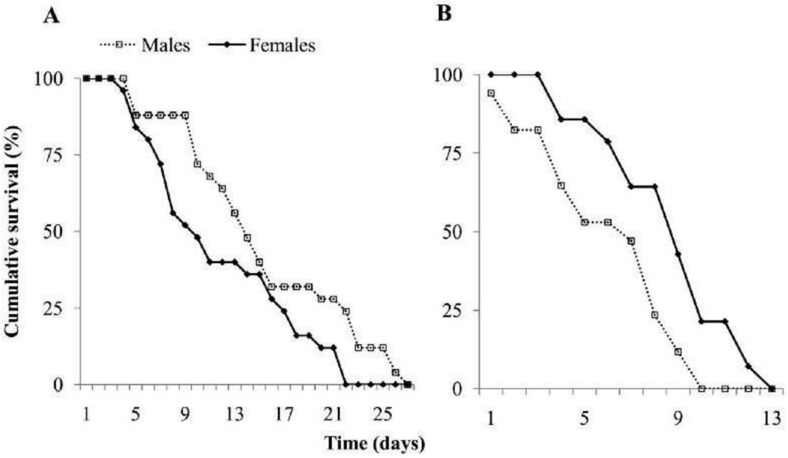

Figure 3. Survival curves of adult (A) *B. nigricans* and (B) *D. appellator* at 25 ± 0.5°C and 16D:8L photoperiod.

days) ($\chi^2 = 6.51$; $df = 1$; $P = 0.01$). The survival of the parasitoid species followed a type II survival curve and showed a constant rate of mortality throughout the entire life as shown in a straight linear line (Figure 3).

3.4. Potential fecundity of female B. nigricans

The potential fecundity of *B. nigricans* varied significantly ($\chi^2 = 171.67$; $df = 3$; $P = 0.0001$) with wasp age and it was the lowest on the day of eclosion. However, the number of mature eggs in the ovaries of three-, six- and nine-day-old wasps was similar (Figure 4).

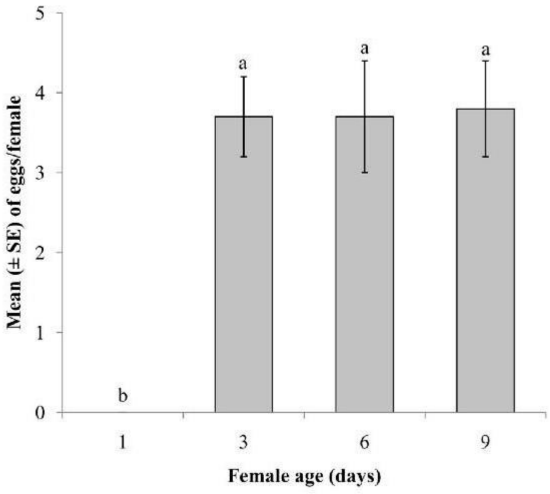

Figure 4. Potential fecundity of *B. nigricans* at different female age. Bars caped with different letters are significantly different ($\alpha = 0.05$, Tukey's test).

4. Discussion

The recent arrival of *T. absoluta* to the Mediterranean region presents its tomato industry with a serious pest problem. Many chemical insecticides have been reported ineffective against *T. absoluta* and it has already developed resistance to many classes of insecticides (Gontijo et al., 2013; Roditakis et al., 2013). The distribution of the pest has also expanded rapidly and alternate management options are limited (Biondi et al., 2018). Therefore, most of the control methods have recently focused on biological control agents. Several parasitoid species have been reported as natural enemies of the pest in the newly invaded areas (Gabarra et al., 2014; Zappalà et al., 2013). Most if not all of the reported species are primary parasitoids of other insects such as Coleoptera, Diptera and Lepidoptera (Doğanlar & Yiğit, 2011; Ferracini et al., 2012; Gabarra et al., 2014; Zappalà et al., 2012). Nevertheless, they have a significant impact in reducing *T. absoluta* populations under laboratory and greenhouse conditions (Biondi et al., 2013; Cabello et al., 2012; Chailleux, Biondi, Han, Tabone, & Desneux, 2013; El-Arnaouty et al., 2014; Ferracini et al., 2012). Braconidae is one of the most species-rich families of *T. absoluta* parasitoids (Doğanlar & Yiğit, 2011; Gabarra et al., 2014; Zappalà et al., 2012). The genus Bracon includes ecto- and endoparasitoids of *T. absoluta* larvae (Desneux et al., 2010; Zappalà et al., 2013). Among these, the gregarious ectoparasitoid *B. nigricans* is widely distributed across Europe and some countries of North Africa and the Middle East (Al-Jboory, Katbeh-bader, & Al-Zaidi, 2012; Gabarra et al., 2014; Zappalà et al., 2012; Zappalà et al., 2013).

Host preference and acceptance by female parasitoids are not only influenced by genes or geographic variations (Vos & Vet, 2004) but also vary with host condition, environmental factors, female parasitoid physiological state (King, 1987) and host nutritional quality (Li et al., 2006). In this study, female *B. nigricans* showed a strong preference and acceptance for the fourth larval instar compared to the other larval instars of *T. absoluta* when exposed simultaneously in a choice experiment. It is similar to the results reported by Biondi et al. (2013) that fourth instar larvae are the most preferred stage by *B. nigricans* females. The impact of *B. nigricans* females on *T. absoluta* larvae is not limited to the parasitism only but also includes paralysis and/or host killing for feeding purposes, which is mainly on larvae of the third instar. The preference of fourth instar larvae for oviposition and third instar larvae for feeding could be explained by the fact that the female parasitoid perceived that the former stage is of a higher nutritional quality for its immature development. This is in accordance to earlier studies has been done by Bell, Marris, Prickett, and Edwards (2005) and Zaviezo and Mills (2000) who demonstrated that variations in host size or stage have significant effects on the clutch size, survival and other various measures of fitness of gregarious parasitoids progeny. Furthermore, females of *Necremnus artynes* (Walker) (Hymenoptera: Eulophidae) prefer third instar *T. absoluta* larvae for oviposition and offspring development and second instar larvae for host-feeding (Calvo, Soriano, Bolckmans, & Belda, 2013). The higher mobility of third instar larvae as a defensive reaction could also have contributed to this stage escaping parasitism as postulated (Gentry & Dyer, 2002). In this study, most of the paralysed and/or dead *T. absoluta* third instar larvae were found outside the mines on the cage floor and free of parasitoid eggs. Generally, host acceptability mirrors host suitability as this determines the rate of offspring emergence (Li et al., 2006; Rehman & Powell, 2010; Smilanich, Dyer, & Gentry, 2009) and sex ratio (Pascua & Pascua, 2004). It was also found in this study where the most accepted host stage for oviposition by *B. nigricans* was also the most suitable stage in terms of parasitism rate and parasitoid offspring emergence. Similar findings have been reported for related parasitoid species. For example, *Eriborus argenteopilosus* (Cameron) (Hymenoptera: Ichneumonidae) had a higher parasitism rate when reared on first and second compared to third and fourth larval instars of its host *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Pascua & Pascua, 2004). Although sex ratio was slightly male-skewed when *B. nigricans* was reared on both host instars, the proportion of female progeny was significantly higher when reared on fourth instar. This suggests that *B. nigricans* can manipulate sex ratio in response to differences in the suitability of the host instars. Ode and Heinz (2002) obtained similar results for *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae), whereby the parasitoid had female-biased offspring when reared on a larger stage (third instar) compared to the smaller (second instar) of its host *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae). Similarly, Ueno (2005) found that sex ratio of *Pimpla luctuosa* (Hymenoptera: Ichneumonidae) is influenced by the size and age of its host *Galleria mellonella* L. (Lepidopteran: Pyralidae). The *T. absoluta* instar *B. nigricans* parasitised did not affect the developmental time of the offspring and the male:female ratio of the offspring. This was also found by Biondi et al. (2013) for *B. nigricans* reared on the same host stage of *T. absoluta*.

Our results clearly indicate that the females of *D. appellator* prefer to parasitise second and third *T. absoluta* instars. The total number of offspring produced and female progeny from these two *T. absoluta* instars were similar. Yazdani, Feng, Glatz, and Keller (2015) reported a significant difference in parasitism of the first three instars of *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), by the congeneric, *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae), with the highest percentage of parasitism on second instar larvae. The related Microgastrine, *Microplitis mediator* (Hymenoptera: Braconidae), was, however, reported to perform similarly on the second and third larval instars of its host, *Mythimna separata* (Lepidoptera: Noctuidae) (Li et al., 2006). There was no difference in the egg to adult developmental time for *D. appellator* on second and third instars *T. absoluta* larvae. The sex ratio of the parasitoids emerging from those instars was also not biased towards males or females. Similarly, developmental time from egg to adult of the larval endoparasitoids, *Dolichogenidea sicaria* and *Cotesia vestalis* (Braconidae, Microgastinae) was also reported unaffected by larval instars two and three of *Plutella xylostella* (Lepidoptera: Plutellidae) (Malcicka & Harvey, 2015). A difference in developmental time caused by the host stage parasitised was also reported for the parasitoid, *Microplitis mediator* Haliday (Hymenoptera: Braconidae), on *Pseudoplusia includes* Walker (Lepidoptera: Noctuidae) (Harvey & Strand, 2003). Developmental time of this parasitoid decreased consistently with instars, from the first instar onwards. Males did, however, develop faster than females regardless of the host stage.

Results from this study on the effect of host instar on fecundity of the parasitoid, *B. nigricans*, confirmed the finding of Biondi et al. (2013) on synovigenity of this parasitoid. The parasitoid eclosed with no developed eggs, but they developed with time and the number of eggs remained unchanged from the third day after eclosing onwards. This is not a typical case of a synovigenic wasp as the egg load is expected to increase with female age to a certain limit. The finding that egg load remained constant and did not increase according to female age may follow the optimal egg load theory. It predicts that the egg load should be higher for females with a high chance of encountering many hosts compared to those encountering a few/no hosts (Ellers, Sevenster, & Driessen, 2000). In other words, the ability of females to supply eggs depends in part on host availability, as this increases the female's egg load (Ellers & Jervis, 2003; Rosenheim, Heimpe, & Mangel, 2000).

Longevity of male and female *B. nigricans* without exposure to the host larvae did not differ and it ranged between 12 and 14 days. In an experiment carried out in Italy under similar conditions to that of this study, Biondi et al. (2013) reported a much longer longevity for the same parasitoid species. Differences in the longevity of the paper wasp *Polistes canadensis* (L.) (Hymenoptera: Vespidae), sourced from different locations but reared under similar conditions, were also reported (Southon, Bell, Graystock, & Sumner, 2015). In this case, the authors speculated that the difference could have been related to genetic variation in the insect populations or differences in environmental conditions. Although females of *D. appellator* lived significantly longer than males, the life span of both sexes was generally short. It is, however, known the koinobiont parasitoid species tend to be short lived (Jervis & Ferns, 2004; Jervis, Ferns, & Heimpel, 2003; Jervis, Heimpel, Ferns, Harvey, & Kidd, 2001).

From the results of this study, it can be concluded that the indigenous parasitoid species (*B. nigricans* and *D. appellator*) in Sudan are able to form new associations with the alien invasive pest *T. absoluta* with very promising results (parasitism up to 55%). Furthermore, the parasitism of the early larval instars by *D. appellator* and later instars by *B. nigricans* is indicative of the synergetic role these parasitoids can play in the suppression of *T. absoluta*. These parasitoids should, therefore, be conserved by using an eco-friendly management approach for control of *T. absoluta* instead of calendar spray of synthetic insecticides. The latter is common practice in Sudan since the pest had invaded the country.

T. absoluta being an alien invasive pest, it is imperative that classical biological control should be considered. Indeed the Microgastrine, *Dolichogenidea gelechiidivoris* Marsh (=*Apanteles Dolichogenidia*) (Hymenoptera: Braconidae) has been used for biological control in South America (Bajonero, Córdoba, Cantor, Rodríguez, & Cure, 2008; García-Suabita, Rodríguez, Cantor, & Hilarión, 2008; Riano, 2012) with parasitism of up to 70% in greenhouse tomatoes (Morales, Muñozl, Rodríguez, & Cantor, 2014). Recently, this parasitoid has been imported by International Centre of Insect Physiology and Ecology (ICIPE) from Peru for testing and subsequent release in Africa for biological control of *T. absoluta*. At ICIPE, laboratory evaluation has shown that this parasitoid prefers first instar larvae with over 70% parasitism (Mohamed, unpub.). The differential host-stage preference of this parasitoid and that of the native ones evaluated in this study is indicative of the synergistic and complementary roles these parasitoids can play in the suppression of *T. absoluta*, hence *D. gelichiidivoris* should be considered for introduction into Sudan. Both indigenous parasitoids can be mass reared and used for augmentative and inundative releases for suppression of *T. absoluta* in both open field and greenhouse tomatoes. However, more research is needed to establish their host range, a study which is currently being undertaken.

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No potential conflict of interest was reported by the authors.

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CHAPTER 7

Phylogeography studies of *Tuta absoluta* populations and identification of two indigenous parasitoids through DNA barcoding

7.1. Abstract

Studies have shown that insect pests greatly influence the management strategies of different populations of the same species. Sequence data from mitochondrial cytochrome oxidase subunit I gene (COI) was used to compare the relationship or the geographic structure of *T. absoluta* and to identify the indigenous parasitoid species of this invasive pest. The phylogenetic analyses included 85 nucleotide target sequences, and an outgroup species from GenBank, the *Tecia solanivora* (NC029386.1). Sequence analysis showed that there was a high genetic homogeneity in *T. absoluta* populations and there is therefore, no cryptic speciation within Sudan populations or among the populations from Uganda, Senegal and Tanzania using the barcode region of the mt COI gene. The identities of the parasitoid species sequences were checked using Basic Local Alignment Search Tool (BLAST). The identity of the parasitoid species (nine individuals) did not match to a named or described species in Barcode of Life Database (BOLD). However, sequences (nine individuals) of another parasitoid species matched 100% to *Dolichogenidea appellator* (Telenga) (Hymenoptera: Braconidae).

7.2. Introduction

The spread of *T. absoluta* from its native area (South America) to Europe, Africa and Asia has resulted in negative economic and biodiversity impacts (Desneux *et al.,* 2010). It has been shown that different populations of the same pest species may respond differently to different management strategies including biocontrol agents (Vass *et al.,* 1993; Kraaijeveld *et al.,* 1998; Fleury *et al.,* 2009). This may also be applicable to *T. absoluta*; therefore knowledge of the population genetic structure of this invasive pest will contribute to its management. The aim of this study was therefore to determine the genetic variability, if any, between *T. absoluta* populations sampled from various localities. To achieve this objective, DNA barcoding based on short genetic sequences of the mitochondrial cytochrome oxidase subunit I gene (COI) was used to compare or examine and compare the relationships between the populations of the same species or groups and to identify the indigenous parasitoid species.

7.3. Material and methods

7.3.1. Sampling

Tuta absoluta infested tomato and eggplant were collected from various agro-ecological zones in Sudan during the seasons of 2014 -2016 (Table 7.1). Larvae of *T. absoluta* in leaves were reared in insect cages under laboratory conditions (see chapter 3.3). *Tuta absoluta* moths were also collected from various sites in Sudan, Senegal, Uganda and Tanzania using sex pheromone traps (Table 7.1). The moths were fixed in 95% alcohol for DNA extraction. Adults of the two parasitoid species, the *Bracon nigricans* (Szépligeti) (Hymenoptera: Braconidae) and *Dolichogenidea appellator* (Telenga) (Hymenoptera: Braconidae) that emerged from parasitized *T. absoluta* larvae were reared on *T. absoluta* larvae in the laboratory according to the rearing methods described in chapter 6. Offspring of *T. absoluta* and parasitoids species were collected from each culture and placed in vials with 95% alcohol. All samples were brought to the *icipe* Molecular Pathology Laboratory in the Arthropod Pathology Unit for processing. Morphological characteristics were documented using a Leica EZD stereomicroscope (Leica Microsystems (UK) Ltd) and samples stored at – 20 °C for DNA extraction.

7.3.2. DNA extraction, PCR and sequencing

Each individual insect sample was surface-sterilized using 3% NaOCl and rinsed three times with distilled water. Genomic DNA was extracted using the Isolate II genomic DNA Kit (Bioline, London, UK), following the manufacturer’s instructions. The purity and concentration of the resultant extracted DNA was determined using Nanodrop 2000/2000c Spectrophotometer (Thermo Scientific, Wilmington, USA). Polymerase chain reaction (PCR) was done to amplify the mitochondrial region using LepF1 5’ ATTCAACCAATCATAAAGATATTGG 3’ and LepR1 5’ TAAACTTCTGGATGTCCAAAAAATCA 3’ primers (Hajibabaei *et al*., 2006). The PCR was carried out in a total reaction volume of 20 µL containing 5X My *Taq* Reaction Buffer (5 mM dNTPs, 15 mM $MgCl_{2,}$ stabilizers and enhancers), 10 µmole of each primer, 0.5 mM $MgCl_2$, 0.25 µL My *Taq* DNA polymerase (Bioline, London, UK) and 15 ng/µL of DNA template. This reaction was set up in the Nexus Mastercycler gradient (Eppendorf). The following cycling conditions were used: initial denaturation for 2 min at 95 °C, followed by 40 cycles of 30 sec at 95 °C, 45 sec annealing at 52 °C and 1 min at 72 °C, then a final elongation step of 10 min at 72 °C. The target gene region was 700 base pairs.

The amplified PCR products were resolved through a 1.2% agarose gel. DNA bands on the gel were analyzed and documented using KETA GL imaging system trans-illuminator (Wealtec Corp, Meadowvale Way Sparks, Nevada, USA). Successfully amplified products were excised and purified using Isolate II PCR and Gel Kit (Bioline, London UK) following the manufacturer’s instructions. The purified samples were shipped to Macrogen Inc. Europe Laboratory, the Netherlands, for bi-directional sequencing.

Table 0.1: *Tuta absoluta* and parasitoid collection sites in Sudan, Senegal Uganda and Tanzania.

Sample site		Species	Host plant	Collection method	No. of specimens	GPS coordinates
Sudan						
	South Darfur State	*T. absoluta*	Eggplant	Trap	5	12° 01’55.4" N 024° 55' 48. 9" E
	South Darfur State	*T. absoluta*	Tomato	Lab culture	5	12° 01’55.4" N 024° 55' 48. 9" E
	South Darfur State	*T. absoluta*	Tomato	Trap	10	12° 01’55.4" N 024° 55' 48. 9" E
	West Darfur State	*T. absoluta*	Tomato	Trap	5	12° 54' 21. 6" N 023° 28' 08.7" E
	Central Darfur State	*T. absoluta*	Tomato	Trap	10	12° 54' 21. 6"N 023° 28' 08.7"E
	Northern State	*T. absoluta*	Tomato	Lab culture	10	21° 19' 14. 6" N 030° 55' 04.4" E
	Northern State	*T. absoluta*	Tomato	Trap	10	18° 47' 44. 55" N 030° 32' 46.63"E
	Nile State	*T. absoluta*	Tomato	Trap	10	16° 40' 56. 88"N 033° 24' 18.23"E
	Kassala State	*T. absoluta*	Tomato	Trap	10	15° 28' 24. 32" N 036° 22' 14.75" E
	Khartoum State	*T. absoluta*	Tomato	Lab culture	5	15° 38' 51. 4" N 032° 31' 03.43" E
	Sennar State	*T. absoluta*	Tomato	Trap	10	12° 51' 53. 2" N 034° 01' 16.5" E
	Sennar State	*T. absoluta*	Tomato	Lab culture	10	13° 37' 57. 0" N 033° 51' 49.9" E
	Gezira State	*T. absoluta*	Tomato	Lab culture	10	
	Gezira State	*B. nigricans*	Tomato	Lab culture	10	14° 16' 03. 2" N 033° 35' 27.9" E
	Gezira State	*D. appellator*	Tomato	Lab culture	10	14° 16' 03. 2" N 033° 35' 27.9" E
	Gezira State	*T. absoluta*	Tomato	Lab culture	10	

	Gezira State	*T. absoluta*	Tomato	Lab culture	5	14° 18' 33. 4" N 033° 34' 19.1"E
Senegal	LacRose	*T. absoluta*	Tomato	Trap	10	14°49.625 N 17°15.117 W
Uganda	Mukono	*T. absoluta*	Tomato	Trap	10	00°28'34.464" N 032°48'19.187" E
Tanzania	Nyamle-Mwanza	*T. absoluta*	Tomato	Trap	10	02°42'52.0" S 033°02'57.0" E

7.3.3. Data analysis

The successful sequences were assembled and edited using Chromas Lite Version 2.1.1 (Thompson *et al*., 1997) and Geneious Version 8 (http://www.geneious.com) (Kearse *et al*., 2012). The primer sequences were identified and removed from the consensus sequences generated from both the forward and reverse reads. Phylogenetic and molecular evolutionary analyses were conducted using MEGA X (Kumar *et al*., 2018) using the Maximum Likelihood method (Tamura and Nei, 1993). The reliability of the tree was assessed using 1000 bootstrap replications. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 83 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 1538 positions in the final dataset. Similarity searches were conducted by querying the consensus sequences via BLAST at the GenBank database hosted by National Centre of Biotechnology Information (NCBI). Basic Local Alignment Search Tool (BLAST) algorithm finds regions of local similarity between sequences, in which consensus sequences were compared to reference sequences in the GenBank database. In addition to this, a query was also done in Barcode of Life Database (BOLD). Estimates of evolutionary divergence analyses between sequences were conducted using the Maximum Composite Likelihood model (Tamura *et al*., 2004). The analysis involved 16 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 1535 positions in the final evolutionary divergence dataset. The evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018).

7.4. Results

Summary of the identities of the processed samples

The evolutionary history by MEGA X yielded a tree with the highest log likelihood of -2778.02 as shown in figure7 7.1. All *T. absoluta* samples from the different localities/regions and host plants clustered together in one branch with very low variabilities. The outgroup included in the analysis, *Tecia solanivora* (Povolný, 1973) distinctly branched on its own. This low variability between the sample sequences was observed in the estimates of evolutionary divergence analyses (Table 7.3). A total of 18 individuals (nine specimens/ species) of *B. nigricans* and *D. appellator* were successfully sequenced and the sequences were deposited at the GenBank with accession numbers FJ413730.1 and HM396614.1 for

B. nigricans and *D. Appellator*, respectively. The BLAST search through the NCBI BLASTN for the parasitoid with the accession number FJ413730.1 (*B. nigricans*) did not match with or link to any sequences in the BOLD. However, BLAST analysis of the parasitoid species with accession number HM396614.1 revealed 100% identity of the sequenced specimens with specimens of *D. appellator* from Israel, Pakistan, Russia and United Arab Emirates (sequences in the BOLD with Barcode Index Number Registry BOLD: AAD2295). The identities of *T. absoluta* and the parasitoid species are provided in Table 7.2.

Table 0.2: **Summary of the identities of the *Tuta absoluta* samples and the parasitoids characterized**

Sample name	**ID from GenBank**	**Accession no.**	**Querry %**	**E-value**	**ID %**	**ID from BOLD**
Eggplant-1–5	*T. absoluta* COI	KY212128.1	100	0	99	*T. absoluta* (100%)
Gazira-2, 3, 4, 7	*T. absoluta* COI	KY212128.1	100	0	99	*T. absoluta* (100%)
Genina-1–5	*T. absoluta* COI	KY212128.1	100	0	100	*T. absoluta* (100%)
Halfa-1–10	*T. absoluta* COI	KY212128.1	100	0	100	*T. absoluta* (100%)
Hillat-1 – 5	*T. absoluta* COI	KY212128.1	100	0	100	*T. absoluta* (100%)
Kassala-1–10	*T. absoluta* COI	KY212128.1	100	0	100	*T. absoluta* (100%)
Khartoum-1–5	*T. absoluta* COI	KY212128.1	100	0	100	*T. absoluta* (100%)
Lawni-1–10	*T. absoluta* COI	KY212128.1	100	0	100	*T. absoluta* (100%)
Nyala2-1–5	*T. absoluta* COI	KY212128.1	100	0	100	*T. absoluta* (100%)
Nyala-1, 4, 6, 7	*T. absoluta* COI	KY212128.1	100	0	100	*T. absoluta* (100%)
Senegal-2–6	*T. absoluta* COI	KY212128.1	100	0	100	*T. absoluta* (100%)
Sinnar-2, 3, 6, 8, 10	*T. absoluta* COI	KY212128.1	100	0	98	*T. absoluta* (99.89%)
Tomato-2, 3, 4, 5, 10	*T. absoluta* COI	KY212128.1	100	0	100	*T. absoluta* (99.89%)
Uganda-1, 3, 4, 5	*T. absoluta* COI	KY212128.1	100	0	100	*T. absoluta* (99.89%)
Tz-1, 5, 6, 9	*T. absoluta* COI	KY212128.1	94	1.00E-112	80	*T. absoluta* (99.89%)
PARA1-1 – 10	*Braconinae* sp. BOLD: AAG8403 voucher BIOUG<CAN>:06-PROBE-4177 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	FJ413730.1	99	0	97	No match
PARA2-1– 10	*Dolichogenidea* sp. jft21 voucher CNCH2666 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	HM396614.1	100	0	100	*Dolichogenidea appellator* (100%)

Table 7.3: Estimates of evolutionary divergence between sequences.

	Gazira -3	**Sinnar -3**	**Genina -3**	**Khartoum -3**	**Eggplant -3**	**Kassala -3**	**Nyala2 -3**	**Senegal -3**	**Uganda -3**	**TZ -5**	**Nyala -7**	**Lawni -3**	**Halfa -3**	**Hillat -3**	**Tomato -3**	**NC_029386.1**
Gazira-3	0.000															
Sinnar-3	0.023	0.000														
Genina-3	0.011	0.018	0.000													
Khartoum-3	0.011	0.018	0.000	0.000												
Eggplant-3	0.011	0.018	0.000	0.000	0.000											
Kassala-3	0.011	0.018	0.000	0.000	0.000	0.000										
Nyala2-3	0.011	0.018	0.000	0.000	0.000	0.000	0.000									
Senegal-3	0.011	0.018	0.000	0.000	0.000	0.000	0.000	0.000								
Uganda-3	0.011	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000							
TZ-5	0.011	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000						
Nyala-7	0.011	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000					
Lawni-3	0.011	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
Halfa-3	0.011	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
Hillat-3	0.011	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
Tomato-3	0.009	0.020	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.000	
NC_029386.1	0.133	0.139	0.123	0.123	0.134	0.123	0.123	0.123	0.123	0.123	0.123	0.123	0.123	0.123	0.121	0.000

***NC029386.1** *Tecia solanivora*. The names denote the locations where *Tuta absoluta* for the respective samples were collected.

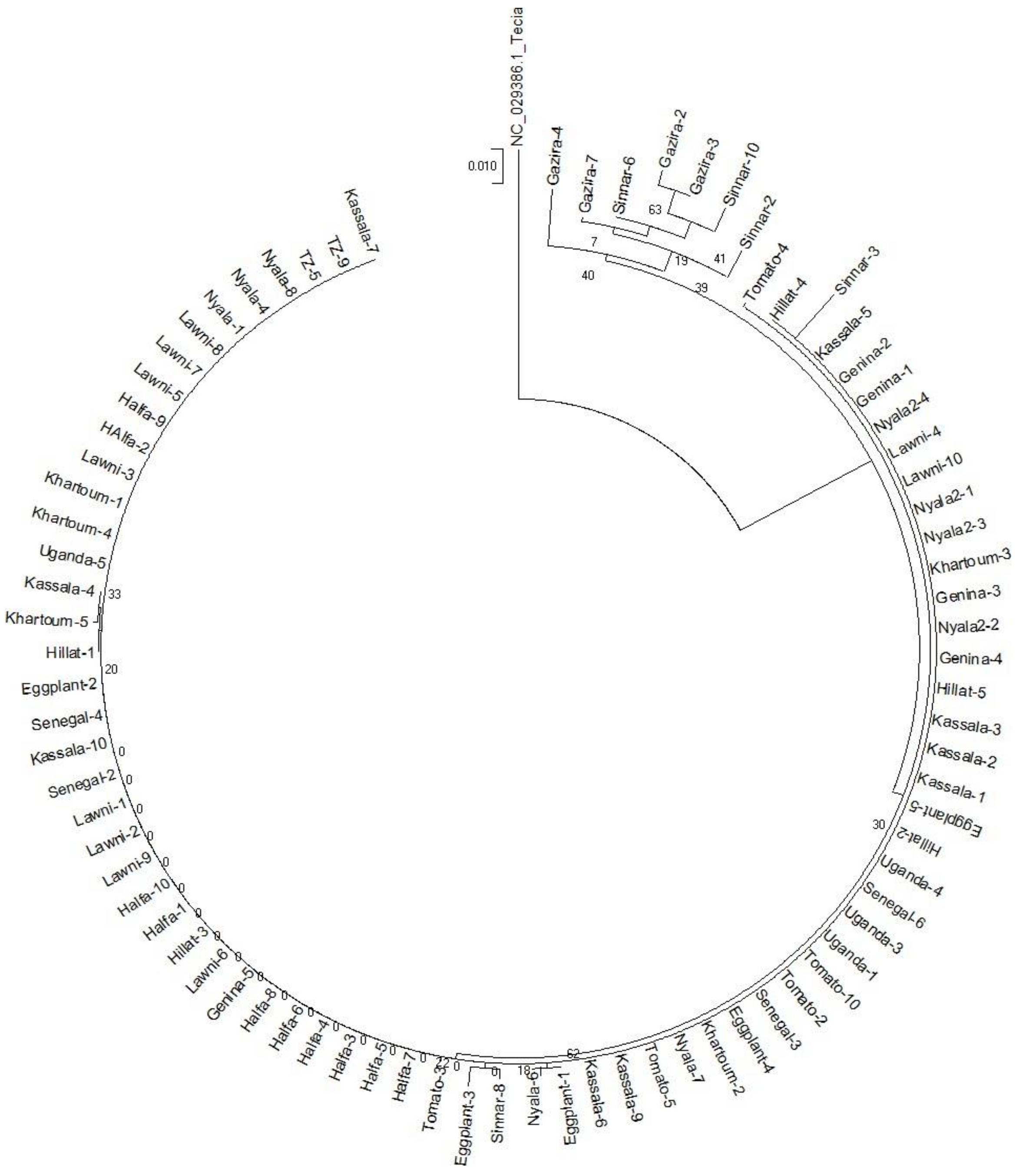

Figure 7.1: Molecular phylogenetic analysis by Maximum Likelihood method, circular topology. **NC_029386.1**: *Tecia solanivora* .

7.5. Discussion

DNA barcoding based on short genetic sequences of the mitochondrial cytochrome oxidase subunit I gene (COI) has been used to study the history of invasive insect populations and to compare or examine the relationship within the different populations or groups (Cifuentes *et al.,* 2011; Hebert *et al.,* 2003; Simon *et al.,* 1994). However, for cryptic species, DNA barcoding may not provide the correct phylogenetic tree due to problems associated with the shared ancestral polymorphisms and multiple substitutions at a single nucleotide site (Simon *et al.,* 1994). In such a case, stringent makers such as simple sequence repeats (SSR), can be deployed.

In this study, the COI sequences of the *T. absoluta* populations from different regions clustered together. These specimens were collected from Sudan (11 different sites) and one site each in Uganda, Senegal and Tanzania, which represent an extensive geographical distribution and great diversity of habitats. The homogeneity in the COI genomic regions of *T. absoluta* populations may indicate that these populations originate from an intermediate introduction from North Africa (Sudan), the point of invasion to other areas (Uganda and Tanzania). In contrast to indigenous populations of a particular species, introduced individuals often are genetically similar, because they frequently contain only a subset of the genetic diversity present in the native population (Cifuentes *et al.,* 2011). The level of genetic homogeneity observed here within *T. absoluta* populations is not uncommon, with examples of other similar results of the Mediterranean and South American populations of the pest (Cherif *et al.,* 2017; Cifuentes *et al.,* 2011).

Results from this study are in contrast with those of Bettaïbi *et al.* (2012), who reported a high genetic diversity within and among populations of *T. absoluta* collected from different regions in Tunisia using RAPD-PCR technology. They suggested that the results indicated that there were several introduction events of *T. absoluta* into Tunisia. Suinaga *et al.* (2004) divided the Brazilian populations of *T. absoluta* into two groups based on their genetic fingerprints. The two groups were identical in their susceptibility to insecticides and these results could therefore indicate genetic diversity in the resistance mechanisms between populations of the pest. Although reductions in genetic diversity are generally considered detrimental, however, other findings provide an example of how low levels of genetic structure can lead to widespread ecological success of invasive species such as *Linepithema humile* (Mayr, 1868) (Hymenoptera: Formicidae) and *T. absoluta* (Cifuentes *et al.,* 2011; Tsutsui *et al.,* 2000; Tsutsui *et al.,* 2001). The spread of *T. absoluta* from its original home of South America to Europe began in Spain in 2006 (EPPO, 2017) and within 10 years, the pest had reached South Africa and India (EPPO, 2017; Visser *et al.,* 2017). Although *T. absoluta* has spread fast over geographical areas, the pest has already developed resistance to different insecticide groups in the newly invaded areas (Haddi *et al.,* 2012; Roditakis *et al.,* 2013).

7.6. Conclusion

DNA barcoding showed that there was low genetic variation in the studied populations of *T. absoluta* from Sudan, Uganda, Senegal and Tanzania. In future, more stringent markers such as the already developed microsatellite markers for *T. absoluta* (Guillemaud *et al.,* 2015) can be used to study the genetic homogeneity of *T. absoluta* populations from Africa.

7.7. References

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CHAPTER 8

General discussion, conclusions and recommendations

8.1. General Discussion

Invasive species have the potential to impact negatively on the economy and biodiversity of the invaded areas (Guillemaud *et al.,* 2011). The South American leaf miner *T. absoluta* that invaded Afro-Eurasia is a major threat to world tomato production (Desneux *et al.,* 2011; Biondi *et al.,* 2018;). This pest causes a decrease in the mass production of tomato and control of the pest increases production costs. Increased pesticide application affects the natural enemies used in tomato IPM programs negatively (Desneux *et al.,* 2011). Due to a lack of effective control measures other than chemical control (Ferracini *et al.,* 2012), insecticide resistant populations of *T. absoluta* had been speculated to be responsible for expansions of the pest into new areas (Cifuentes *et al.,* 2011). Thus in the newly invaded areas, most research focuses on IPM control measures and knowledge on various IPM related aspects is therefore crucial for its implementation.

Results from this study showed that *T. absoluta* infestation differ between the seasons with the infestation being higher during November to April. However, very low infestations were recorded during the fall season. Environmental conditions such as temperature and rainfall and the availability of suitable host plants are the important factors influencing the development of the pest populations under field conditions (Cocco *et al.,* 2015). Heavy rains significantly reduce the pest populations (Chermiti and Abbes, 2012), whereas, tomato crops that overlap during a season are likely to maintain *T. absoluta* populations throughout the season (Abbes *et al.,* 2012a).

This study also confirmed Solanaceous plants to be the primary hosts of *T. absoluta* since it has been found on only four plant species during the field survey all belonging to this family only (chapter 3). Laboratory experiments also confirmed that French bean, pepper, bell pepper and faba bean are not suitable hosts for *T. absoluta* development. Females laid only a few eggs on French bean and pepper in laboratory experiments. Only 3% of larvae completed their development on French bean. No larvae developed into adults on pepper. Morphological structures (such as trichomes) of French bean leaves negatively affected the performance and survival of *T. absoluta* larval instars.

The results obtained from the survey of indigenous parasitoid species and predators of *T. absoluta* conducted in different agro-ecological zones and on cultivated and uncultivated solanaceous plants provided basic knowledge about the parasitoids and predator species as well as their parasitism rates. Six hymenopteran parasitoid species belonging to the Braconidae, Eupelmidae, Pteromalidae and Ichneumonidae families were reared from larvae collected from tomato, eggplant, and black nightshade during this study in Sudan. The number of parasitoid species found during this study is relatively low compared to similar surveys conducted in other countries such as Italy (Zappalà *et al.,* 2012), Spain (Gabarra *et al.,* 2014) and Turkey (Doğanlar and Yiğit, 2011). The low parasitism rate by these indigenous parasitoids under field conditions found in this study indicates their current inability to reduce *T. absoluta* populations below economically important infestation levels. This could be as a result of the local farming practices such as pesticides use which cause undesirable effects on parasitoids. Promising results were, however, obtained under laboratory conditions regarding the possible use of *B. nigricans* and *D. appellator* for control of *T. absoluta*. *Nesidiocoris tenuis* and *Chrysoperla* sp. were identified as *T. absoluta* predators. Although, the performance of these species was not determined in this study, it is known that *N. tenius* is an important natural enemy of *T. absoluta* in newly invaded areas (Desneux *et al.,* 2010; Mollá *et al.,* 2011; Abbes *et al.,* 2012b; Biondi *et al.,* 2018). Two of the native parasitoid species have adapted to the exotic pest, namely *B. nigricans* and *D. appellator*. Their efficacy of control was demonstrated in laboratory experiments and inclusion of these parasitoids into an integrated management programme of *T. absoluta* in the newly invaded areas in Sudan is therefore suggested.

8.2. Recommendations

It is recommended that:

1. Additional field surveys should be conducted to determine the indigenous natural enemies which have adapted to *T. absoluta* and that can be used for control of the pest. Areas with no or little pesticide applications should be targeted.
2. Studies on the population incidence and dynamics of *T. absoluta* on tomato and eggplant should be done on a year-round basis, to include all production seasons (June-October and March - July) and in more sites.
3. The host range of *B. nigricans* and *D. appellator* should be studied as well as their efficacy of control under greenhouse and field conditions. These species should also be mass reared and evaluated for possible inclusion in an IPM program for control of this pest instead of calendar spray of synthetic insecticides.
4. Microsatellite markers should be used to study the origin of the invasive *T. absoluta* populations in Sudan.

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Appendix A

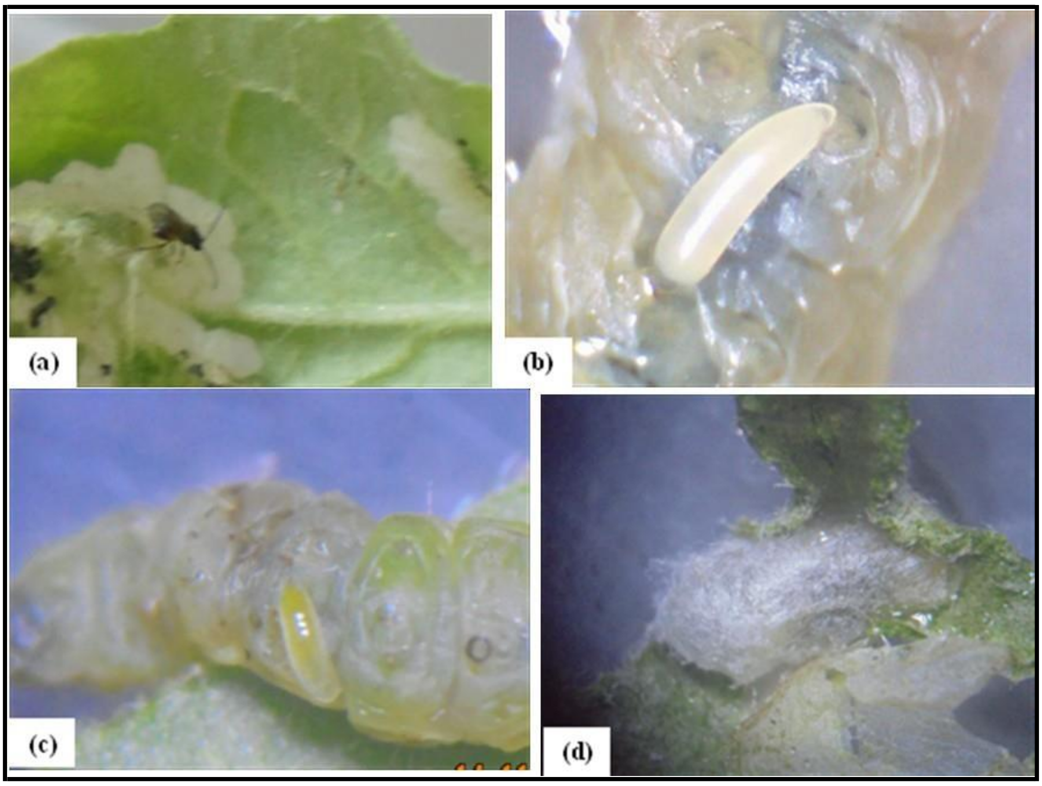


Figure 8.1: (a) *B. nigricans* female attacking *T. absoluta* larvae, (b) *B. nigricans* egg on *T. absoluta* larva, (c) *B. nigricans* larva feeds on the 4th larval instar of *T. absoluta* and (d) the cocoon of *B. nigricans*.

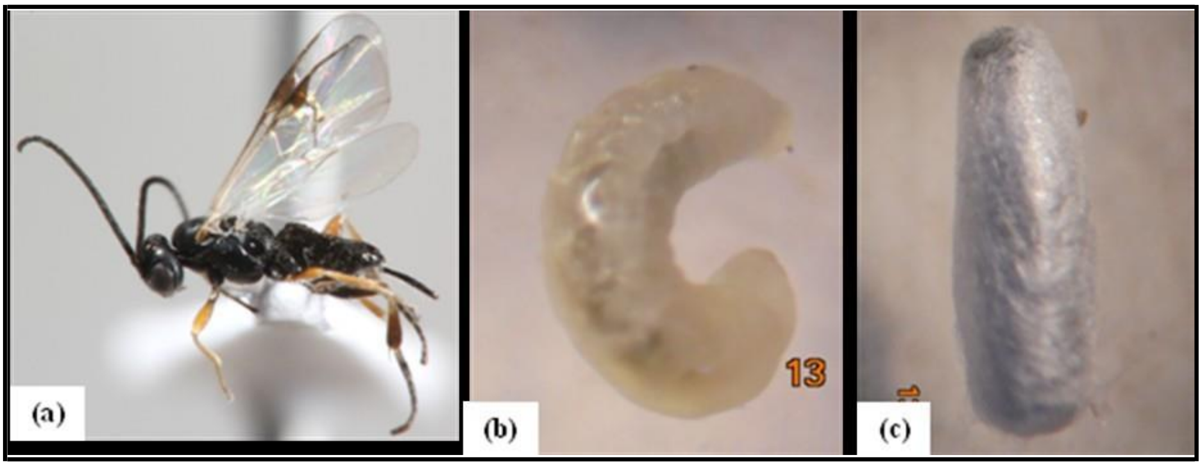


Figure 8.2: (a) Female of *Dolichogenidea* sp., (b) *Dolichogenidea* sp. larva and (c) the cocoon of the parasitoid.

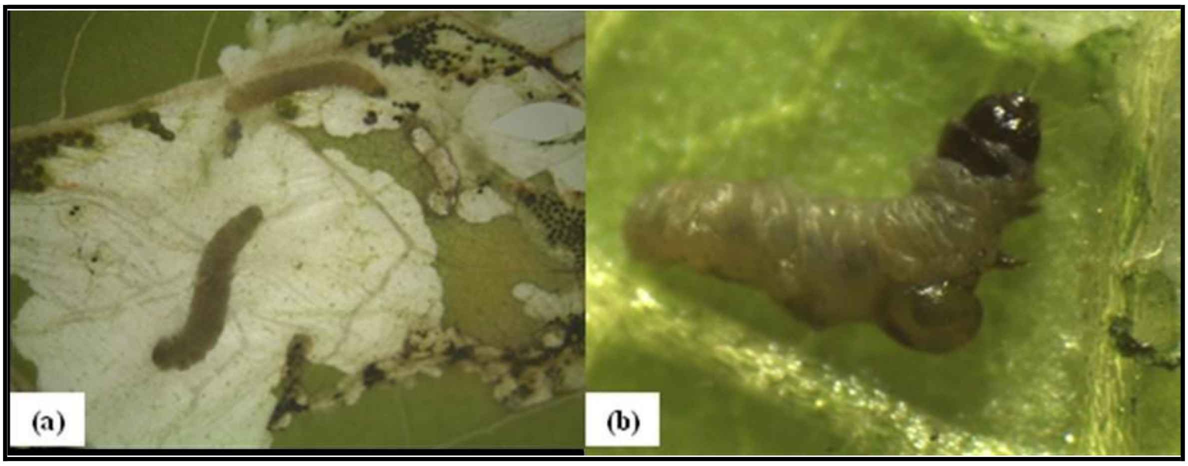
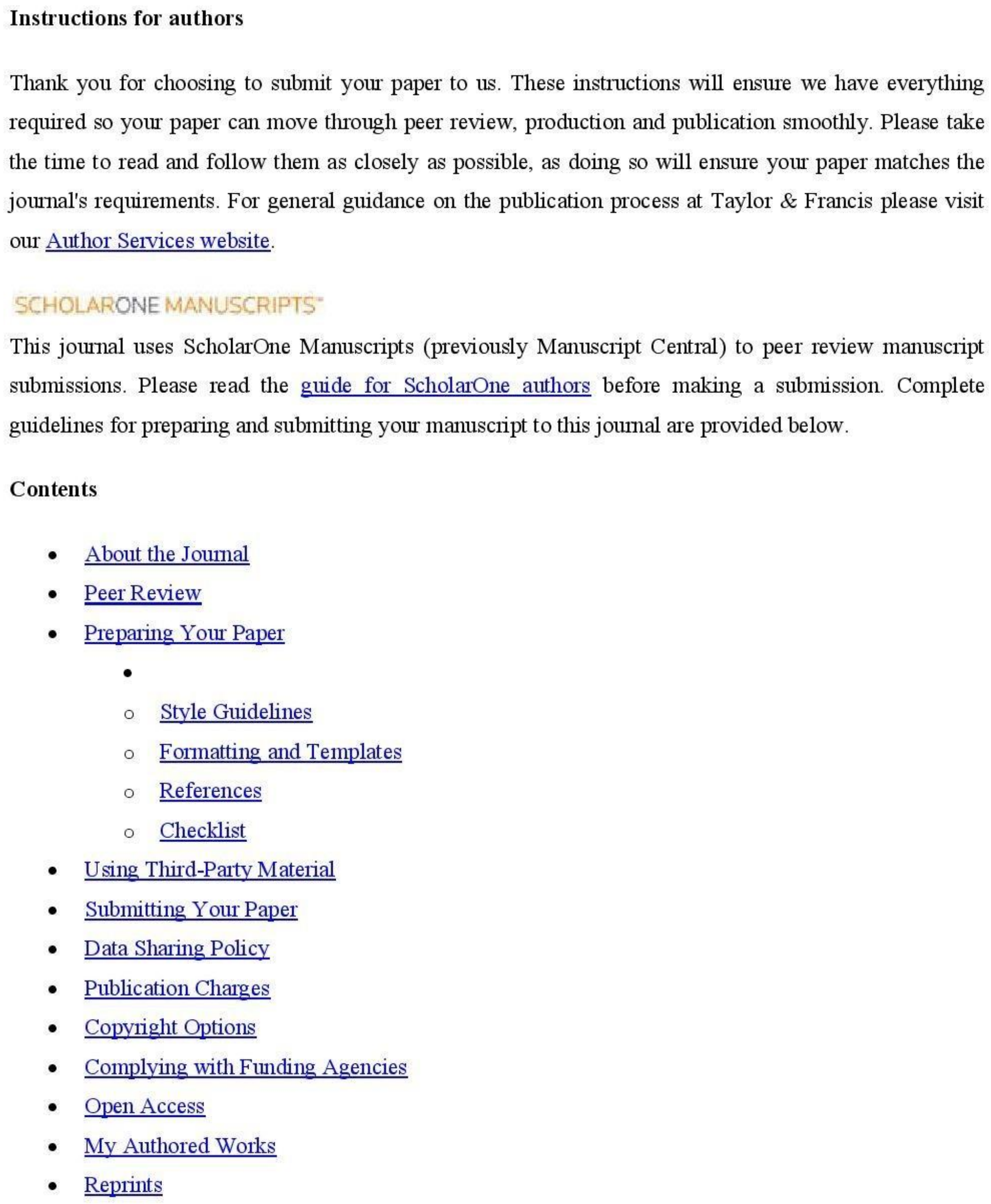


Figure 8.7: (a) Third instar larvae of *T. absoluta* inside a *Phaseolus vulgaris* leaf and (b) the third instar larva entrapped by the trichomes on the leaf surface of *Phaseolus vulgaris*.

Appendix A

Appendix B

Biocontrol Science and Technology (Published article)



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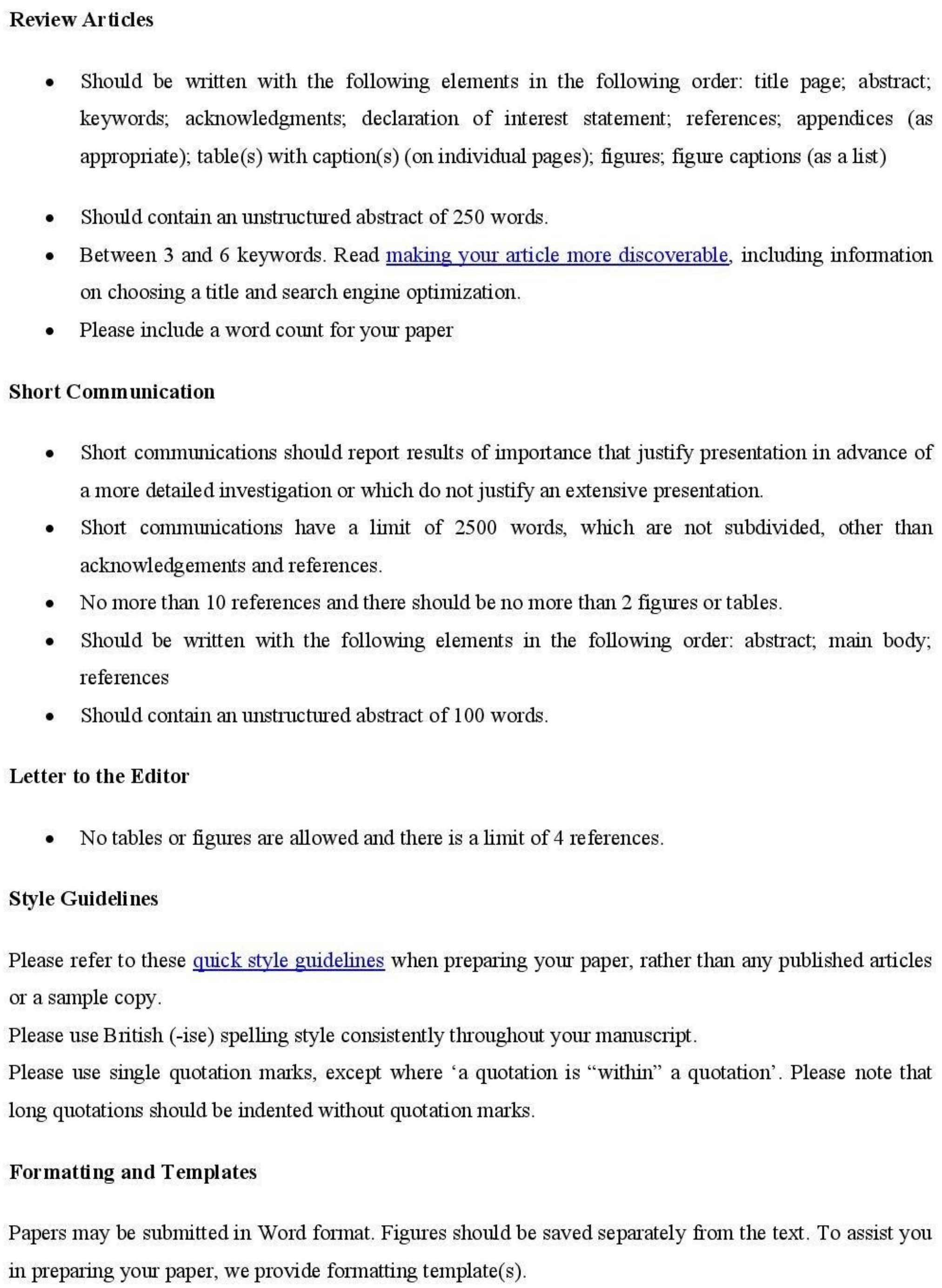

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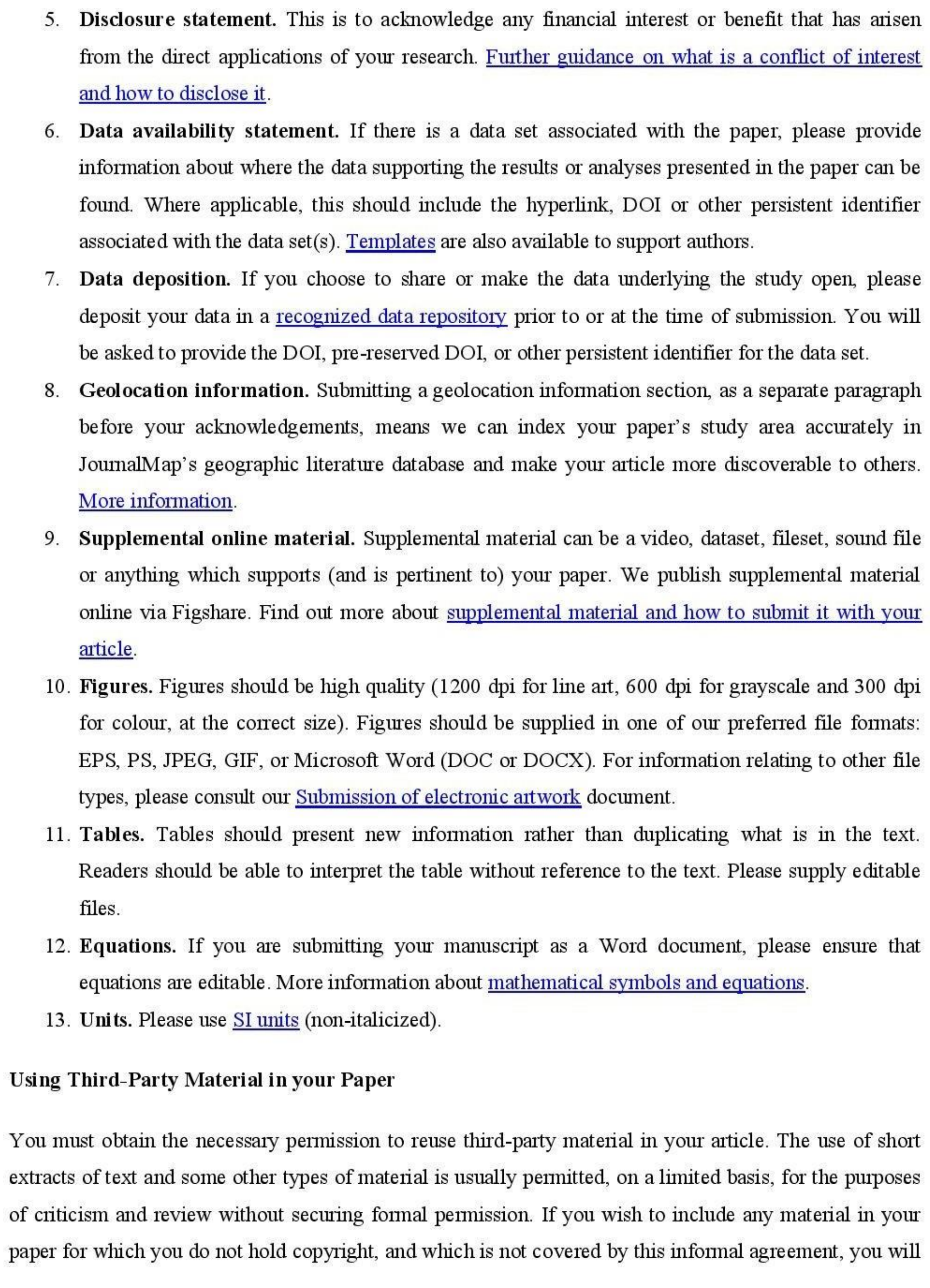

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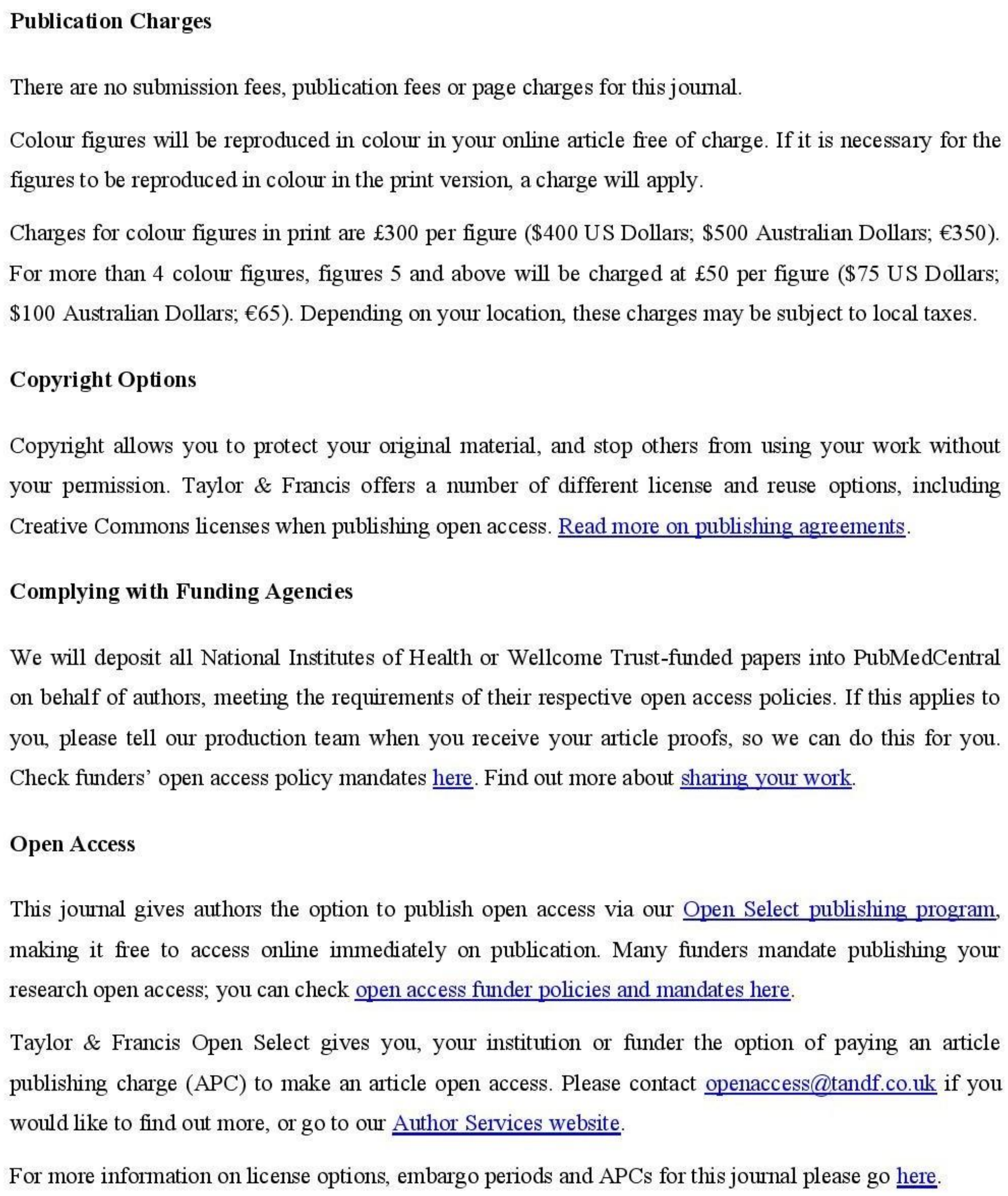

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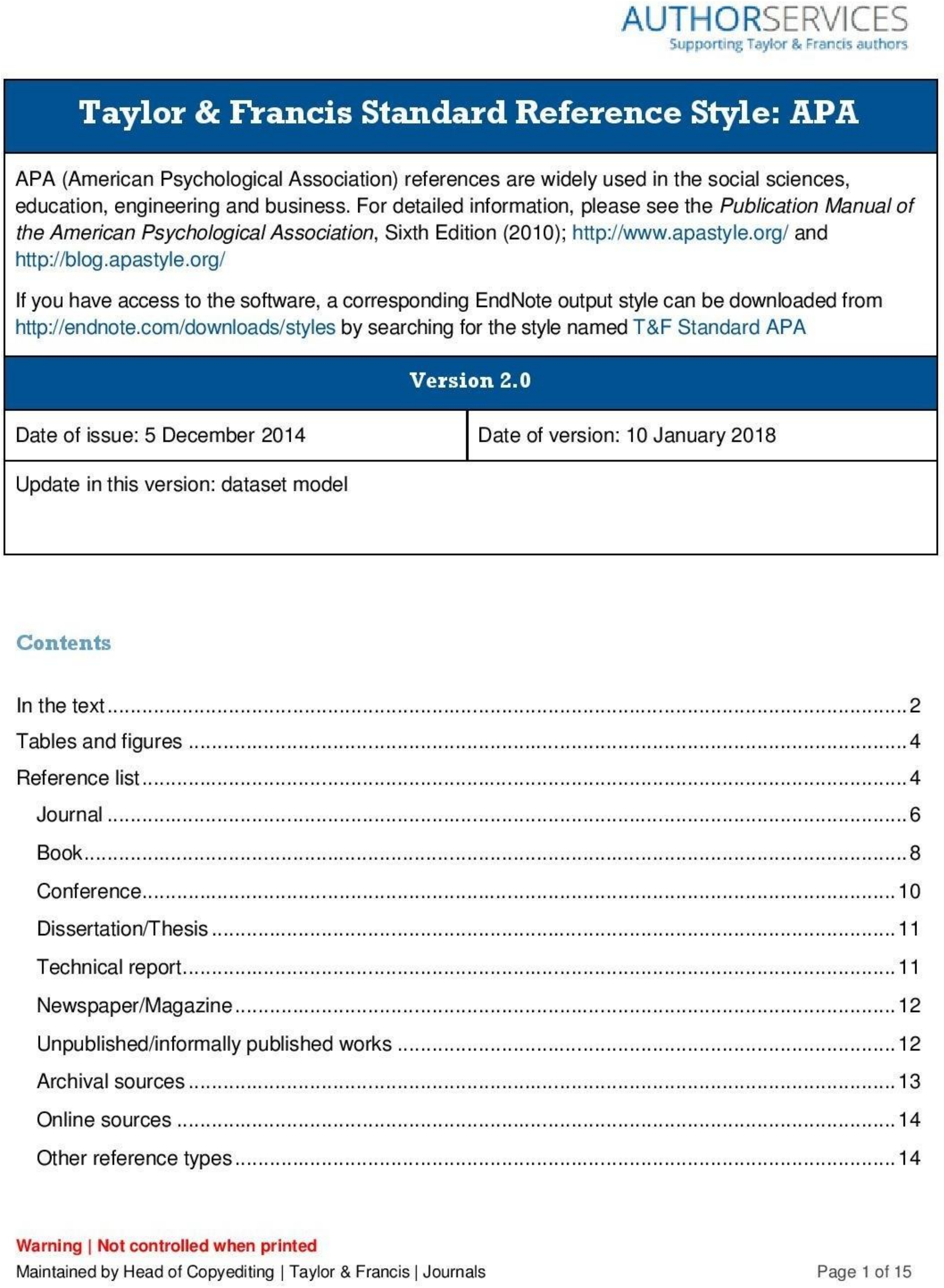

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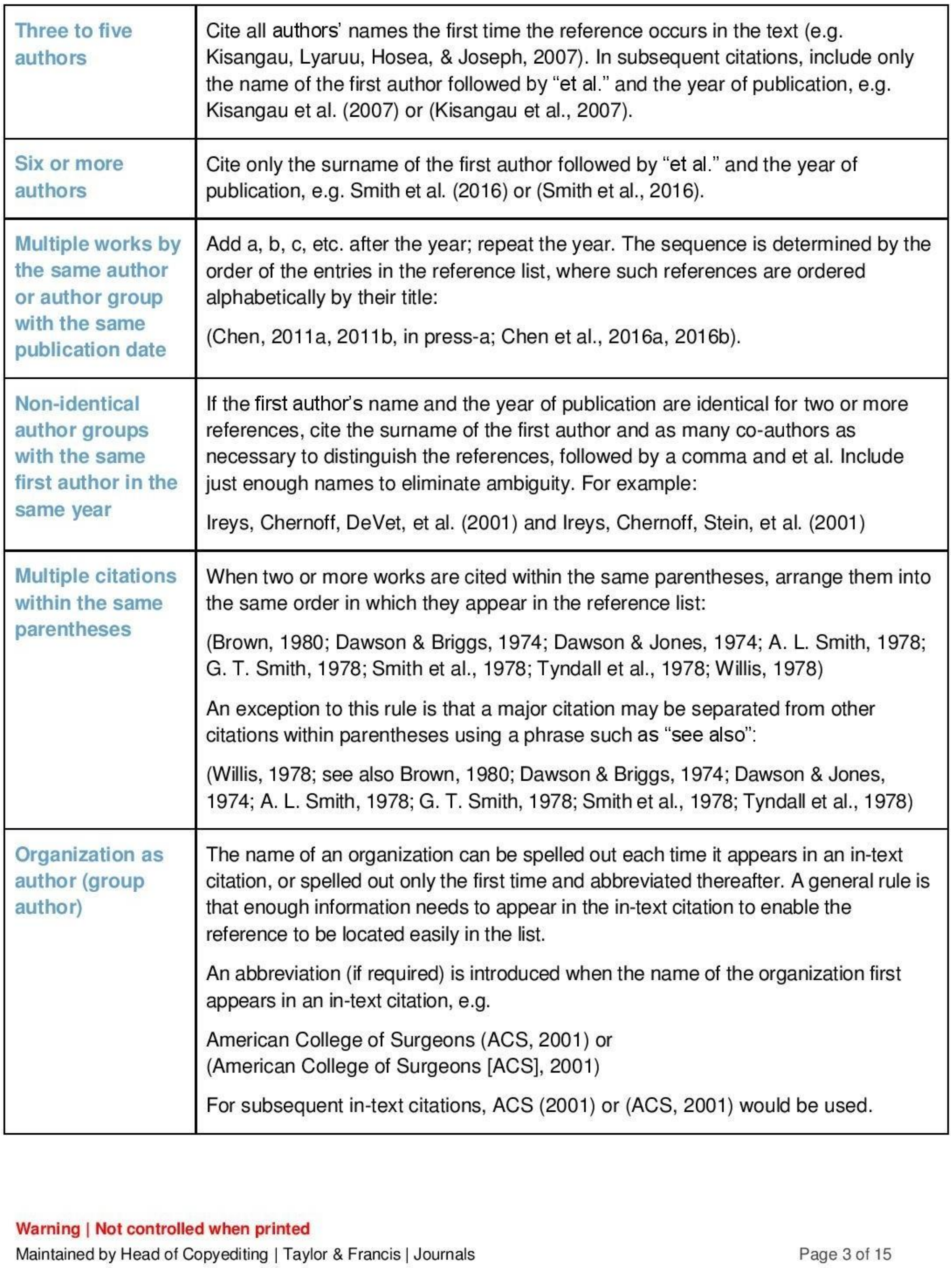
Version 2.0

Date of issue: 5 December 2014	Date of version: 10 January 2018
Update in this version: dataset model	

Contents

In the text	
Placement	In-text citations generally consist of the surname(s) of the author(s), the year of publication of the work cited, and page number(s) if necessary, enclosed within parentheses. For example: The most recent report (Smith, 2016) on the use of … If the author's name forms part of the discussion, the parenthesis can be limited to the year of publication. For example: Smith (2016) found that the use of … If *both* the author's name *and* the year form part of the discussion, no parentheses need be added. For example: In 2016, Smith's report on the use of … If a citation appears within parenthetical text, place the year within commas (not square brackets). For example: (see Table 3 of U.S. Department of Labor, 2007, for more detail) Even if a reference includes a month and a day of the month, include only the year in the in-text citation.
Page, chapter, etc. number	(Smith, 2016, p. 6) or (Chen, 2016, Chapter 5) Page number ranges are preceded by "pp." and a space, and linked with an en dash, e.g. "pp. 156–163".
With a quotation	This is the text, and Smith (2016) says "quoted text" (p. 1), which supports my argument. This is the text, and this is supported by "quoted text" (Smith, 2016, p. 1). This is a displayed quotation. (Smith, 2016, p. 1)
One author	Smith (2016) or (Smith, 2016). Arrange multiple works by the same author in different years in chronological order, separated by a comma (e.g. Smith, 1990, 1995, in press). If the primary authors of two or more works in the reference list have the same surname, include the first author's initials in all in-text citations even if the year of publication differs (J. Dawson, 1990; M. Dawson, 1986).
Two authors	Smith and Jones (2016) or (Smith & Jones, 2016). If both authors of a work have the same surname, include the first author's initials in all in-text citations (e.g. M. A. Light & Light, 2008).



Three to five authors	Cite all authors' names the first time the reference occurs in the text (e.g. Kisangau, Lyaruu, Hosea, & Joseph, 2007). In subsequent citations, include only the name of the first author followed by "et al." and the year of publication, e.g. Kisangau et al. (2007) or (Kisangau et al., 2007).
Six or more authors	Cite only the surname of the first author followed by "et al." and the year of publication, e.g. Smith et al. (2016) or (Smith et al., 2016).
Multiple works by the same author or author group with the same publication date	Add a, b, c, etc. after the year; repeat the year. The sequence is determined by the order of the entries in the reference list, where such references are ordered alphabetically by their title: (Chen, 2011a, 2011b, in press-a; Chen et al., 2016a, 2016b).
Non-identical author groups with the same first author in the same year	If the first author's name and the year of publication are identical for two or more references, cite the surname of the first author and as many co-authors as necessary to distinguish the references, followed by a comma and et al. Include just enough names to eliminate ambiguity. For example: Ireys, Chernoff, DeVet, et al. (2001) and Ireys, Chernoff, Stein, et al. (2001)
Multiple citations within the same parentheses	When two or more works are cited within the same parentheses, arrange them into the same order in which they appear in the reference list: (Brown, 1980; Dawson & Briggs, 1974; Dawson & Jones, 1974; A. L. Smith, 1978; G. T. Smith, 1978; Smith et al., 1978; Tyndall et al., 1978; Willis, 1978) An exception to this rule is that a major citation may be separated from other citations within parentheses using a phrase such as "see also": (Willis, 1978; see also Brown, 1980; Dawson & Briggs, 1974; Dawson & Jones, 1974; A. L. Smith, 1978; G. T. Smith, 1978; Smith et al., 1978; Tyndall et al., 1978)
Organization as author (group author)	The name of an organization can be spelled out each time it appears in an in-text citation, or spelled out only the first time and abbreviated thereafter. A general rule is that enough information needs to appear in the in-text citation to enable the reference to be located easily in the list. An abbreviation (if required) is introduced when the name of the organization first appears in an in-text citation, e.g. American College of Surgeons (ACS, 2001) or (American College of Surgeons [ACS], 2001) For subsequent in-text citations, ACS (2001) or (ACS, 2001) would be used.

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No identified author	If a work has no identified author, begin the in-text citation with the first few words of the reference list entry (usually the title, e.g. "Editorial," 2000). If the author is designated as "Anonymous", cite the work as such in the text (Anonymous, 1998).
Multiple dates	For in-text citations to publications with a range of dates, give the first and last years of publication linked with an en dash: (Author, 1959–1963). For in-text citations to reprinted publications, give the date of the original and of the reprint linked by a solidus/forward slash: (Author, 1970/1988).
Unknown date	For in-text citations to publications with no date, use "n.d." within parentheses: (Author, n.d.)
Classical or religious work	Works such as the Bible and the Qur'an are cited only in the text. Identify in the first in-text citation the version used, e.g. 1 Cor. 13:1 (King James Version)
Personal communication	Personal communications include private letters, memos, personal interviews, telephone conversations, email, and messages from online discussion groups, etc. Where they do not provide recoverable data, personal communications are cited only in the text and not included in the reference list. Include the initials as well as the surname of the communicator and provide as exact a date as possible, for example: T. K. Lutes (personal communication, April 18, 2001) (V.-G. Nguyen, personal communication, September 28, 1998)

Tables and figures	
	References in a table are usually most appropriately put in footnotes to the table. If references must appear within the field of a table, use a separate column or row for them and supply an appropriate heading to identify them. Do not use references within figures, charts, graphs or illustrations. If such references are needed to support the data or methods, put them in the caption.

Reference list	
Order	At the end of a document, list the references to sources that have been cited in the text, including those found in tables and figures, under the heading "References". Place references in alphabetical order by the surname of the first author followed by the initials of the author's given name. Arrange references with the same

	author(s) by year of publication, beginning with the earliest. If several items have the same first author, both alone and with co-authors, arrange the single-author items before any multi-author items. Arrange the multi-author publications alphabetically by the surname of the second author or, if the second author is the same, by the surname of the third author, etc. Items by the same author(s) with the same publication date are arranged alphabetically by title (excluding “A”, “An” or “The”) unless they are identified as belonging to a series, in which case arrange them in series order. Add a lower-case letter (a, b, c, etc.) after the year: Smith, J. (2016a). Smith, J. (2016b). When organizations serve as authors, alphabetize by the first significant word of the name. Full official names should be used in the list (e.g. American Psychological Association, not APA). The name of a parent body precedes that of a subdivision (e.g. University of Michigan, Department of Psychology). If no authors are present, move the title to the author position and alphabetize the entry by the first significant word of the title. If a work is actually signed “Anonymous”, begin the reference with and alphabetize by the word Anonymous in the reference list.
Form of author name	Begin with the surname, followed by the initials, e.g. Author, A. A. Separate successive author names from one another by a comma and a space, e.g. Author, A. A., Author, B. B., & Author, C. C. If the reference list includes more than one author with the same surname and first initial, the authors’ full first names may be given in square brackets, e.g. Smith, J. [Jane]. (2012). Smith, J. [John]. (2016). If an author’s first name is hyphenated, retain the hyphen and add a full stop (period) after each initial, e.g. Latour, J.-B. Place any family designation of rank after the initials, e.g. Author, A. A., Jr.
Date of publication	The year of publication is required for all references. The month is also required when citing a journal that has no volume or issue number, or a presentation at a conference; the month and day of the month are required when citing a magazine, a newsletter or a newspaper. For articles *accepted for publication* but not yet published, use (in press). If no date of publication is available, use (n.d.).

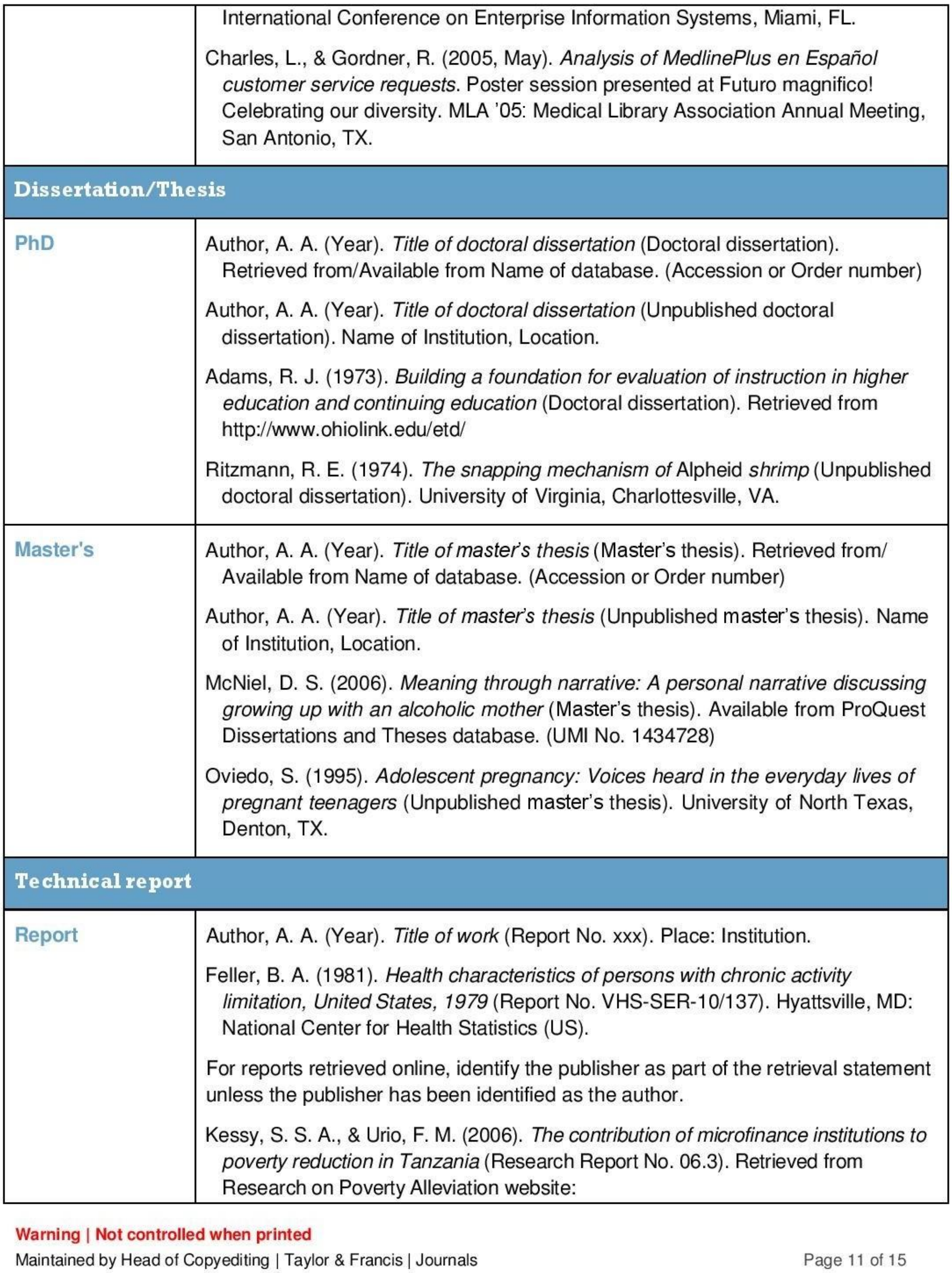
Title	If the original version of a non-English work is used as a source, cite the original version. Give the original title and, in square brackets, the English translation of the title. Capitalize non-English titles according to the conventions of the particular language. If the English translation of a non-English work is used as a source, cite the English translation. Give the English title without square brackets.
Journal	
Volume and issue numbers	The issue number can be omitted if the journal is paginated consecutively through the volume, but it is not incorrect to include it. Enclose issue information in parentheses. Link multiple volume or issue numbers with an en dash.
Page numbers	List the first and last pages of the article, linked with an en dash, e.g. “156–163”.
DOIs	There is no need for authors to include DOI numbers for *published* articles in a manuscript; these will be added as links in any online version of the article by the typesetter as part of the production process.
Basic format (with one author)	Author, A. A. (Year). Title of article: And subtitle. *Journal Title*, *volume*(issue), pages. Fauci, A. S. (2002). Smallpox vaccination policy: The need for dialogue. *New England Journal of Medicine*, *346*(17), 1319–1320.
Two authors	Light, M. A., & Light, I. H. (2008). The geographic expansion of Mexican immigration in the United States and its implications for local law enforcement. *Law Enforcement Executive Forum Journal*, *8*, 73–82.
Three to seven authors	Include all authors' names in the reference list. Good, C. D., Johnsrude, I. S., Ashburner, J., Henson, R. N. A., Firston, K. J., & Frackowiak, R. S. J. (2001). A voxel-based morphometric study of ageing in 465 normal adult human brains. *NeuroImage, 14,* 21–36.
More than seven authors	List the *first six* names, followed by an ellipsis …, then the *last* author’s name. Gilbert, D. G., McClernon, F. J., Rabinovich, N. E., Sugai, C., Plath, L. C., Asgaard, G., ... Botros, N. (2004). Effects of quitting smoking on EEG activation and attention last for more than 31 days and are more severe with stress, dependence, DRD2 A1 allele, and depressive traits. *Nicotine and Tobacco Research*, *6*, 249–267.

Organization as author (group author)	American College of Surgeons, Committee on Trauma, Ad Hoc Subcommittee on Outcomes, Working Group. (2001). Practice management guidelines for emergency department thoracotomy. *Journal of the American College of Surgeons*, *193*(3), 303–309.
No identified author	Editorial: "What is a disaster" and why does this question matter? [Editorial]. (2006). *Journal of Contingencies and Crisis Management*, *14*, 1–2.
No volume or issue number	Sampat, P. (2000, January–February). Groundwater shock: The polluting of the world's major freshwater stores. *World Watch*, 10–22.
Article in a supplement	Ochi, K., Sugiura, N., Komatsuzaki, Y., Nishino, H., & Ohashi, T. (2003). Patency of inferior meatal antrostomy. *Auris Nasus Larynx*, *30*(Suppl.), S57–S60.
Not in English	Guimard, P., & Florin, A. (2007). Les évaluations des enseignants en grande section de maternelle sont-elles prédictives des difficultés de lecture au cours préparatoire? [Are teacher ratings in kindergarten predictive of reading difficulties in first grade?]. *Approche Neuropsychologique des Apprentissages chez l'Enfant*, *19*, 5–17.
Article published online ahead of placement in an issue	Author, A. (Year). Title of article: And subtitle. *Journal Title*. Advance online publication. [Retrieved from URL] or [DOI] Von Ledebur, S. C. (2007). Optimizing knowledge transfer by new employees in companies. *Knowledge Management Research & Practice*. Advance online publication. doi:10.1057/palgrave/kmrp.8500141 If the DOI of the article is not provided, include the URL of the article or the journal's home page. No retrieval date is needed. Do not add a period after the URL.
Not the Version of Record (including Author Manuscript Online, Advanced Author Version, etc.)	Author, A. (in press). Title of article. *Journal Title*. Retrieved from URL Briscoe, R. (in press). Egocentric spatial representation in action and perception. *Philosophy and Phenomenological Research*. Retrieved from http://cogprints.org/5780/1/ECSRAP.F07.pdf
Other article types	Author, A. (Year). Title of article [Article type]. *Journal Title, Volume*(issue), pages. Woolf, N. J., Young, S. L., Fanselow, M. S., & Butcher, L. L. (1991). MAP-2 expression in cholinoceptive pyramidal cells of rodent cortex and hippocampus is altered by Pavlovian conditioning [Abstract]. *Society for Neuroscience Abstracts*, *17*, 480.

Supplemental material	Marshall-Pescini, S., & Whiten, A. (2008). Social learning of nut-cracking behavior in East African sanctuary-living chimpanzees (*Pan troglodytes schweinfurthii*) [Supplemental material]. *Journal of Comparative Psychology*, *122*, 186–194.
Special issue or special section	Haney, C., & Wiener, R. L. (Eds.). (2004). Capital punishment in the United States [Special issue]. *Psychology, Public Policy, and Law*, *10*(4). Greenfield, P., & Yan, Z. (Eds.). (2006). Children, adolescents, and the Internet [Special section]. *Developmental Psychology*, *42*, 391–458.
Monograph	Ganster, D. C., Schaubroeck, J., Sime, W. E., & Mayes, B. T. (1991). The nomological validity of the Type A personality among employed adults [Monograph]. *Journal of Applied Psychology*, *76*, 143–168. For a monograph with an issue number, include any serial number or supplement/part number in the issue number parenthesis, e.g. *80*(3, Pt. 2).
Book	
Place of publication	Always list the city, and for the sake of consistency always include the two-letter state or province abbreviation for US and Canadian cities. Include the country name for other countries only where this is necessary to avoid ambiguity, e.g. Cambridge, MA: Harvard University Press. Cambridge, UK: Cambridge University Press. If more than one place of publication is given, use the first one listed (or the one set in the most prominent font).
Publisher	Abbreviate well-known publishers' names, e.g. "John Wiley & Sons, Ltd." may become simply "Wiley"; but retain the words "Books" and "Press". If the author and the publisher are the same, use the word "Author" as the name of the publisher.
Page numbers	List the first and last pages of a chapter or part being cited, linked with an en dash and preceded by "pp." and a space, e.g. "pp. 156–163". It is not necessary to list the extent (total pagination) of books, conference proceedings and other monographs.
Basic format (with one author)	Author, A. A. (Year). *Title of book: And subtitle*. Place: Publisher. Bandura, A. J. (1977). *Social learning theory*. Englewood Cliffs, NJ: Prentice Hall.
Two authors	Van de Velde, R., & Degoulet, P. (2003). *Clinical information systems: A component-based approach*. New York, NY: Springer.

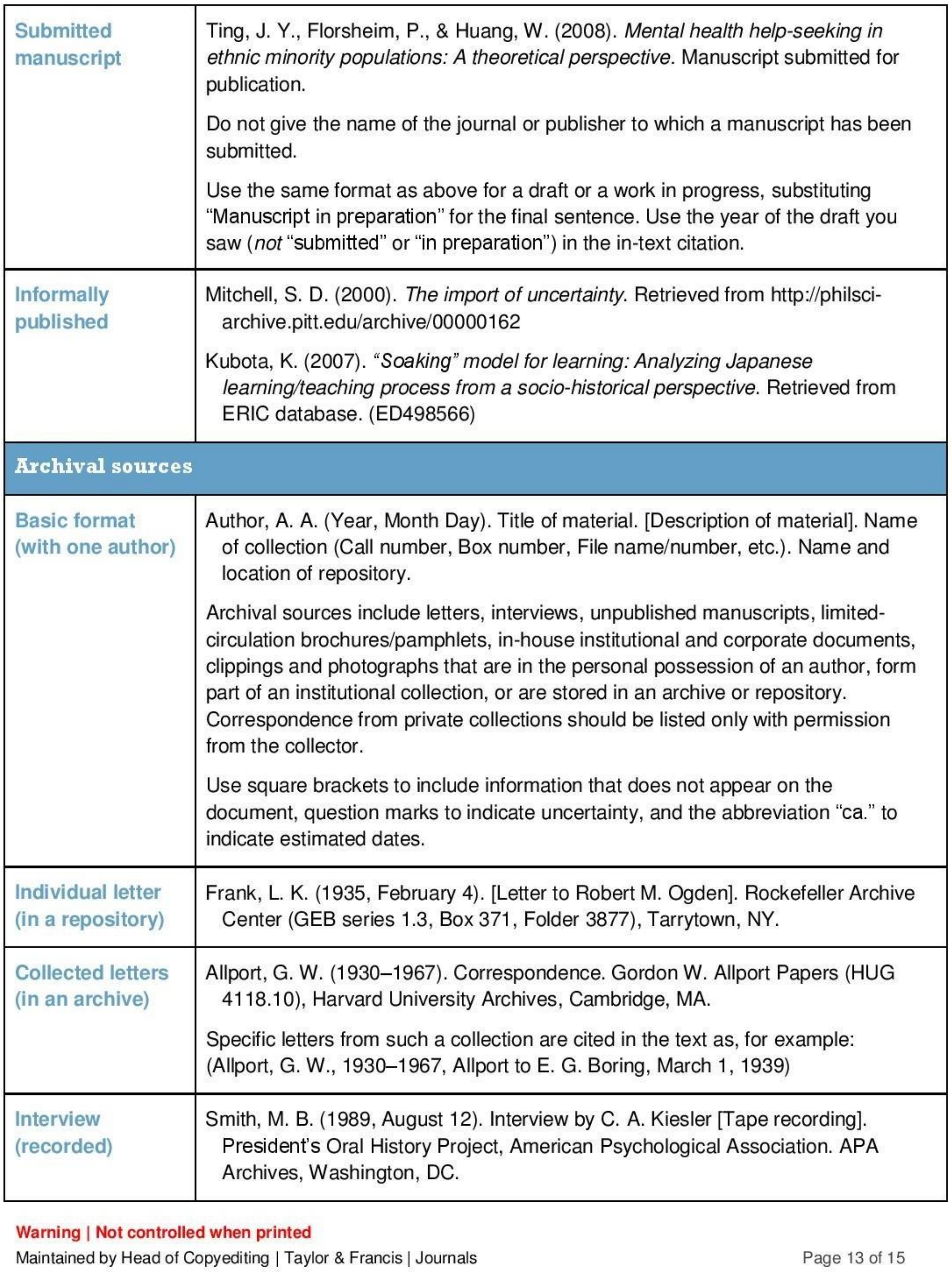
Three to seven authors	Include all authors' names in the reference list. Ferrozzi, F., Garlaschi, G., & Bova, D. (2000). *CT of metastases*. New York, NY: Springer.
More than seven authors	List the *first six* names, followed by an ellipsis …, then the *last* author's name. Wenger, N. K., Sivarajan Froelicher, E., Smith, L. K., Ades, P. A., Berra, K., Blumenthal, J. A., ... Rogers, F. J. (1995). *Cardiac rehabilitation*. Rockville, MD: Agency for Health Care Policy and Research (US).
Organization as author (group author)	Advanced Life Support Group. (2001). *Acute medical emergencies: The practical approach*. London: BMJ Books. American Psychological Association. (2010). *Publication manual of the American Psychological Association* (6th ed.). Washington, DC: Author.
No author	*Handbook of geriatric drug therapy*. (2000). Springhouse, PA: Springhouse.
Unknown date of publication	Lederer, J. (n.d.). *Alimentation et cancer* [*Diet and cancer*]. Brussels: Nauwelaerts.
Edition	Schott, J., & Priest, J. (2002). *Leading antenatal classes: A practical guide* (2nd ed.). Boston, MA: Books for Midwives.
Edited	VandenBos, G. R. (Ed.). (2007). *APA dictionary of psychology*. Washington, DC: American Psychological Association.
Chapter in an edited book	Author, A. A. (Year). Chapter title. In E. E. Editor (Ed.), *Title of book: And subtitle* (pp. pages). Place: Publisher. Haybron, D. M. (2008). Philosophy and the science of subjective well-being. In M. Eid & R. J. Larsen (Eds.), *The science of subjective well-being* (pp. 17–43). New York, NY: Guilford Press. Nash, M. (1993). Malay. In P. Hockings (Ed.), *Encyclopedia of world cultures* (Vol. 5, pp. 174–176). New York, NY: G. K. Hall.
A single volume from a multi-volume work	Katz, I., Gabayan, K., & Aghajan, H. (2007). A multi-touch surface using multiple cameras. In J. Blanc-Talon, W. Philips, D. Popescu, & P. Scheunders (Eds.), *Lecture notes in computer science: Vol. 4678. Advanced concepts for intelligent vision systems* (pp. 97–108). Berlin: Springer-Verlag.
Multiple volumes from a multi-volume work	Koch, S. (Ed.). (1959–1963). *Psychology: A study of science* (Vols. 1–6). New York, NY: McGraw-Hill.

Not in English	Real Academia Española. (2001). *Diccionario de la lengua española* [Dictionary of the Spanish language] (22nd ed.). Madrid: Author.
Translated	Flaws, B. (Trans.). (2004). *The classic of difficulties: A translation of the Nan Jing* (3rd ed.). Boulder, CO: Blue Poppy Press. Luzikov, V. N. (1985). *Mitochondrial biogenesis and breakdown*. (A. V. Galkin, Trans.). New York, NY: Consultants Bureau.
Reprint	Piaget, J. (1988). Extracts from Piaget's theory (G. Gellerier & J. Langer, Trans.). In K. Richardson & S. Sheldon (Eds.), *Cognitive development to adolescence: A reader* (pp. 3–18). Hillsdale, NJ: Erlbaum. (Reprinted from *Manual of child psychology*, pp. 703–732, by P. H. Mussen, Ed., 1970, New York, NY: Wiley)
Online (e-book)	Schiraldi, G. R. (2001). *The post-traumatic stress disorder handbook: A guide to healing, recovery, and growth* [Adobe Digital Editions version]. doi: 10.1036/0071393722 O'Keefe, E. (n.d.). *Egoism & the crisis in Western values*. Retrieved from http://www.onlineoriginals.com/showitem.asp?itemID=135
Conference	
Proceedings	Antonioli, G. E. (Ed.). (1997, September). *Pacemaker leads 1997. Proceedings of the 3rd international symposium on pacemaker leads*, Ferrara, Italy. Bologna: Monducci Editore. Callaos, N., Margenstern, M., Zhang, J., Castillo, O., Doberkat, E. E. (Eds.). (2003, July). *SCI 2003. Proceedings of the 7th world multiconference on systemics, cybernetics and informatics*, Orlando, FL. Orlando, FL: International Institute of Informatics and Systematics.
Paper in proceedings	Lee, D. J., Bates, D., Dromey, C., Xu, X., & Antani, S. (2003, June). An imaging system correlating lip shapes with tongue contact patterns for speech pathology research. In M. Krol, S. Mitra, & D. J. Lee (Eds.), *CMBS 2003. Proceedings of the 16th IEEE symposium on computer-based medical systems* (pp. 307–313). Los Alamitos, CA: IEEE Computer Society.
Symposium contribution	Muellbauer, J. (2007, September). Housing, credit, and consumer expenditure. In S. C. Ludvigson (Chair), *Housing and consumer behavior*. Symposium conducted at the meeting of the Federal Reserve Bank of Kansas City, Jackson Hole, WY.
Presentation	Liu, S. (2005, May). *Defending against business crises with the help of intelligent agent based early warning solutions*. Paper presented at the Seventh



	International Conference on Enterprise Information Systems, Miami, FL. Charles, L., & Gordner, R. (2005, May). *Analysis of MedlinePlus en Español customer service requests*. Poster session presented at Futuro magnifico! Celebrating our diversity. MLA ’05: Medical Library Association Annual Meeting, San Antonio, TX.
Dissertation/Thesis	
PhD	Author, A. A. (Year). *Title of doctoral dissertation* (Doctoral dissertation). Retrieved from/Available from Name of database. (Accession or Order number) Author, A. A. (Year). *Title of doctoral dissertation* (Unpublished doctoral dissertation). Name of Institution, Location. Adams, R. J. (1973). *Building a foundation for evaluation of instruction in higher education and continuing education* (Doctoral dissertation). Retrieved from http://www.ohiolink.edu/etd/ Ritzmann, R. E. (1974). *The snapping mechanism of* Alpheid *shrimp* (Unpublished doctoral dissertation). University of Virginia, Charlottesville, VA.
Master's	Author, A. A. (Year). *Title of master’s thesis* (Master’s thesis). Retrieved from/ Available from Name of database. (Accession or Order number) Author, A. A. (Year). *Title of master’s thesis* (Unpublished master’s thesis). Name of Institution, Location. McNiel, D. S. (2006). *Meaning through narrative: A personal narrative discussing growing up with an alcoholic mother* (Master’s thesis). Available from ProQuest Dissertations and Theses database. (UMI No. 1434728) Oviedo, S. (1995). *Adolescent pregnancy: Voices heard in the everyday lives of pregnant teenagers* (Unpublished master’s thesis). University of North Texas, Denton, TX.
Technical report	
Report	Author, A. A. (Year). *Title of work* (Report No. xxx). Place: Institution. Feller, B. A. (1981). *Health characteristics of persons with chronic activity limitation, United States, 1979* (Report No. VHS-SER-10/137). Hyattsville, MD: National Center for Health Statistics (US). For reports retrieved online, identify the publisher as part of the retrieval statement unless the publisher has been identified as the author. Kessy, S. S. A., & Urio, F. M. (2006). *The contribution of microfinance institutions to poverty reduction in Tanzania* (Research Report No. 06.3). Retrieved from Research on Poverty Alleviation website:

	http://www.repoa.or.tz/documents_storage/Publications/Reports/06.3_Kessy_and_Urio.pdf
Working paper or issue brief	Employee Benefit Research Institute. (1992, February). *Sources of health insurance and characteristics of the uninsured* (Issue Brief No. 123). Washington, DC: Author.
Newspaper/Magazine	
Date of publication	Full dates of publication are required, including the month (for magazine articles) and day of the month (for newspaper articles).
Print edition	Chamberlin, J., Novotney, A., Packard, E., & Price, M. (2008, May). Enhancing worker wellbeing: Occupational health psychologists convene to share their research on work, stress, and health. *Monitor on Psychology*, *39*(5), 26–29. Schwartz, J. (1993, September 30). Obesity affects economic, social status. *The Washington Post*, pp. A1, A4. Precede page numbers for *newspaper* articles with p. or pp. If an article appears on discontinuous pages, give all page numbers and separate them with a comma.
Online edition	Clay, R. (2008, June). Science vs. ideology: Psychologists fight back about the misuse of research. *Monitor on Psychology*, *39*(6). Retrieved from http://www.apa.org/monitor/ Brody, J. E. (2007, December 11). Mental reserves keep brain agile. *The New York Times*. Retrieved from http://www.nytimes.com Give the URL of the home page when the online version of the article is available by search to avoid non-working URLs.
Newsletter article, no author named	Six sites meet for comprehensive anti-gang initiative conference. (2006, November/December). *OJJDP News @ a Glance*. Retrieved from http://www.ncjrs.gov/html/ojjdp/news_at_glance/216684/topstory.html Alphabetize works with no author by the first significant word in the title. In the text, use a short title (unless the full title is short) enclosed in quotation marks: (“Six Sites Meet,” 2006).
Unpublished/informally published works	
Unpublished manuscript	Blackwell, E., & Conrod, P. J. (2003). *A five-dimensional measure of drinking motives*. Unpublished manuscript, Department of Psychology, University of British Columbia, Vancouver, Canada.



Submitted manuscript	Ting, J. Y., Florsheim, P., & Huang, W. (2008). *Mental health help-seeking in ethnic minority populations: A theoretical perspective*. Manuscript submitted for publication. Do not give the name of the journal or publisher to which a manuscript has been submitted. Use the same format as above for a draft or a work in progress, substituting "Manuscript in preparation" for the final sentence. Use the year of the draft you saw (*not* "submitted" or "in preparation") in the in-text citation.
Informally published	Mitchell, S. D. (2000). *The import of uncertainty*. Retrieved from http://philsci-archive.pitt.edu/archive/00000162 Kubota, K. (2007). *"Soaking" model for learning: Analyzing Japanese learning/teaching process from a socio-historical perspective*. Retrieved from ERIC database. (ED498566)
Archival sources	
Basic format (with one author)	Author, A. A. (Year, Month Day). Title of material. [Description of material]. Name of collection (Call number, Box number, File name/number, etc.). Name and location of repository. Archival sources include letters, interviews, unpublished manuscripts, limited-circulation brochures/pamphlets, in-house institutional and corporate documents, clippings and photographs that are in the personal possession of an author, form part of an institutional collection, or are stored in an archive or repository. Correspondence from private collections should be listed only with permission from the collector. Use square brackets to include information that does not appear on the document, question marks to indicate uncertainty, and the abbreviation "ca." to indicate estimated dates.
Individual letter (in a repository)	Frank, L. K. (1935, February 4). [Letter to Robert M. Ogden]. Rockefeller Archive Center (GEB series 1.3, Box 371, Folder 3877), Tarrytown, NY.
Collected letters (in an archive)	Allport, G. W. (1930–1967). Correspondence. Gordon W. Allport Papers (HUG 4118.10), Harvard University Archives, Cambridge, MA. Specific letters from such a collection are cited in the text as, for example: (Allport, G. W., 1930–1967, Allport to E. G. Boring, March 1, 1939)
Interview (recorded)	Smith, M. B. (1989, August 12). Interview by C. A. Kiesler [Tape recording]. President's Oral History Project, American Psychological Association. APA Archives, Washington, DC.

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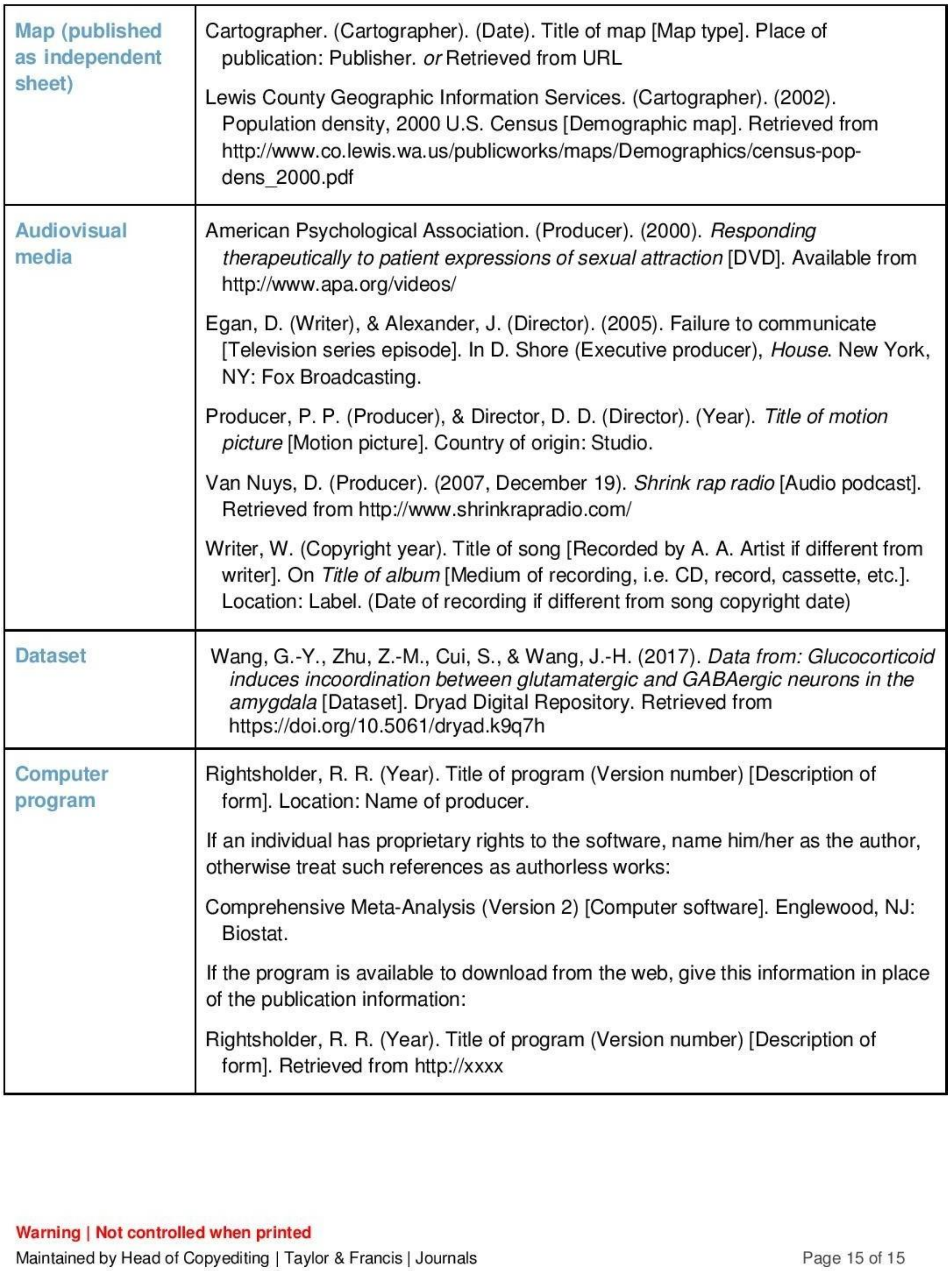
Corporate document	Subcommittee on Mental Hygiene Personnel in School Programs. (1949, November 5–6). *Meeting of Subcommittee on Mental Hygiene Personnel in School Programs*. David Shakow Papers (M1360). Archives of the History of American Psychology, University of Akron, Akron, OH.
Limited-circulation publication	Sci-Art Publishers. (1935). *Sci-Art Publications* [Brochure]. Cambridge, MA: Author. A. A. Roback Papers (HUGFP 104.50, Box 2, Folder “Miscellaneous Psychological Materials”), Harvard University Archives, Cambridge, MA.
Photograph	[Photographs of Robert M. Yerkes]. (ca. 1917–1954). Robert Mearns Yerkes Papers (Box 137, Folder 2292), Manuscripts and Archives, Yale University Library, New Haven, CT.
Online sources	
Website	When citing an entire website, it is sufficient just to give the address of the site in the text: The BBC (http://www.bbc.co.uk).
Web page	If the format is out of the ordinary (e.g. lecture notes), add a description in square brackets: Author, A. A. (Year, Month Day). Title of document [Format description]. Retrieved from http://URL
Message posted to an electronic mailing list	Smith, S. (2006, January 5). Re: Disputed estimates of IQ [Electronic mailing list message]. Retrieved from http://tech.groups.yahoo.com/group/ForensicNetwork/message/670
Other reference types	
Review	Reviewer, R. R. (Year). Title of review [Review of the publication *Title of the publication*, by A. A. Author]. *Periodical Title, Volume*(issue), pages. Schatz, B. R. (2000, November 17). Learning by text or context? [Review of the book *The social life of information*, by J. S. Brown & P. Duguid]. *Science*, *290*, 1304.
Patent	Inventor, A. A. (Year of issue). *Patent Number*. Place: Office Issuing the Patent. Smith, I. M. (1988). *U.S. Patent No. 123,445*. Washington, DC: U.S. Patent and Trademark Office. In the text, cite the patent number and the year of issue: (U.S. Patent No. 123,445, 1988) *or* U.S. Patent No. 123,445 (1988)



Map (published as independent sheet)	Cartographer. (Cartographer). (Date). Title of map [Map type]. Place of publication: Publisher. *or* Retrieved from URL Lewis County Geographic Information Services. (Cartographer). (2002). Population density, 2000 U.S. Census [Demographic map]. Retrieved from http://www.co.lewis.wa.us/publicworks/maps/Demographics/census-pop-dens_2000.pdf
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Dataset	Wang, G.-Y., Zhu, Z.-M., Cui, S., & Wang, J.-H. (2017). *Data from: Glucocorticoid induces incoordination between glutamatergic and GABAergic neurons in the amygdala* [Dataset]. Dryad Digital Repository. Retrieved from https://doi.org/10.5061/dryad.k9q7h
Computer program	Rightsholder, R. R. (Year). Title of program (Version number) [Description of form]. Location: Name of producer. If an individual has proprietary rights to the software, name him/her as the author, otherwise treat such references as authorless works: Comprehensive Meta-Analysis (Version 2) [Computer software]. Englewood, NJ: Biostat. If the program is available to download from the web, give this information in place of the publication information: Rightsholder, R. R. (Year). Title of program (Version number) [Description of form]. Retrieved from http://xxxx

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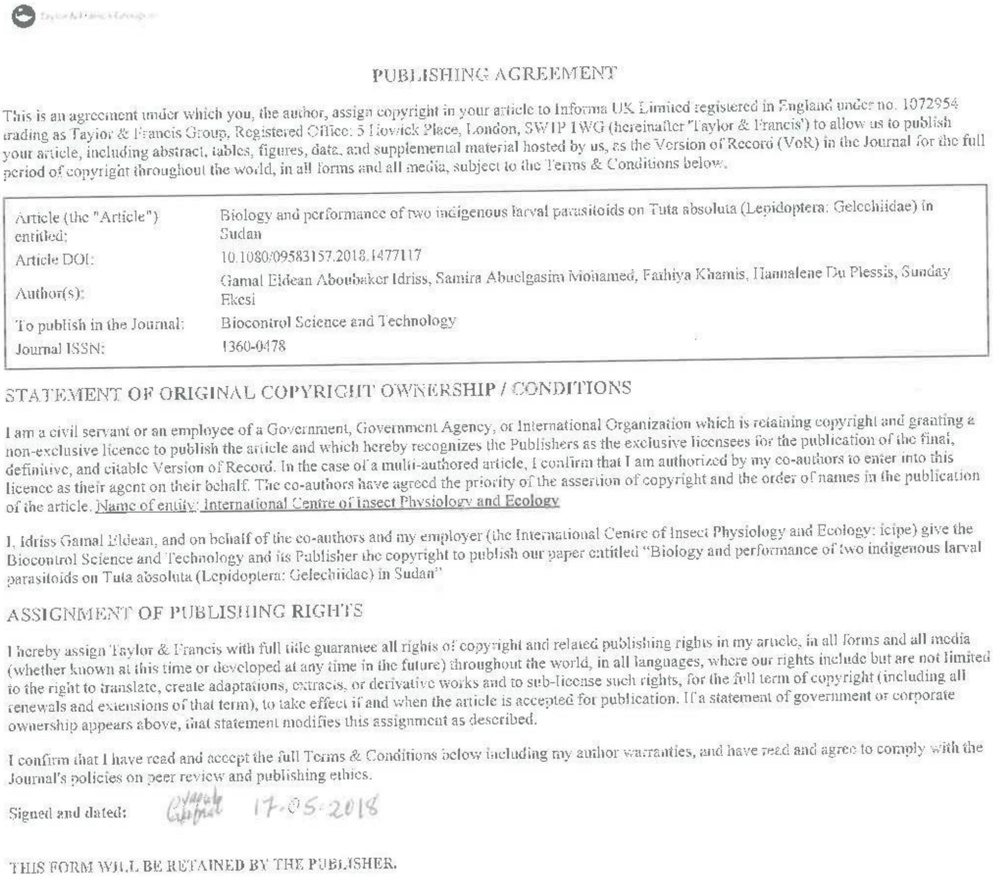

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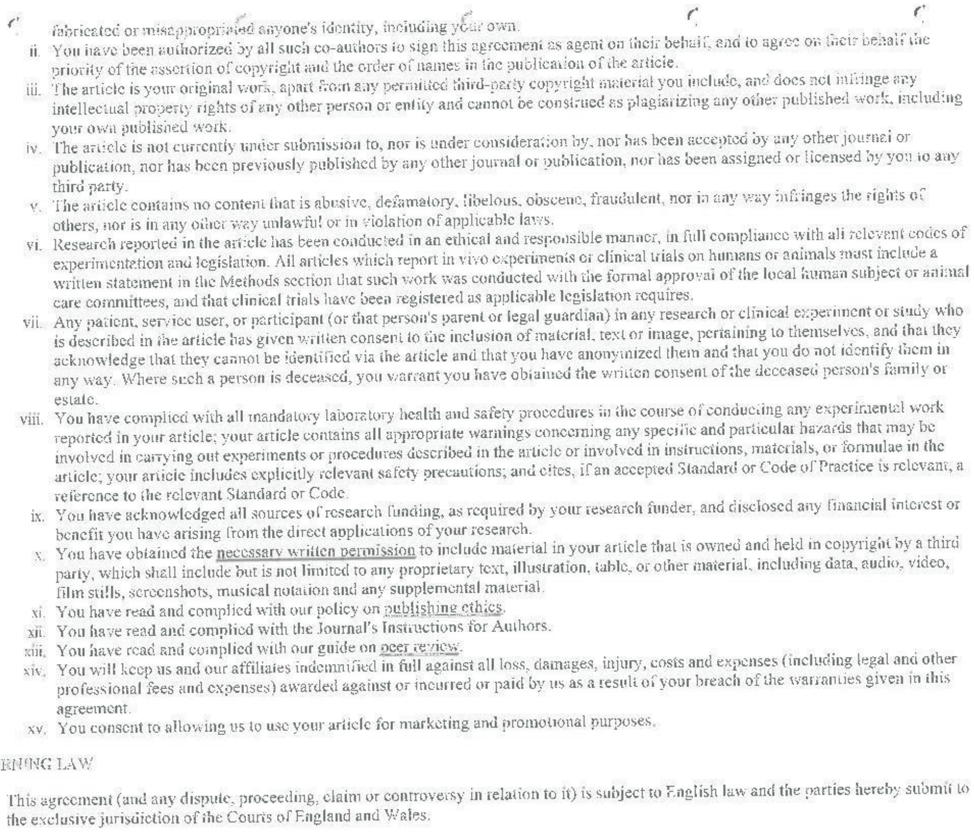

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