



Efficacy of entomopathogenic nematodes for control of *Tuta absoluta* in South Africa

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DECLARATION BY THE CANDIDATE

I, O. Coleman, declare that the work presented in this MSc thesis is my own work, that it is not been submitted for any degree or examination at any other University and that all the sources I have used or cited have been acknowledged by the complete reference.

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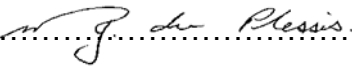
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We declare that the work presented in this thesis was carried out by the candidate under our supervision and we approve this submission.

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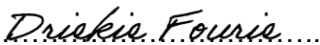


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ABSTRACT

The South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one of the most devastating pests of tomato (*Solanum lycopersicon* L.) in South America, Europe, the Middle East and Africa. Current management tactics of *T. absoluta* consist mainly of monitoring with sex pheromone traps and application of insecticides. Resistance to various insecticide groups has, however, been reported in Asia, Europe and South America. Development of integrated pest management (IPM) strategies for this pest is therefore important. There is currently no tomato cultivar commercially available which is resistant to *T. absoluta*, and parasitoids from only four families are known as biological control agents of *T. absoluta* in Africa. A variety of insect pests are controlled with entomopathogens such as fungi, bacteria and nematodes, which are used as biopesticides. Although entomopathogenic nematodes (EPNs) were initially applied as soil applications against pests, investigations to use EPNs as foliar applications also received renewed interest. In Europe, *Heterorhabditis bacteriophora* Poinar, 1976, *Steinernema feltiae* (Filipjev, 1934) Wouts, Mráľcek, Gerdin and Bedding, 1982, and *Steinernema carpocapsae* (Weiser, 1955) Wouts, Mráľcek, Gerdin and Bedding, 1982 have been reported to effectively control *T. absoluta* as foliar applications. The Agricultural Pest Act 36 of South Africa, prohibits importation of exotic species without a full impact study and permit. A search for native biological control agents for *T. absoluta* is therefore warranted. The aim of this study was to evaluate the efficacy of four native EPN species, viz. *Steinernema jeffreyense* Malan, Knoetze and Tiedt, 2015, *Steinernema yirgalemense* Nguyen, Tesfamariam, Gözel, Gaugler and Adams, 2005, *Heterorhabditis baujardi* Phan, Subbotin, Nguyen and Moens, 2003 and *Heterorhabditis noenieputensis* Malan, Knoetze and Tiedt *et al.*, 2014 against *T. absoluta* in South Africa. Fourth instar *T. absoluta* larvae and pupae were exposed to IJs of the four EPN species *in vitro*. All four EPNs were found to be highly effective in controlling the larvae, with 100% larval morality caused, but pupae were less susceptible. Following the successful *in vitro* assays using the EPNs against *T. absoluta* larvae, greenhouse trials were conducted. Efficacy of *S. jeffreyense* and *S. yirgalemense* applied to the foliage of tomato seedlings for control of third and fourth instar *T. absoluta* larvae, was evaluated at four concentrations, viz. 250, 500, 1 000 and 2 000 IJs mL⁻¹ distilled water containing 0.05% adjuvant (Nu-Film-P). High mortality rates of *T. absoluta* larvae in tomato leaves were recorded with both species at application rates of 1 000 and 2 000 IJs mL⁻¹. Results from this study identified *S. jeffreyense* and *S. yirgalemense* as promising biocontrol agents of *T. absoluta* under greenhouse tomato production in South Africa, which could be included in IPM of this pest. By applying an IPM system and not relying on chemical control only, resistance to insecticides in South Africa, may be prevented or delayed.

Key words: Biological control, biopesticide, EPNs, Integrated Pest Management, tomato leafminer

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Chapter 1: Introduction and Literature review

1.1. General introduction

Tomato (*Solanum lycopersicon* L.) is one of the most widely cultivated and economically important crops worldwide (Tropea Garzia *et al.*, 2012). It has been cultivated on more than 5.7 million hectares, with a global production of approximately 233 million tons reported in 2018 (FAOSTAT, 2018). The total tomato production in the United States of America (USA) during this period was 14 million tons and the South African production, 580 000 tons (FAOSTAT, 2018).

Tomato fruit is rich in lycopene (Falara *et al.*, 2011; Fernández-Ruiz *et al.*, 2011), a carotenoid with several important health benefits for humans (Clinton, 1998). It is a powerful antioxidant that acts as an anti-carcinogen (Kelley and Boyhan, 2006). Tomato is also rich in vitamins and minerals and one medium ripe tomato can provide up to 40% of the recommended daily allowance (RDA) of Vitamin C and 20% of RDA Vitamin A (Kelley and Boyhan, 2006). The B vitamins, potassium, iron and calcium needed in the human diet, are also found in tomato (Kelley and Boyhan, 2006). The commercial value of the fruit includes processed foods as well as selling it on the fresh market (Kelley and Boyhan, 2006).

Weeds are serious competitors for environmental resources and if left unchecked can reduce crop yield and quality considerably (COPR, 1983; Ozores-Hampton *et al.*, 2001). Fungal and bacterial diseases, viruses and mycoplasma-like diseases also contribute to lower yields and production of tomato crops (COPR, 1983). Various ecto- and endoparasitic nematodes, Phylum Nematoda (Rudolphi, 1808) Lankester, 1877, are economically important pests of tomato, causing excessive damage to crops worldwide (Greco and Di Vito, 2011; Seid *et al.* 2015; Jones *et al.*, 2017). Ectoparasites listed as major pests include species of stubby-root (*Paratrichodorus* Siddiqi, 1974) and dagger (*Xiphinema* Cobb, 1913) nematodes as well as genera and species of the stunt nematodes (Dolichodoridae Chitwood, 1950) (Greco and Di Vito, 2011). By contrast the migratory, endoparasitic lesion nematodes *Pratylenchus* Filipjev, 1936 and *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936, sedentary counterpart species of cyst (*Globodera* Skarbilovich, 1959), root-knot (*Meloidogyne* Göldi, 1887) nematodes, the false root-knot (*Nacobbus aberrans* (Thorne, 1935) Thorne and Allen, 1944) and reniform (*Rotylenchulus reniformis* Linford and Oliveira, 1940) nematodes are major pests of tomato (Greco and Di Vito, 2011). Root-knot nematodes are, however, regarded as the most damaging genus that infect roots of tomato crops. The four species generally known to cause damage in tropical and subtropical areas being *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, *Meloidogyne enterolobii* Yang and Eisenback, 1983, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949. In

temperate parts of the world *Meloidogyne chitwoodi* Golden, O'Bannon, Santo and Finley, 1980, *Meloidogyne fallax* Karssen, 1996 and *Meloidogyne hapla* Chitwood, 1949 cause damage to the crop (Greco and Di Vito, 2011; Seid *et al.* 2015).

There are between 100 and 200 insect pest species globally recorded on tomato (Attwa *et al.*, 2015). In South Africa, more than 45 insects were listed as pests on tomato (Table 1.1) (Visser, 2015).

Table 1.1: Most common tomato insect pests in South Africa from Visser (2015).

Order	Pest species common name	Scientific name
Coleoptera	Family Scarabaeidae	
	Fruit and root chafer beetles	<i>Anoplocheilus friguratus</i> Boheamn <i>Dischista cincta</i> (DeGeer) <i>Pachnoda sinuata</i> (Fabricius) <i>Pedinorrhina trivittata</i> (Schaum) <i>Porphyronata Hebraeae</i> (Olivier) <i>Tephraea dichroa</i> (Schaum) <i>Tephraea leucomelona</i> (Gory and Percheron)
	Family Coccinellidae	
	Potato ladybird Solanum ladybird	<i>Epilachna dregei</i> Mulsant <i>Henosepilachna hirta</i> (Thunberg)
Diptera	Family Agromyzidae	
	Potato leaf miner American leaf miner	<i>Liriomyza huidobrensis</i> (Blanchard) <i>Liriomyza trifolii</i> (Brugess)
Hemiptera	Family Aphididae	
	Black bean aphids Potato aphid Green peach aphid	<i>Aphis fabae</i> Scopoli <i>Macrosiphum euphorbiae</i> (Thomas) <i>Myzus persicae</i> (Sulzer)
	Family Petatomidae	
	Green vegetable bug	<i>Nezara viridula</i> (Linnaeus)
	Family Lygaeidae	
	Milkweed bugs	<i>Spilostethus pandurus</i> (Scopoli)
	Family Coreidae	
	Large black tip wilter Common tip wilter	<i>Anoplocnemis curvipes</i> (Fabricius) <i>Elasmopoda valga</i> (Linnaeus)
	Family Aphididae	
	Sweet potato whitefly Greenhouse whitefly	<i>Bemisia tabaci</i> (Gennadius) <i>Trialeurodes vaporariorum</i> (Westwood)
Lepidoptera	Family Noctuidae	
	African bollworm	<i>Helicoverpa armigera</i> (Hübner)
	Black cutworm Brown cutworm	<i>Agrotis ipsilon</i> (Hufnagel) <i>Agrotis longidentifera</i> (Hampson)

	Common cutworm Grey cutworm	<i>Agrotis segetum</i> (Denis and Schiffermüller) <i>Agrotis subalba</i> Walker
	Lesser armyworm	<i>Spodoptera exigua</i> (Hübner)
	Potato tuber moth	<i>Phthorimaea operculella</i> (Zeller) <i>Scrobipalpa aptatella</i> (Walker)
	Tomato semi-looper Plusia semi-looper	<i>Chrysodeixis acuta</i> (Walker) <i>Thysanoplusia orichalcea</i> (Fabricius)
	Tomato moth	<i>Spodoptera littoralis</i> (de Boisduval)
Thysanoptera	Family Thripidae	
	Western flower thrips Kromnek thrips Onion thrips	<i>Frankliniella occidentalis</i> (Pergande) <i>Frankliniella schultzei</i> (Trybom) <i>Thrips tabaci</i> Lindeman

Since the list was compiled by Visser in 2015, the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) invaded South Africa.

1.2. *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

Tuta absoluta was first detected in South Africa in August 2016 (Visser *et al.*, 2017). The pest originates from South America (Desneux *et al.*, 2010) and has initially been introduced into Spain late in 2006 after which it spread quickly in the European countries (Loni *et al.*, 2011). It also spread throughout the Mediterranean Basin, parts of Northern Africa and the Middle East (Urbaneja *et al.*, 2012) and downwards into southern Africa (Biondi *et al.*, 2018). It has been reported as one of the most devastating pests on tomato in South America (Desneux *et al.*, 2010), Europe (Loni *et al.*, 2011), the Middle East and Africa (Abbes *et al.*, 2016).

A map showing the distribution of *T. absoluta* in 2018 is provided in Figure 1.1. The distribution of *T. absoluta* now extends to latitudes that are well above its original habitat (South America) (Fig. 1.1), indicating the ability of this pest to adapt and acclimatise. For a species to establish in any newly invaded area, it needs climatic conditions suitable for its survival, availability of food sources as well as the ability to survive other stress related factors that occurs with transmission to a new environment (Renault *et al.*, 2018). *Tuta absoluta* is considered a typical invasive species, due to its capacity to develop very quickly in suitable agro-ecological conditions, its ability to spread rapidly and to cause economic damage in new areas (Desneux *et al.*, 2010; Tropea Garzia *et al.*, 2012). Since its invasion, it has caused serious crop losses and has become an agricultural threat to crops in these areas (Desneux *et al.*, 2010).

Tuta absoluta is an oligophagous insect and feeds almost exclusively on plants belonging to the Solanaceae family (Siqueira *et al.*, 2000; Sannino and Espinosa, 2010a). The Solanaceae includes important crops such as potato (*Solanum tuberosum* L.), eggplant (*Solanum melongena* L.) and pepper (*Capsicum annuum* L.) (Tropea Garzia *et al.*, 2012; Cocco *et al.*, 2015, Hernández-Ruiz and Arnao, 2016). *Tuta absoluta* feeds mainly on tomato plants but other cultivated solanaceous species can also act as hosts for this pest (Cocco *et al.*, 2015). The widespread cultivation of tomato, potato and other naturally occurring solanaceous crops around the world together with the mild European to subtropical African climates, allowed *T. absoluta* moths to be introduced and established in new areas (Sannino and Espinosa, 2010b).

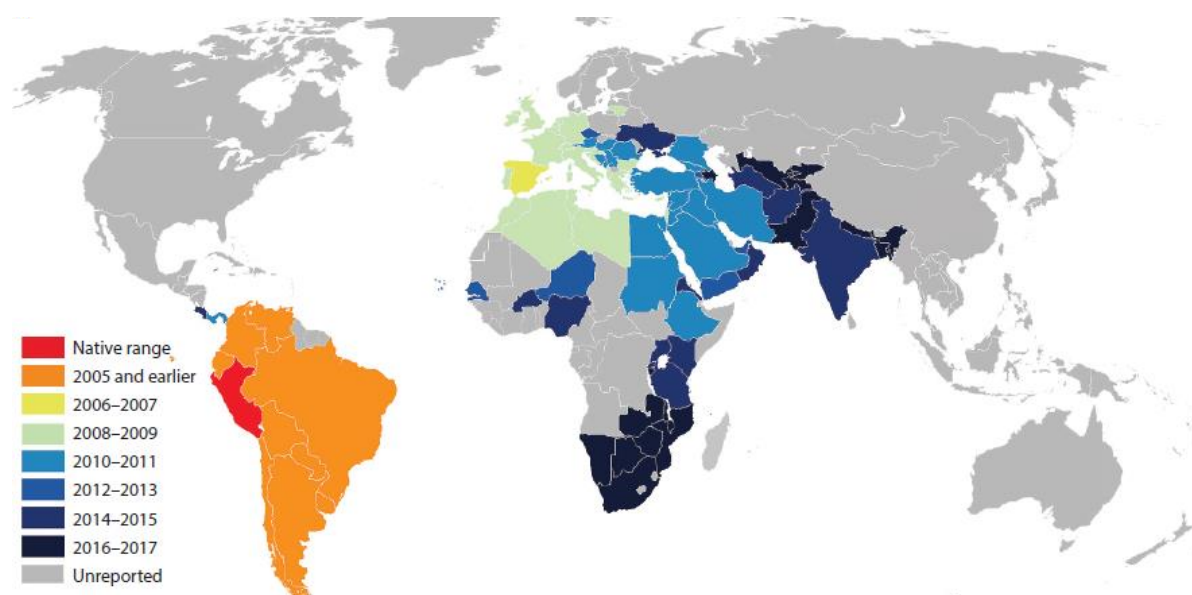


Figure 1.1: Geographic distribution of *Tuta absoluta* in 2018 (From Bondi *et al.*, 2018).

Despite its preference for Solanaceous vegetables, some weeds have also been reported to act as hosts for this insect pest in several countries (Unlu, 2012). Reported host plants of *T. absoluta* are provided in Table 1.2.

Table 1.2: Host plants reported for *Tuta absoluta*.

Plant family	Host plants	Common name	Reference
Crop host plants			
Solanaceae	<i>Lycopersicon esculentum</i> Linnaeus	Tomato	Park <i>et al.</i> (2004) Desneux <i>et al.</i> (2010) Hernández-Ruiz and Arnao (2016)
	<i>Solanum tuberosum</i> Linnaeus	Potato	Megido <i>et al.</i> (2013)
	<i>Solanum melongena</i> Linnaeus	Eggplant	Megido <i>et al.</i> (2013)
	<i>Nicotiana tabacum</i> Linnaeus	Tobacco	Megido <i>et al.</i> (2013)
	<i>Physalis peruviana</i> Linnaeus	Cape gooseberry	USDA-APHIS (2011).
	<i>Capsicum annuum</i> Linnaeus	Pepper	USDA-APHIS (2011)
	<i>Solanum muricatum</i> Linnaeus	Sweet pepper	Desneux <i>et al.</i> (2010)
Alternative host plants			
Fabaceae	<i>Phaseolus vulgaris</i> Linnaeus	Common beans	Mohamed <i>et al.</i> (2015)
	<i>Vicia faba</i> Linnaeus	Broad bean	Mohamed <i>et al.</i> (2015)
	<i>Vigna unguiculata</i> (Linnaeus) Walpers	Cowpea	Mohamed <i>et al.</i> (2015)
	<i>Medicago sativa</i> Linnaeus	Alfalfa	Mohamed <i>et al.</i> (2015)
Solanaceae	<i>Lycium barbarum</i> Linnaeus	Goji berry	Mohamed <i>et al.</i> (2015)
Brassicaceae	<i>Raphanus raphanistrum</i> Linnaeus	Wild radish	Mohamed <i>et al.</i> (2015)
Wild host plants			
Solanaceae	<i>Solanum nigrum</i> Linnaeus	Night shade	Desneux <i>et al.</i> (2010)
	<i>Solanum elaeagnifolium</i> Cavanilles	Silverleaf nightshade	Desneux <i>et al.</i> (2010) Mekki (2007)
	<i>Solanum bonariense</i> Linnaeus		Desneux <i>et al.</i> (2010)
	<i>Solanum sisymbriifolium</i> Lamarck		Desneux <i>et al.</i> (2010)
	<i>Solanum saponaceum</i> Welwitsch		Desneux <i>et al.</i> (2010) Bayram <i>et al.</i> (2015)
	<i>Solanum hirtum</i> Vahl		Cocco <i>et al.</i> (2015)

	<i>Datura stramonium</i> Linnaeus	Jimson weed	Desneux <i>et al.</i> (2010), Mohamed <i>et al.</i> (2015)
	<i>Lycium chilense</i> Miers ex Bertero	Coralillo	Mohamed <i>et al.</i> (2015) Varela <i>et al.</i> (2018)
	<i>Datura ferox</i> Linnaeus	Long spined thorn apple	Desneux <i>et al.</i> (2010)
	<i>Nicotiana glauca</i> Graham	Tree tobacco	Mohamed <i>et al.</i> (2015)
Amaranthaceae	<i>Chenopodium album</i> Linnaeus	Lambs-quarters	Mohamed <i>et al.</i> (2015)
Convolvulaceae	<i>Convolvulus arvensis</i> Linnaeus	Bindweed	Mohamed <i>et al.</i> (2015)

Alternative host plants maintain this insect pest in the absence of tomato crops. For successful integrated pest management (IPM), the presence and control of these alternative host plants should be taken into consideration (Tropea Garzia *et al.*, 2012).

1.2.1. Biology of *Tuta absoluta* and damage symptoms

Tuta absoluta is a holometabolic insect with four development stages namely eggs, larvae, pupae and moths (Fig. 1.2) (Desneux *et al.*, 2010). Spreading of *T. absoluta* is attributed to the uncontrolled trading of infested plants and fruit, but spreading by moths also contribute to the repeated and continued infestation in more enclosed areas (Sannino and Espinosa, 2010b). Active flight or passive spreading through wind currents are also means of dispersal (Desneux *et al.*, 2010). The high population growth potential of *T. absoluta* whereby many generations are produced per season as well as greenhouse crops that create conditions for continuous feeding and reproducing, also contribute to the rapid distribution of the pest. Uncontrolled outbreaks of an introduced pest such as *T. absoluta* can also be as a result of the lack of natural enemies in newly infested countries as well as the development of resistance to commonly used insecticides (Sannino and Espinosa, 2010b).

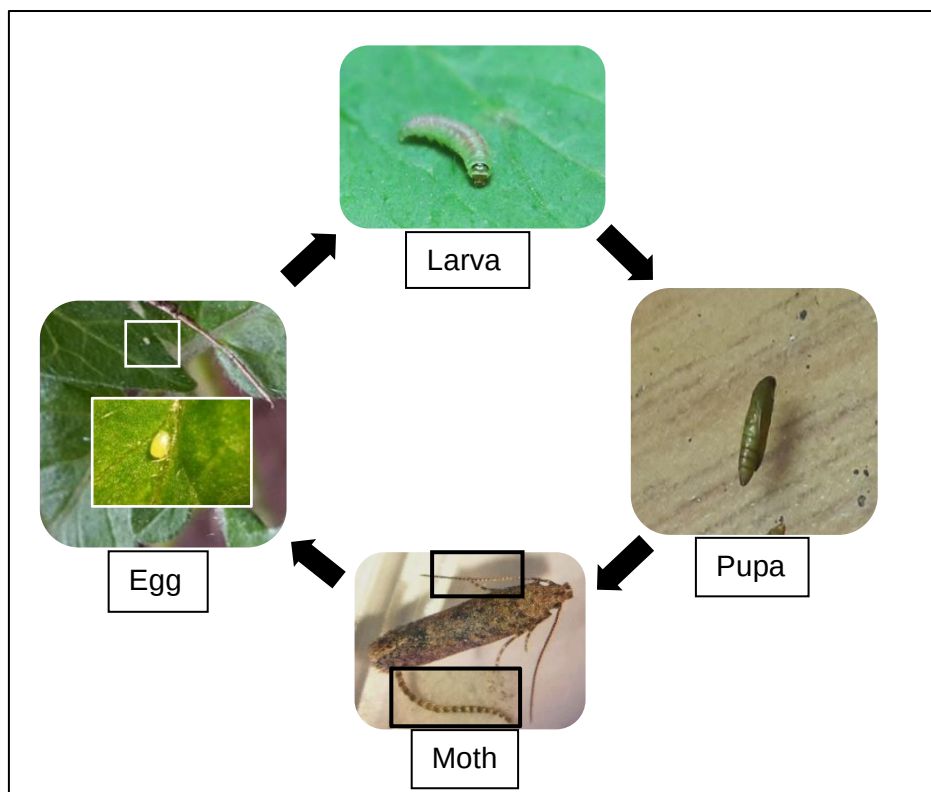


Figure 1.2: Life cycle of *Tuta absoluta* (Photo's: Odette Coleman, NWU).

Tuta absoluta completes 10-12 generations per year in South America and overwinters in all its life stages (Cocco *et al.*, 2015). The life cycle of *T. absoluta* is completed in approximately 24 days at 27 °C but the development time can vary between 13-65 days depending on temperature (Desneux *et al.*, 2010). Moths are 6-7 mm in length with silver to grey scales and the antennae are filiform (Fig. 1.3A) (Desneux *et al.*, 2010). Female moths live for 10-15 days and males for 6-7 days, but moths can live for up to 40 days at 10 °C (Cuthbertson *et al.*, 2013). The females can mate once a day for 4-5 h, and up to six times during their life span (Desneux *et al.*, 2010; Tropea Garzia *et al.*, 2012). The longer life span of female moths allows them to become sexually mature before the males emerge (Tropea Garzia *et al.*, 2012). On tomato, eggs are laid on leaves, usually underneath, or on the stems (Desneux *et al.*, 2010). In cases of severe infestation, eggs are laid on the fruit as well (Cocco *et al.*, 2015). Most eggs are laid in the seven days following the first mating, with 70% of the total oviposition occurring during this period (Tropea Garzia *et al.*, 2012). A female can oviposit up to 260 eggs during her lifespan (Tropea Garzia *et al.*, 2012).

Larval development time at 27 °C is completed in 11-13 days (Sannino and Espinosa, 2010c). Fully developed, fourth instar larvae (Fig. 1.3B) drop to the soil or pupate in a cocoon on leaves of the host plant (Desneux *et al.*, 2010). Pupae are 5-6 mm in length, cylindrical shaped, greenish after pupation and become darker in colour with time (Cocco *et al.*, 2015). The larvae

have a very distinctive black mark behind the head capsule (Sannino and Espinosa, 2010a), are endophytic and briefly move around before mining into the plant (Cocco *et al.*, 2015).

Tuta absoluta attacks any stage of tomato plants, from seedlings to mature plants. They feed on the mesophyll tissue of leaves (Fig. 1.3C), flowers, stems and fruit (Fig. 1.3D) (Ubaneja *et al.*, 2012). Mines in leaf stalks and stems can cause plant death (Sannino and Espinosa, 2010c). Larvae tunnel into the leaves. These tunnels expand rapidly and become large chambers leaving the two outer membranes (Fig. 1.3C) (Sannino and Espinosa, 2010c). This damage reduces the photosynthetic capacity of the plant, resulting in fewer fruit and a lower yield (Pereyra and Sánchez, 2006; Ubaneja *et al.*, 2012).



Figure 1.3: (A) *Tuta absoluta* moth, (B) fourth instar larva, (C) damage caused by larvae to leaves and (D) larval damage to fruit (Photo's: Odette Coleman, NWU).

1.3. Control strategies for *Tuta absoluta*

The best option for control of *T. absoluta* is with an IPM strategy (González-Cabrera *et al.*, 2011). It can consist of a single method or a combination of methods to produce an effective management strategy (Kogan, 1998), in order to maintain pest populations below the economic injury level (Dent, 2000). These methods include chemical, biological and cultural control, and host plant resistance (Dent, 2000). Although IPM strategies include chemical control in tomato fields, the aim should be to reduce the overall usage and still maintain effective control (Miranda *et al.*, 2005). More environmentally friendly control measures are also needed to reduce the potential harmful effects that might disrupt existing IPM programs (Urbaneja *et al.*, 2012).

1.3.1. Chemical control

The production cost of tomato has increased substantially in countries where crops are infested with *T. absoluta* due to additional pest control and monitoring strategies (Tropea Garzia *et al.*, 2012). For example, production costs more than tripled in the main tomato production areas of South America due to the increased use of insecticides to control *T. absoluta* (Guedes and Picanço, 2012). The number of insecticide applications increased from

10-12 to over 30 per cultivation period, with approximately four to six insecticide applications per week in commercial fields (Guedes and Picanço, 2012). The year-round cultivation of tomato crops and the short *T. absoluta* life cycle of 26-38 days in fields, provide for multiple overlapping generations and a challenge to effectively control the pest. It subsequently results in increased levels of infestation and higher losses (Guedes and Picanço, 2012).

Tuta absoluta is mainly controlled with insecticides (Loni *et al.*, 2011). Effective chemical control is problematic due to the endophytic feeding of this pest that takes place between the two lamellas of a leaf (Gözel and Kasap, 2015). The efficacy of insecticides applied to tomato crops is compromised when an insect population is already well established before the reproductive stage (Guedes and Picanço, 2012). The main concern with extensive use of insecticides is the quick development of resistance (Guedes and Picanço, 2012; Urbaneja *et al.*, 2012). The high reproduction capacity, short generation cycle of *T. absoluta* as well as the intensive use of insecticides, enhance and account for the higher risk of resistance development to insecticides (Gözel and Kasap, 2015).

Insecticides from four groups with different modes of action, namely diamides, avermectins, spinosyns and oxadiazines are the most extensively used in Europe (Roditakis *et al.*, 2018). Excessive use of insecticides results in selection pressure where resistant genotypes are favoured reducing the efficacy of insecticides (Roditakis *et al.*, 2018). The use of insecticides is therefore not a sustainable management strategy as shown by the efficacy of organophosphates to control *T. absoluta* which has gradually decreased in many countries since the 1980s (Desneux *et al.*, 2010). The only insecticides that were initially available for *T. absoluta* control in Argentina were organophosphates, but they were replaced by pyrethroids in 1970 (Lietti *et al.*, 2005). In 1980, as an alternative to pyrethroids, cartap and thiocyclam that were highly effective, were introduced (Lietti *et al.*, 2005). Only a limited number of products within these groups, were available to control *T. absoluta* before 1990 increasing the risk of resistance development. Resistance to organophosphates and pyrethroids was reported in Chile and Brazil (Desneux *et al.*, 2010). Abamectin, cartap and permethrin were subsequently introduced followed by chitin synthesis inhibitors which was then relied on by producers. This resulted in extreme resistance development against chitin synthesis inhibitors, indoxacarb and spinosad (Guedes and Picanço, 2012).

Tuta absoluta resistance development and susceptibility to insecticides in different tomato production localities are not universal and the loss in effectiveness does not occur in all regions (Lietti *et al.*, 2005). Resistance to deltamethrin and abamectin was reported in *T. absoluta* populations in open fields and greenhouses in Argentina in 2010 (Desneux *et al.*, 2010). Roditakis *et al.* (2018) conducted a follow up baseline susceptibility study on European *T. absoluta* and the efficacy of key insecticides. In his study, resistance to chlorantraniliprole was

reported from Italy, Greece and Israel. In a few cases resistance to oxadiazines, avermectins and spinosyns was also detected (Roditakis *et al.*, 2018). Registered agrochemicals that can be used against *T. absoluta* in South Africa are spinetoram, indoxacarb, spinosad, cypermethrin as well as the mixtures, emamectin benzoate and lufenuron and chlorantraniliprole and lambda-cyhalothrin (DAFF, 2017). No resistance to insecticides by *T. absoluta* has been reported in South Africa to date.

Insecticide resistance management (IRM) is applied to delay or prevent resistance development to insecticides or to regain susceptibility to insecticides in an insect pest population (Spark and Nauen, 2015). To reduce selection pressure to an insecticide, methods such as rotation or alteration of insecticides, mixtures and mosaics can be implemented (Spark and Nauen, 2015). Following a basic IPM strategy and implementing IRM can reduce selection pressure which will reduce resistance development enabling products to maintain field efficacy (Roditakis *et al.*, 2018). The use of chemical insecticides is not a sustainable management strategy due to the proven development of resistance by *T. absoluta*, but also due to the potential effect it has on non-target organisms occurring in fields where tomato crops are planted. Moreover, its adverse effects on animals, humans and the environment lead to numerous pesticides being banned from world markets (PAN, 2019).

1.3.2. Cultural control

Cultural control is a strategy that aims to manipulate the environment to render it unfavourable for pests to live or breed in. This method interferes with the ability of the pests to colonise, reproduce or survive (Dent, 2000). Cultural control is the management of an agro-ecosystem to favour crop production and to avoid or reduce pest populations (Bajwa and Kogan, 2004). The main focus of cultural control is therefore to enhance the processes that limit pest invasion and population growth in agro-ecosystems and also limits the potential of an organism to reach pest status (Bajwa and Kogan, 2004). Preventive measures that could be implemented are the destruction of crop remains and the removal of naturally occurring alternative hosts in and around the tomato production area. Such actions, however, increase labour and can become costly (Sannino and Espinosa, 2010d; Guedes and Picanço, 2012).

Crop rotation should also be applied and consists of seasonal alteration of one or more crops within a field with the aim to disrupt the biology of the pest that depends on feeding on one of those crops (All, 1999). Overwintering insect pests will then have to colonise a non-host crop which will reduce colonisation of the pest in a field (Dent, 2000). Crop rotation with non-host plants is effective in interrupting the pest cycle and lower pest numbers. Tilling practices can destroy hibernating insect individuals (Sannino and Espinosa, 2010d). Continuous cultivation of tomato crops in tomato production areas throughout the year reduces the effectiveness of

this control method because of the continuous availability of hosts (Guedes and Picanço, 2012).

Other cultural control management strategies include methods aimed at preventing the pest from mating. Development of a population will be affected and high pest numbers will be prevented. For these methods, either synthetic pheromones for male annihilation and mating disruption are used, or the sterile insect technique (SIT) is applied (Sannino and Espinosa, 2010d; Guedes and Picanço, 2012; Megido *et al.*, 2012). By monitoring insect pests to detect when increases in pest populations occur, an informed decision for application of control measures can be made. Pheromone traps baited with female sex pheromones are very effective in monitoring the increase in population size of *T. absoluta* and can be used to decide on the best time for control applications in the field (Sannino and Espinosa, 2010d). Correct timing of application is important because early application, before the major outbreak, would not be effective (Sannino and Espinosa, 2010d). The traps are also used for mass capturing to lower numbers of the insect pest (Sannino and Espinosa, 2010d). An important factor with sex pheromone based management and SIT is that the control is aimed at target insect pests which reproduce sexually (Megido *et al.*, 2013). As soon as asexual or parthenogenetic reproduction occur, the efficacy of these control methods are reduced which is the case with *T. absoluta* since females can reproduce asexually (Megido *et al.*, 2012; 2013).

1.3.3. Host plant resistance

Host plant resistance to *T. absoluta* has not been successfully implemented because tomato yield is lower in resistant cultivars (Guedes and Picanço, 2012). Cultivated tomato is highly susceptible to *T. absoluta* and even though there are genetic sources of resistance located in germplasm banks, accessions of *S. lycopersicum* (wild tomato) are the most promising sources of resistance (Bondi *et al.*, 2018). Density of granular trichomes on the leaves and the insecticidal compounds produced by these trichomes fend off *T. absoluta*. Allelochemicals are mainly focussed on to impair egg laying as well as feeding by larvae (Guedes and Picanço, 2012; Bondi *et al.*, 2018). Tomato cultivars with induced resistance mechanisms activated by larval feeding is also considered because feeding on the plant could activate plant defences against various other insect pests (Bondi *et al.*, 2018). Breeding tomato cultivars resistant to this pest remains a highly considered control management strategy but the development of such varieties is still in progress (Guedes and Picanço, 2012; Bondi *et al.*, 2018). Research on breeding lines with acyl sugars have been conducted and it was found that a high content of these chemicals are the main provider for host plant resistance (Dias *et al.*, 2013; Bondi *et al.*, 2018). Acyl sugars are easily introduced into elite tomato lines with a simple inheritance that are essentially monogenic, containing modifier genes with additive effect (Dias *et al.*, 2013).

1.3.4. Biological control

Biological control is the use of living organisms that include invertebrates and a wide variety of microbial pathogens, including fungi, bacteria and viruses, as pest control agents to maintain low pest population density. It may operate alone or as a component of integrated pest management (Greathead and Waage, 1983). It is an environmentally safe measure to manage *T. absoluta* in the countries where it is present. It also includes natural occurring predators and parasitoids that attack this pest (González-Cabrera *et al.*, 2011). Applied by humans, biological control is the deliberate exploitation of natural enemies to control and regulate insect pest populations. It is therefore a human activity to control pests in a more environmentally friendly manner (Hagler, 2000). It is actions of parasites, predators or pathogens that maintain the population density of a pest organism at a density lower than what it would have been in their absence (Ruberson *et al.*, 1999). It can also be defined as the manipulation of natural enemies to control pests in crop systems (Dent, 2000). These enemies can occur naturally or they can be introduced into the cropping system.

The strategies used for biological control are the introduction, inundation, augmentation and inoculation of natural enemies as well as their conservation (Greathead and Waage, 1983; Dent, 2000). A biological control strategy could be a long term sustainable strategy to manage *T. absoluta* (Desneux *et al.*, 2010).

Classical biological control is the introduction of exotic natural enemies to control a newly invaded exotic insect pest (Lacey *et al.*, 2001). It presents a permanent control solution with few risks and provides a highly cost effective approach. Introduction of exotic natural enemies of insect pests may take two to three years since it requires intensive research on the target pest and its native complex of natural enemies, as well as selection, collection, rearing and release of ideal candidates (Dent, 2000). This is a long process and not an immediate solution, but when effectively introduced, it would provide long term control of a newly introduced insect pest (Dent, 2000). The introductions of exotic pathogens are not as popular as the introduction of predators and parasitoids (Lacey *et al.*, 2001). Inoculation and augmentation are used in cases where the natural enemies are absent or where the population densities are too low to be effective. The numbers of natural enemies may be increased with the release of laboratory reared insects (Dent, 2000). Augmentation is, however, only a temporary approach and provides only temporary control to keep the population below an economic threshold level. An inoculation biological control approach is a seasonal application which lasts for the duration of the crop, making it the preferred approach over augmentation (Dent, 2000). Inundation is the mass release of the biological control agent, which kills the host fast, but it is usually not persistent (Dent, 2000). Biological control agents used for inundation are pathogens,

consisting of viruses, bacteria, fungi as well as EPNs which are formulated into a biopesticide that can be used as an alternative to chemical insecticides (Dent, 2000).

Many natural enemies had been described and successfully implemented to control *T. absoluta* in regions where this pest naturally occurs (Urbaneja *et al.*, 2012). Biological control agents were reported to be successful in effectively suppressing *T. absoluta* populations (Van Damme *et al.*, 2016). Both predators and parasitoids attack *T. absoluta* in European and North African countries (Zappalà *et al.*, 2013). Parasitoids attack the egg, larval and pupal stages of this pest (Desneux *et al.*, 2010). Predators are mainly species from the Miridae family and are used as part of IPM strategies (Zappalà *et al.*, 2013). These predators prey mostly on the eggs and to a limited extend on the larvae of the leaf miner (Van Damme *et al.*, 2016). The most commonly used predators against *T. absoluta* in European greenhouses are *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) and *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) (Van Damme *et al.*, 2016). More than 70 species of generalist natural enemies have been reported to attack *T. absoluta* in the western Palaearctic region (Zappala *et al.*, 2013).

The efficacy of entomopathogens for control of *T. absoluta* is not well documented in South America (Desneux *et al.*, 2010), but Lacey *et al.* (2001) reported entomopathogens to provide good control of insect pests compared to other biological control agents. The entomopathogen, *Bacillus thuringiensis* has been reported to have great potential for controlling *T. absoluta* in tomato greenhouses and fields in the Mediterranean Basin (González-Cabrera *et al.*, 2011). *Bacillus thuringiensis* is applied with other biological agents to control the early instars of *T. absoluta*, for example with the predators, *M. pygmaeus* and *N. tenuis* (Van Damme *et al.*, 2016). In these instances, *B. thuringiensis* acts as a complimentary biological control agent.

Another group of biological control agents with potential to target the larval stages of *T. absoluta*, is EPNs (Van Damme *et al.*, 2016). Entomopathogenic nematodes are already applied successfully against weevils, for example the black vine weevil *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae), the root weevil *Diaprepes abbreviatus* Linnaeus (Coleoptera: Curculionidae), and the sweet potato weevil *Cylas formicarius* (Fabricius) (Coleoptera: Brentidae), fungus gnats, *Bradysia* spp. Winnertz (Diptera: Sciaridae), *Lycoriella* spp. Frey (Diptera: Sciaridae), grubs (the japanese beetle *Popillia japonica* Newman (Coleoptera: Scarabaeidae) and the garden chafer *Phyllopertha horticola* (Linnaeus) (Coleoptera: Scarabaeidae) (Půža, 2015). It is also applied against pest organisms that reside in the soil or spend part of their life cycles in the soil (Půža, 2015; Van Damme *et al.*, 2016). Although EPNs are soil-dwelling organisms, they do have the potential to control pests that attack the foliar parts of plants (Van Damme *et al.*, 2016). Their efficacy increased when they are applied in areas that are protected from adverse environmental conditions, e.g.

greenhouses, or if the pest lives in a cryptic habitat such as within a leaf (Van Damme *et al.*, 2016). The success rate of EPNs is lower with foliar application because of the exposure to abiotic factors such as UV radiation, desiccation and extreme temperatures. The use of adjuvants increases leaf coverage and persistence of the infective juveniles (IJs), which in turn enhances the use of EPNs against these foliar pests (Zolfagharian *et al.*, 2016; Kamali *et al.*, 2018; Platt *et al.*, 2019a). Nematode species from the families Steinernematidae Travassos, 1927 and Heterorhabditidae Poinar, 1976 are considered as possible control agents for *T. absoluta* (Gözel and Kasap, 2015).

1.4. Entomopathogenic nematodes

Entomopathogenic nematodes occur naturally in soil and act as obligate parasites by attacking different life stages of insects. They therefore have the benefit of not being harmful to animals and plants (Shapiro-Ilan *et al.*, 2006; Zolfagharian *et al.*, 2016; Devi and Nath, 2017). This is a diverse group of organisms that can be found almost anywhere in the world, in any biome such as cultivated fields, forests, grasslands, deserts and even on beaches or in the ocean (Devi and Nath, 2017). These nematodes are safe to use in the environment and are exempted from registration in European countries such as Austria and Germany, North America including the USA and the United Kingdom (UK) (Van Zyl and Malan, 2014; Devi and Nath, 2017; Malan and Ferreira, 2017). Many countries are, however, required to undergo a registration process (Ehlers, 2005). The introduction of non-endemic EPNs in South Africa is also not exempted from registration as in most of the European countries, North America or the UK (Van Zyl and Malan, 2014). Malan and Ferreira (2017) stated that: "according to the amended Act 18 of 1989 (South African Agricultural Pests Act No. 36 of 1947), the introduction of exotic animals such as non-endemic EPN is only allowed under permit, which has to be accompanied by a full impact study". Research to determine the efficacy of native EPN species is therefore important. Nearly 40 nematode families are associated with insects (Gaugler and Kaya, 1990), but not all cause host mortality and only 23 of these families contain species described as EPNs (Lacey and Georgis, 2012). Research is focused on species which have the potential to act as biological control agents, and these are found in the families Mermithidae Braun, 1883, Tetradenematidae Cobb 1919, Allantonematidae Pereira, 1931, Phaenopsitylenchidae Blinova and Korenchenko, 1986, Sphaerulariidae Lubbock, 1861, Steinernematidae and Heterorhabditidae (Goodey, 1960; Lacey *et al.*, 2001; Poinar *et al.*, 2002; Stock and Hunt, 2005). *Heterorhabditis* spp. Poinar, 1976 and *Steinernema* spp. Travassos, 1927 are the best known EPNs that are widely available and used for biological control of many insect pests worldwide (Griffin, 2012).

In the family Steinernematidae, the genus *Steinernema* contains at least 88 identified species (Abate *et al.*, 2017), while the genus *Neosteinerema* has only one identified species (Devi

and Nath, 2017). The family Heterorhabditidae has only one genus, namely *Heterorhabditis* with 19 identified species (Stock, 2015; Abate *et al.*, 2017). The use of these EPNs against crop pests has shown promise (Griffin, 2012). *Steinernema* spp. and *Heterorhabditis* spp. have both parasitoid and pathogenic attributes. Chemoreceptors, a parasitoid characteristic, enables them to actively search for their host while the association with mutualistic bacteria is a pathogenic characteristic (Lacey *et al.*, 2001). The mutualistic bacteria contained within EPNs is the main reason for these nematodes to be such attractive biopesticides in management of insect pests (Akhursts, 1993). The bacteria associated with *Steinernema* spp. are *Xenorhabdus* spp., that are motile, gram-negative enterobacteria, and γ -proteobacteria, while *Photorhabdus* spp. are gram-negative rods that occur in *Heterorhabditis* spp. (Chaston *et al.*, 2011, Poinar and Grewal, 2012). Although each nematode species is associated with a specific bacterium, the bacterium can be associated with more than one nematode species (Lacey *et al.*, 2001). *Steinernema* spp. are associated with 20 *Xenorhabdus* spp., while four *Photorhabdus* spp. are described to be associated with *Heterorhabditis* spp. (Devi and Nath, 2017). These associations are synergistic, with *Steinernema* spp. carrying the bacteria in a specialized vesicle in their intestine (Bird and Akhurst, 1983), whereas in *Heterorhabditis* spp. the bacteria occur in the foregut and midgut region of the non-feeding IJs (Boermare *et al.*, 1996). Infective juveniles, the only free living stage of *Heterorhabditis* and *Steinernema*, is adapted to survive outside the insect host (Mahmoud, 2016) using stored reserves of energy to search for and enter the host (Griffin *et al.*, 2005). They enter their insect hosts through natural openings such as the mouth, anus and spiracles (Kaya and Gaugler 1993; Griffin *et al.*, 2005).

Once the IJs have entered the haemocoel of an insect, the symbiotic bacteria are released and suppress the insect's defence systems. The bacterium multiply and produces a diverse group of components namely bacteriocins, antibiotics, antimicrobials and a scavenger deterrent compound (Chaston *et al.*, 2011). This compound suppresses the growth of antagonistic microorganisms to provide a safe niche, and breaks down the host tissue which causes death of the insect host usually within 24-48 h after infection, and ultimately produces food (Chaston *et al.*, 2011; Devi and Nath, 2017). The EPNs act by feeding on the bacteria and the degrading host tissue, they multiply, develop and reproduce (Griffin, 2012; Mahmoud, 2016). Depending on the size of the host, the nematode completes one generation or more. In the case of *Steinernema*, the first generation develops into males and females, while in *Heterorhabditis* the first generation nematodes are hermaphrodites (Hazir *et al.*, 2003; Griffin *et al.*, 2005). When nutrients are depleted, a high nematode population density induces the development of new non-feeding IJs. They therefore leave the host and move to the soil to search for a new host, continuing the life cycle (Chaston *et al.*, 2011; Mahmoud, 2016). The

bacterium-nematode complex is highly virulent and kills its hosts quickly (Lacey *et al.*, 2001). The general life cycle of EPNs is illustrated in Figure 1.4 and the advantages and disadvantages associated with the use of EPNs as biocontrol agents are provided in Table 1.3.

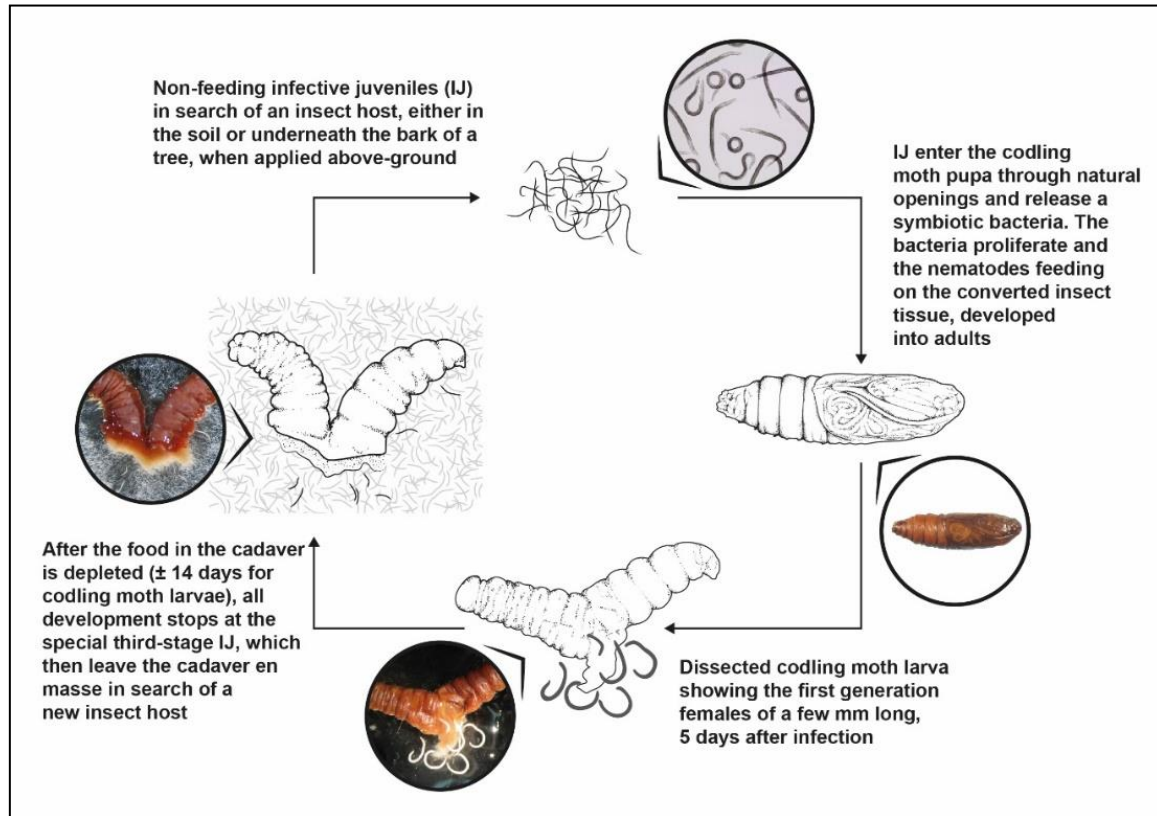


Figure 1.4: An illustration of the typical life cycle of entomopathogenic nematodes (Hannes Visagie, North-West University, South Africa; photo's supplied by Antoinette Malan, University of Stellenbosch; illustration cited by Malan and Ferreira, 2017).

Table 1.3: Advantages and disadvantages of EPNs used commercially to control insect pests.

Advantages	References
Easily massed produced <i>in vivo</i> and <i>in vitro</i> and formulated as a biopesticide.	Lacey <i>et al.</i> (2001) Gözel and Gozel (2016)
Exempted from registration in many countries.	Gözel and Gozel (2016)
Compatible with many pesticides.	Kaya and Gaugler (1993)
Amendable to genetic selection.	Kaya and Gaugler (1993)
A broad host range of insect pests.	Gözel and Gozel (2016)
Able to seek or ambush an insect host and rapidly kill it.	Gözel and Gozel (2016)
Can be applied with conventional application equipment.	Lacey <i>et al.</i> (2001) Gözel and Gozel (2016)
Increase biodiversity in managed ecosystems, resulting in increase of other natural enemy activities.	Lacey <i>et al.</i> (2001)
Not harmful to humans or higher organisms.	Lacey <i>et al.</i> (2001) Gözel and Gozel (2016)
Pesticide residues in food is reduced.	Lacey <i>et al.</i> (2001)
Disadvantages	References
Formulation and quality control is challenging.	Gözel and Gozel (2016)
Limited shelf life and refrigerated storage required.	Gözel and Gozel (2016)
Environmental limitations for survival and infestation are factors like adequate moisture, temperatures, harsh environmental conditions.	Gözel and Gozel (2016)
Has a broad host range.	Lacey <i>et al.</i> (2001)

1.4.1. Entomopathogenic nematodes as biological control agents

The potential use of EPNs as biopesticides is an environmentally friendly control option. Various EPN species are globally used against a wide range of insect pest species and success has been achieved against insect pests occurring in different habitats (Gözel and Gozel, 2016). The success of EPNs for control of insects varied according to the host species as well as the prevailing environmental conditions where they were applied. Commercially available EPNs applied against insect pest worldwide are listed in Table 1.4.

The commercialised EPN species listed in Table 1.4 are those that are currently the most commonly used to successfully control target insect pests. Their wide geographic distribution enables them to be applied where law restriction only permits naturally occurring species to be used for biological control (Půža *et al.* 2016; Abate *et al.*, 2017). More than a decade ago, Kaya *et al.* (2006) listed many insect pests controlled by EPNs specifically in Asia and Central and South America, which included species not listed above, such as *Steinernema arenarium* (Artyukhovsky, 1967) Wouts, Mráček, Gerdin and Bedding, 1982, *Steinernema bicornutum* Tallosi, Peters and Ehlers, 1995, *Steinernema longicaudum* Shen and Wang, 1992,

Steinernema monticolum Stock, Choo and Kaya, 1997 and *Steinernema thermophilum* Ganguly and Singh, 2000, *Steinernema kushidai* Mamiya, 1988, *Steinernema scarabaei* Stock and Koppenhöfer, 2003, *Heterorhabditis downesi* Stock, Griffin and Brunell, 2002, *Heterorhabditis indica* Poinar, Karunakar and David, 1992, and *Heterorhabditis marelata* Liu and Berry, 1996. *Heterorhabditis zealandica* Poinar, 1990 can also be considered for economic use having effectively controlled insects in bioassays (Gözel and Gozel, 2016). However, EPN's have the potential to control many more insect pests as proved in laboratory and field studies (Grewal *et al.*, 2001).

Table 1.4: Entomopathogenic nematode species commercially available, according to Abate *et al.* (2017), with their associated symbiotic bacteria, the geographic distribution and a list of insect pests successfully controlled (Gözel and Gozel, 2016).

EPN species	Symbiotic bacteria	Geographic distribution	Targeted insect pest (Gözel and Gozel, 2016)
<i>Steinernema carpocapsae</i> (Weiser, 1955) Wouts, Mráček, Gerdin and Bedding, 1982	<i>Xenorhabdus nematophila</i> (Půža <i>et al.</i> , 2016)	Worldwide (Půža <i>et al.</i> , 2016)	<i>Agrotis ipsilon</i> (Hufnagel) (Lepidoptera: Noctuidae) <i>Amyelois transitella</i> (Walker) (Lepidoptera: Pyralidae) <i>Chrysoteuchia topiaria</i> (Zeller) (Lepidoptera: Crambidae) <i>Cosmopolites sordidus</i> (Germar) (Coleoptera: Curculionidae) <i>Ctenocephalides felis</i> (Bouché) (Siphonaptera: Pulicidae) <i>Cylas formicarius</i> (Fabricius) (Coleoptera: Brentidae) <i>Cydia pomonella</i> (Linnaeus) (Lepidoptera: Tortricidae) <i>Diabrotica</i> spp. Chevrollet (Coleoptera: Chrysomelidae) <i>Helicoverpa zea</i> (Boddie) (Lepidoptera: Noctuidae) <i>Hylobius abietis</i> Linnaeus (Coleoptera: Curculionidae) <i>Liriomyza</i> spp. Mik (Diptera: Agromyzidae) <i>Macronoctua onusta</i> Grote (Lepidoptera: Noctuidae) <i>Opogona sacchari</i> (Bojer) (Lepidoptera: Tineidae) <i>Otiorhynchus sulcatus</i> (Fabricius) (Coleoptera: Curculionidae) <i>Platyptilia carduidactyla</i> (Riley) (Lepidoptera: Pterophoridae) <i>Spodoptera</i> spp. Guenée (Lepidoptera: Noctuidae) <i>Scapteriscus</i> spp. (Scudder) (Orthoptera: Gryllotalpidae) <i>Scatella</i> spp Robineau-Desvoidy (Diptera: Ephydriidae) <i>Sphenophorus</i> spp. Schönherr (Coleoptera: Curculionidae) <i>Synanthedon</i> spp. Hübner (Lepidoptera: Sesiidae) <i>Tipula</i> spp Linnaeus (Diptera: Tipulidae) Turf and ornamental scarub grubs (Coleoptera: Scarabaeidae)

<i>Steinernema feltiae</i> (Filipjev, 1934) Wouts, Mráček, Gerdin and Bedding, 1982	<i>Xenorhabdus bovienii</i> (Půža <i>et al.</i> , 2016)	Worldwide (Půža <i>et al.</i> , 2016)	<i>Bradysia</i> spp. Winnertz (Diptera: Sciaridae) <i>C. sordidus</i> <i>C. pomonella</i> <i>C. formicarius</i> , <i>Liriomyza</i> spp <i>H. zea</i> <i>Scatella</i> spp <i>Spodoptera</i> spp. <i>Synanthedon</i> spp
<i>Steinernema glaseri</i> Steiner, 1929	<i>Xenorhabdus poinarii</i> (Půža <i>et al.</i> , 2016)	Europe, Russia, USA, Argentina, Azores, China, Korea, Spain (Půža <i>et al.</i> , 2016)	<i>C. sordidus</i> <i>O. sulcatus</i> Turf and ornamental scarab grubs (Scarabaeidae),
<i>Steinernema kraussei</i> (Steiner, 1923) Travassos, 1927	<i>Xenorhabdus bovienii</i> (Půža <i>et al.</i> , 2016)	Europe, Russia, Canada (Půža <i>et al.</i> , 2016)	
<i>Steinernema riobrave</i> Cabanillas, Poinar and Raulston, 1994	<i>Xenorhabdus cabanillasii</i> (Půža <i>et al.</i> , 2016)	North America (Půža <i>et al.</i> , 2016)	<i>Spodoptera</i> spp. <i>Aethina tumida</i> Murray (Coleoptera: Nitidulidae), <i>Conotrachelus nenuphar</i> Herbst (Coleoptera: Curculionidae) <i>Diaprepes abbreviatus</i> (Linnaeus) (Coleoptera: Curculionidae) <i>Pachnaeus</i> spp Schönherr (Coleoptera: Curculionidae) <i>H. zea</i> <i>Scapteriscus</i> spp.

<i>Steinernema scapterisci</i> Nguyen and Smart, 1992	<i>Xenorhabdus innexi</i> (Půža <i>et al.</i> , 2016)	South America (Hominick <i>et al.</i> , 1996)	<i>Scapteriscus</i> spp.
<i>Heterorhabditis bacteriophora</i> Poinar, 1976	<i>Photorhabdus luminescens</i> , <i>Photorhabdus temperata</i> (Půža <i>et al.</i> , 2016)	Worldwide (Půža <i>et al.</i> , 2016)	<i>Bradysia</i> spp. <i>C. formicarius</i> <i>Diabrotica</i> spp. <i>D. abbreviatus</i> <i>Macronoctua onusta</i> Grote (Lepidoptera: Noctuidae) <i>O. sacchari</i> <i>O. sulcatus</i> <i>Pachnaeus</i> spp. <i>Sphenophorus</i> spp <i>Synanthedon</i> spp. <i>Vitacea polistiformis</i> (Harris) (Lepidoptera: Sesiidae) Turf and ornamental scarab grubs (Scarabaeidae)
<i>Heterorhabditis megidis</i> Poinar, Jackson and Klein, 1988	<i>Photorhabdus temperata</i> (Půža <i>et al.</i> , 2016)	North America, Asia, Europe (Půža <i>et al.</i> , 2016)	<i>Otiorhynchus ovatus</i> (Linnaeus) (Coleoptera: Curculionidae) <i>O. sulcatus</i>

1.4.2. Entomopathogenic nematodes used for the control of *Tuta absoluta*

Three nematode species indigenous to Spain has been evaluated for control of *T. absoluta* larvae and pupae under laboratory and greenhouse conditions (Batalla-Carrera *et al.*, 2010). These species were effective in controlling the larvae (78.6-100% mortality), but not the pupae (<10% pupa mortality).

Steinernema carpocapsae (Weiser, 1955) Wouts, Mráček, Gerdin and Bedding, 1982, *Steinernema feltiae* (Filipjev, 1934) Wouts, Mráček, Gerdin and Bedding, 1982, and *Heterorhabditis bacteriophora* Poinar, 1976 were also evaluated in laboratory bioassays for the control of *T. absoluta* larvae, pupae and adults and were highly effective as biocontrol agents of this pest in Spain (Garcia-del-Pino *et al.*, 2013). Soil application of *S. carpocapsae*, *S. feltiae* and *H. bacteriophora* for the control of final instar larvae that enter the soil to pupate, was evaluated by catching emerging adults with first flight in a trapping adhesive for *T. absoluta*. High mortality of larvae was reported, but no pupal mortality. A high percentage adults emerged where *S. carpocapsae* was applied, while the emergence rate was very low where *S. feltiae* was applied. Four species of nematodes native to Turkey, viz. *Steinernema affine* Bovien, 1937 (isolate 46), *S. carpocapsae* (isolate 1133), *S. feltiae* (isolate 879) and *H. bacteriophora* (isolate 1144) were evaluated for control of *T. absoluta* larvae in a field in Turkey (Gözel and Kasap, 2015). *Steinernema feltiae* proved to be the most effective with high levels of control, but the other three species provided little control of *T. absoluta* larvae in tomato fields (Gözel and Kasap, 2015).

1.4.3. Entomopathogenic nematodes in South Africa

The discovery of EPNs in South Africa took place during 1953 (Harington, 1953). Entomopathogenic nematodes were found in all life stages, except in the eggs of the black maize beetle, *Heteronychus arator* Fabricius (Coleoptera: Scarabidae), near Grahamstown, Eastern Cape Province in a maize field (Harington, 1953). The first survey of EPNs took place 33 years (1988) after the initial discovery in South Africa (Malan and Ferreira, 2017). The susceptibility of crop pests to native EPN species is continuously investigated in South Africa (Malan and Ferreira, 2017). All EPN species isolated, and the insect pest species used for susceptibility testing in South Africa, are provided in Table 1.5. There are currently 19 identified EPN species in South Africa, viz. 13 *Steinernema* spp. and 7 *Heterorhabditis* spp. The most recent studies were conducted on grapevine (Platt *et al.*, 2019b), avocado, litchi, macadamia (Steyn *et al.*, 2019) and blueberries (Dlamini *et al.*, 2020).

Table 1.5: Entomopathogenic nematodes isolated, associated bacteria and insect pests that has been tested for susceptibility.

EPN spp. identified in South Africa	Associated bacteria	Insect pests (Malan and Ferreira, 2017)
<i>Steinernema beitlechemi</i> Çimen, Puža, Nermuť, Hatting, Ramakuwela, Faktorová and Hazir, 2016 (Çimen, Půža, Nermuť, Hatting, Ramakuwela, Faktorová <i>et al.</i> , 2016; Abate <i>et al.</i> , 2018)	<i>Xenorhabdus khoisanae</i> (Çimen, Půža, Nermuť, Hatting, Ramakuwela, Faktorová <i>et al.</i> , 2016; Abate <i>et al.</i> , 2018)	
<i>Steinernema bertusi</i> Katumanyane, Malan, Tiedt and Hurley 2019 (Katumanyane <i>et al.</i> , 2019)	Unknown	
<i>Steinernema biddulphi</i> Çimen, Půža, Nermuť, Hatting, Ramakuwela and Hazir, 2016 (Çimen, Půža, Nermuť, Hatting, Ramakuwela and Hazir, 2016)	New <i>Xenorhabdus</i> spp. still being identified (Çimen, Půža, Nermuť, Hatting, Ramakuwela and Hazir, 2016)	
<i>Steinernema citrae</i> Stokwe, Malan, Nguyen and Tiedt, 2011 (Malan <i>et al.</i> , 2014; Malan and Ferreira, 2017; Abate <i>et al.</i> , 2018)	<i>Xenorhabdus bovienii</i> (Abate <i>et al.</i> , 2018)	<i>Cydia pomonella</i> (Linnaeus) Codling moth (Lepidoptera: Tortricidae) <i>Phlyctinus callosus</i> (Schönherr) Banded fruit weevil (Coleoptera: Curculionidae) <i>Planococcus citri</i> (Risso) Citrus mealybug (Hemiptera: Pseudococcidae) <i>Planococcus ficus</i> (Signoret) Vine mealybug (Hemiptera: Pseudococcidae) <i>Thaumatotibia leucotreta</i> (Meyrick) False codling moth (Lepidoptera: Tortricidae)

<i>Steinernema fabii</i> Abate, Malan, Tiedt, Wingfield, Slippers and Hurley, 2016 (Abate <i>et al.</i> , 2016; Malan and Ferreira, 2017)	<i>Xenorhabdus khoisanae</i> (Abate <i>et al.</i> , 2018)	
<i>Steinernema innovationi</i> Çimen, Lee, Hatting, Hazir and Stock, 2014 (Çimen <i>et al.</i> , 2015; Malan and Ferreira, 2016; Abate <i>et al.</i> , 2018)	Unknown (Abate <i>et al.</i> , 2018)	
<i>Steinernema jeffreyense</i> Malan, Knoetze and Tiedt, 2015 (Malan <i>et al.</i> , 2016a; Malan and Ferreira, 2017)	Unknown	<i>T. leucotreta</i>
<i>Steinernema khoisanae</i> Nguyen, Malan and Gozel, 2006 (Malan <i>et al.</i> 2014)	<i>Xenorhabdus khoisanae</i> (Malan and Ferreira, 2017)	<i>Ceratitis capitata</i> (Wiedemann) Mediterranean fruit fly (Diptera: Tephritidae) <i>Ceratitis rosa</i> Karsch Natal fruit fly (Diptera: Tephritidae) <i>C. pomonella</i> <i>P. callosus</i> <i>P. citri</i> <i>P. ficus</i> <i>T. leucotreta</i>
<i>Steinernema litchii</i> Steyn, Knoetze, Tiedt and Malan, 2017 (Steyn, Knoetze <i>et al.</i> , 2017)	Unknown	
<i>Steinernema nguyenii</i> Malan, Knoetze and Tiedt, 2016 (Malan <i>et al.</i> , 2016b; Malan and Ferreira, 2017)	Unknown	
<i>Steinernema sacchari</i> Nthenga, Knoetze, Berry, Tiedt and Malan, 2014 (Nthenga <i>et al.</i> , 2014; Malan and Ferreira, 2017; Abate <i>et al.</i> , 2018)	<i>Xenorhabdus khoisanae</i> (Abate <i>et al.</i> , 2018)	

<i>Steinernema tophus</i> Çimen, Lee, Hatting, Hazir and Stock, 2014 (Çimen <i>et al.</i> , 2014; Malan and Ferreira, 2017; Abate <i>et al.</i> , 2018)	Unknown (Abate <i>et al.</i> , 2018)	
<i>Steinernema yirgalemense</i> Nguyen, Tesfamariam, Gözel, Gaugler and Adams, 2005 (Malan <i>et al.</i> , 2014)	<i>Xenorhabdus indica</i> (Malan and Ferreira, 2017)	<i>C. capitata</i> <i>C. rosa</i> <i>Cryptolaemus montrouzieri</i> (Mulsant) mealybug ladybird (Coleoptera: Coccinellidae) <i>C. pomonella</i> <i>P. callosus</i> <i>P. citri</i> <i>P. ficus</i> <i>Pseudococcus viburni</i> (Signoret) Obscure mealybug (Hemiptera: Pseudococcidae) <i>T. leucotreta</i>
<i>Heterorhabditis bacteriophora</i> Poinar, 1976 (Malan <i>et al.</i> , 2014; Malan and Ferreira, 2017; Abate <i>et al.</i> , 2018)	<i>Photorhabdus luminescens</i> subsp. <i>Laumondii</i> (Abate <i>et al.</i> , 2018)	<i>C. capitata</i> <i>C. rosa</i> <i>C. pomonella</i> <i>P. callosus</i> <i>P. citr</i> <i>P. ficus</i> <i>P. viburni</i> <i>T. leucotreta</i>
<i>Heterorhabditis baujardi</i> Phan, Subbotin, Nguyen, and Moens, 2003 (Steyn, Malan <i>et al.</i> , 2017)	<i>Photorhabdus luminescens</i> subsp. <i>luminescens</i> (Abate <i>et al.</i> , 2018)	

<i>Heterorhabditis indica</i> Poinar, Karunakar and David, 1992 (James <i>et al.</i> , 2018)	Unknown	
<i>Heterorhabditis noenieputensis</i> Malan, Knoetze and Tiedt, 2014 (Malan <i>et al.</i> , 2014; Malan and Ferreira, 2017)	<i>Photorhabdus luminescence</i> subsp. <i>noenieputensis</i> (Malan and Ferreira, 2017)	<i>C. pomonella</i> <i>P. callosus</i> <i>P. citri</i> <i>P. ficus</i>
<i>Heterorhabditis safricana</i> Malan, Nguyen, de Waal and Tiedt, 2008 (Malan and Ferreira, 2017; Abate <i>et al.</i> , 2018; Hatting <i>et al.</i> , 2019)	<i>Photorhabdus luminescens</i> subsp. <i>Laumondii</i> (Abate <i>et al.</i> , 2018)	<i>P. citri</i>
<i>Heterorhabditis taysaerae</i> Shamseldean, Abou-El-Sooud, Ab-El-Gawad and Saleh, 1996 (Steyn, Malan <i>et al.</i> , 2017; Abate <i>et al.</i> , 2018)	Unknown (Abate <i>et al.</i> , 2018)	
<i>Heterorhabditis zealandica</i> Poinar, 1990 (Steyn, Malan <i>et al.</i> , 2017)	<i>Photorhabdus zealandica</i> (Malan and Ferreira, 2017)	<i>C. montrouzieri</i> <i>C. pomonella</i> <i>P. callosus</i> <i>P. citri</i> <i>P. ficus</i> <i>P. viburni</i> <i>T. leucotrete</i>

Heterorhabditis bacteriophora is an EPN commonly isolated from South African soils (Malan and Ferreira, 2017). *Steinernema feltiae* is an exotic nematode in Africa and has only once being reported as naturally occurring on the African continent, in Algeria (Malan and Ferreira, 2017). In South Africa, only one EPN species, *H. bacteriophora*, is registered for use and is commercially available as Cryptonem® (Hatting *et al.*, 2019). This product is registered against the False codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), Codling moth, *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), weevils and gnats (Hatting *et al.*, 2019). No research on the control of *T. absoluta* with native South African EPN species has been done to date.

1.5. Aim and Objectives

The aim of this study was to determine the efficacy of selected EPN species native to South Africa for the control of *T. absoluta*.

The objectives of this study were:

- 1) To evaluate the efficacy of four native EPN species, viz. *Steinernema jeffreyense* Malan, Knoetze and Tiedt, 2015, *Steinernema yirgalemense* Nguyen, Tesfamariam, Gözel, Gaugler and Adams, 2005, *Heterorhabditis baujardi* Phan, Subbotin, Nguyen and Moens, 2003 and *Heterorhabditis noenieputensis* Malan, Knoetze and Tiedt, 2014 against *T. absoluta* in South Africa.
- 2) To evaluate the efficacy of two native EPN species, viz. *Steinernema jeffreyense* and *Steinernema yirgalemense* applied as foliar sprays for the control of *T. absoluta* larvae in tomato plants under controlled greenhouse conditions.

1.6. References

- ABATE, B.A., MALAN, A.P., TIEDT, L.R., WINGFIELD, M.J., SLIPPERS, B. and HURLEY, B.P.** 2016. *Steinernema fabii* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. *Nematology* 18:235-255.
- ABATE, B.A., SLIPPERS, B., WINGFIELD, M.J., MALAN, A.P. and HURLEY, B.P.** 2018. Diversity of entomopathogenic nematodes and their symbiotic bacteria in South African plantations and indigenous forests. *Nematology* 20(4):355-371.
- ABATE, B.A., WINGFIELD, M.J., SLIPPERS, B. and HURLEY, B.P.** 2017. Commercialisation of entomopathogenic nematodes: should import regulations be revised? *Biocontrol Science and Technology*, 27(2):149-168.
- ABBES, K., HAFSI, A., ELIMEM, M., HARBI, A. and CHERMITI, B.** 2016. Bioassay of three solanaceous weeds as alternative hosts for the invasive tomato leafminer *Tuta*

absoluta (Lepidoptera: Gelechiidae) and insights on their carryover potential. *African Entomology* 24(2):334-342.

AKHURST, R.J. 1993. Bacterial symbionts of entomopathogenic nematodes: The power behind the throne. pp. 127-136. In: *Nematodes and the biological control of insect pests*. (Eds. RA Bedding, R.A., Akhurst, R.J. and Kaya, H.K.) CSIRO Publications: East Melbourne.

ALL, J.N. 1999. Cultural approaches to managing arthropod pests. pp. 395-415. In: *Handbook of pest management*. (Ed. Ruberson, J.R.). CRC Press: Boca Raton.

ATTWA, W.A., OMAR, N.A., EBADAH, I.M.A., EL-WAHAB, A., MOAWAD, T.E., HANAA, S.M. and SADEKM, E. 2015. Life table parameters of the tomato leaf miner, *Tuta absoluta* (Meyrick) and potato tuber moth *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) on tomato plants in Egypt. *Agricultural Science Research Journal* 5(1):1-5.

BAJWA, W.I. and KOGAN, M. 2004. Cultural practices: springboard to IPM. pp. 21-38. In: *Integrated pest management, potential, constraints and challenges*. (Eds. Koul, O., Dhaliwal, G.S. and Cuperus, G.W.). CAB International: Wallingford.

BATALLA-CARRERA, L., MORTON, A. and GARCIA-DEL-PINO, F. 2010. Efficacy of entomopathogenic nematodes against the tomato leafminer *Tuta absoluta* in laboratory and greenhouse conditions. *Biocontrol* 55:523-530.

BAYRAM, Y., BÜYÜK, M., ÖZASLAN, C., BEKTAŞ, Ö., BAYRAM, N., MUTLU, Ç., ATEŞ, E. and BÜKÜN, B. 2015. New host plants of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Turkey. *Journal of Tekirdag Agricultural Faculty* 12(2):43-46.

BIONDI, A., GUEDES, R.N.C., WAN, F.H. and DESNEUX, N. 2018. Ecology, worldwide spread, and management of the invasive South American tomato pinworm, *Tuta absoluta*: past, present, and future. *Annual Review of Entomology* 63:239-258.

BIRD, A.F. and AKHURST, R.J. 1983. The nature of the intestinal vesicle in nematodes of the family Steinernematidae. *International Journal for Parasitology* 13(6):599-606.

BOEMARE, N., LAUMOND, C. and MAULEON, H. 1996. The entomopathogenic nematode-bacterium complex: biology, life cycle and vertebrate safety. *Biocontrol Science and Technology* 6:333-346.

CENTER FOR OVERSEAS PEST RESEARCH (COPR). 1983. Pest control in tropical tomatoes. Centre for Overseas Pest Research: London.

CHASTON, J.M., SUEN, G., TUCKER, S.L., ANDERSEN, A.W., BHASIN, A., BODE, E., BODE, H.B., BRACHMANN, A.O., COWLES, C.E., COWLES, K.N., DARBY, C., DE LEÓN, L., DRACE, K., DU, Z., GIVAUDAN, A., HERBERT TRAN, E.E., JEWELL, K.A.,

KNACK, J.J., KRASOMIL-OSTERFELD, K.C., KUKOR, R., LANOIS, A., LATREILLE, P., LEIMGRUBER, N.K., LIPKE, C.M., LIU, R., LU, X., MARTENS, E.C., MARRI, P.R., ME´DIGUE, C., MENARD, M.L., MILLER, N.M., MORALES-SOTO, N., NORTON, S., OGIER, J., ORCHARD, S.S., PARK, D., PARK, Y., QUROLLO, B.A., SUGAR, D.R., RICHARDS, G.R., ROUY, Z., SLOMINSKI, B., SLOMINSKI, K., SNYDER, H., TJADEN, B.C., VAN DER HOEVEN, R., WELCH, R.D., WHEELER, C., XIANG, B., BARBAZUK, B., GAUDRIAULT, S., GOODNER, B., SLATER, S.C., FORST, S., GOLDMAN, B.S. and GOODRICH-BLAIR, H. 2011. The entomopathogenic bacterial endosymbionts *Xenorhabdus* and *Photorhabdus*: Convergent lifestyles from divergent genomes. *PLoS ONE* 6(11):1-13.

ÇIMEN, H., LEE, M.-M., HATTING, J., HAZI, S. and STOCK, S.P. 2014. *Steinernema tophus* sp. n. (Nematoda: Steinernematidae), a new entomopathogenic nematode from South Africa. *Zootaxa*: <http://dx.doi.org/10.11646/zootaxa.0000.0.0>

ÇIMEN, H., LEE, M.-M., HATTING, J., HAZI, S. and STOCK, S.P. 2015. *Steinernema innovationi* n. sp. (Panagrolaimomorpha: Steinernematidae), a new entomopathogenic nematode species from South Africa. *Journal of Helminthology* 89:415-427.

ÇIMEN, H., PŮŽA, V., NERMUŤ, J., HATTING, J., RAMAKUWELA, T., FAKTOROVÁ, L. and HAZIR, S. 2016. *Steinernema beitlechemi* n. sp., a new entomopathogenic nematode (Nematoda: Steinernematidae) from South Africa. *Nematology* 18:439-453.

ÇIMEN, H., PŮŽA, V., NERMUŤ, J., HATTING, J., RAMAKUWELA, T. and HAZIR, S. 2016. *Steinernema biddulphi* n. sp., a new entomopathogenic nematode (Nematoda: Steinernematidae) from South Africa. *Journal of Nematology* 48(3):148-158.

CLINTON, S.K. 1998. Lycopene: Chemistry, biology, and implications for human health and disease. *Nutrition Reviews* 56(2):35-51.

COCCO, A., DELIPERI, S., LENTINI, A., MANNU, R. and DELRIO, G. 2015. Seasonal phenology of *Tuta absoluta* (Lepidoptera: Gelechiidae) in protected and open-field crops under Mediterranean climatic conditions. *Phytoparasitica* 43:713-724.

CUTHBERTSON, A.G., MATHERS, J.J., BLACKBURN, L.F., KORYCINSKA, A., LUO, W., JACOBSON, R.J., and NORTHING, P. 2013. Population development of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under simulated UK glasshouse conditions. *Insects* 4(2):185-197.

DENT, D. 2000. Insect pest management. 2nd edition. CAB International: Wallingford.

DEPARTMENT: AGRICULTURAL, FORESTRY AND FISHERY (DAFF). 2017. Guideline for registered agrochemicals to control Tomato Leaf Miner (*Tuta absoluta*) in South Africa. <http://www.nda.agric.za/daaDev/sideMenu/plantHealth/docs/Guideline%20for%20registered>

%20agrochemicals%20to%20control%20Tuta%20absoluta%20-%20Tomato%20Leaf%20Miner%2004%20May%202017.pdf. Date of access 24 Aug 2018.

DESNEUX, N., WAJNBERG, E., WYCKHUYS, K.A.G., BURGIO, G., ARPAIA, S., NARVÁEZ-VASQUEZ, C.A., GONZÁLEZ-CABRERA, J., RUESCAS, C.D., TABONE, E., FRANDON, J., PIZZOL, J., PONCET, C., CABELLO, T. and URBANEJA, A. 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. *Journal of Pest Science* 83:197-215.

DEVI, G. and NATH, D. 2017. Entomopathogenic nematodes: A tool in biocontrol of insect pests of vegetables-A review. *Agricultural Reviews* 38(2):137-144.

DIAS, D.M., RESENDE, J.T.V., FARIA, M.V., CAMARGO, L.K.P., CHAGAS, R.R. and LIMA, I.P. 2013. Selection of processing tomato genotypes with high acyl sugar content that are resistant to the tomato pinworm. *Genetic and Molecular Research* 12:381-389.

DLAMINI, T.M., ALLSOPP, E. and MALAN, A.P. 2020. Application of *Steinernema yirgalamense* to control *Frankliniella occidentalis* (Thysanoptera: Thripidae) on blueberries. *Crop Protection* 128:105-106.

EHLERS, R.-U. 2005. Forum on safety and regulation. p 107-114. In: *Nematodes as biocontrol agents*. (Eds. Grewal, P.S., Ehlers R.-U, and Shapiro-Ilan, D.I.). CAB International: Wallingford.

FALARA, V., AKHTAR, T.A., NGUYEN, T.T.H., SPYROPOULOU, E.A., BLEEKER, P.M., SCHAUVINHOLD, I., MATSUBA, Y., BONINI, M.E., SCHILMILLER, A.L., LAST, R.L., SCHUURINK, R.C. and PICHERSKY, E. 2011. The tomato terpene synthase gene family. *Plant Physiology* 157:770-789.

FERNÁNDEZ-RUIZ, V., OLIVES, A.I., CÁMARA, M., DE CORTES SÁNCHEZ-MATA, M. and TORIJA, M.E. 2011. Mineral and trace elements content in 30 accessions of tomato fruits (*Solanum lycopersicum* L.) and wild relatives (*Solanum pimpinellifolium* L., *Solanum cheesmaniae* L. Riley, and *Solanum habrochaites* S. Knapp and DM Spooner). *Biological Trace Element Research* 141:329-339.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAOSTAT). 2018. FAOSTAT statistics database. <http://www.fao.org/faostat/en/#data/QC> Date of access 23 May 2018.

GARCIA-DEL-PINO, F., ALABERN, X. and MORTON, A. 2013. Efficacy of soil treatments of entomopathogenic nematodes against the larvae, pupae and adults of *Tuta absoluta* and their interaction with the insecticides used against this insect. *BioControl* 58(6):723-731.

- GAUGLER, R AND H. K. KAYA.** 1990. *Entomopathogenic Nematodes in Biological Control*. CRC Press: Boca Raton.
- GONZÁLEZ-CABRERA, J., MOLLA´, O., MONTO´N, H. and URBANEJA, A.** 2011. Efficacy of *Bacillus thuringiensis* (Berliner) in controlling the tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *BioControl* 56:71-80.
- GOODEY, J.B.** 1960. The classification of the Aphelenchoidea Fuchs, 1937. *Nematologica* 5(2):111-126.
- GÖZEL, Ç. and KASAP, İ.** 2015. Efficacy of entomopathogenic nematodes against the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in tomato field. *Türkish Journal of Entomology* 39(3):229-237.
- GÖZEL, U. and GOZEL, C.** 2016. Entomopathogenic Nematodes in Pest Management. pp 55-69. In: *Integrated Pest Management (IPM): Environmentally Sound Pest Management*. (Eds. Gill, H.K. and Goyal, G.). InTech: <http://dx.doi.org/10.5772/63894>
- GREATHEAD, D.J. and WAAGE, J.K.** 1983. *Opportunities for biological control of agricultural pests in developing countries*. The World Bank: Washington.
- GRECO, N. and DI VITO, M.** 2011. Main nematode problems of tomato. *Acta Horticulturae* 914:243-249.
- GREWAL, P.S., DE NARDO, E.A.B. and AGUILLERA, M.M.** 2001. Entomopathogenic nematodes: Potential for exploration and use in South America. *Neotropical Entomology* 30(2):191-205.
- GRIFFIN, C.T.** 2012. Perspectives on the behavior of entomopathogenic nematodes from dispersal to reproduction: traits contributing to nematode fitness and biocontrol efficacy. *Journal of Nematology* 44(2):177-184.
- GRIFFIN, C.T., BOEMARE, N.E. and LEWIS, E.E.** 2005. Biology and behaviour. pp. 47-64. In: *Nematodes as biological control agents*. (Eds. Grewal, P.S., Ehlers, R.U. and Shapiro-Ilan, D.I.). CAB International: Wallingford.
- GUEDES, R.N.C. and PICANÇO, M.C.** 2012. The tomato borer *Tuta absoluta* in South America: pest status, management and insecticide resistance. *Bulletin OEPP/EPPO Bulletin* 42(2):211–216.
- HAGLER, J.R.** 2000. Biological control of insects. pp. 207-241. In: *Insect pest management, techniques for environmental protection*. (Eds. Rechcigl, J.E. and Rechcigl, N.A.). Lewis Publishers: London.

- HARINGTON, J.S.** 1953. Observations on the biology, the parasites and the taxonomic position of the black maize beetle – *Heteronychus sanctae-helenae* Blanch. *South African Journal of Science* 50:11-14.
- HATTING, J.L., MOORE, S.D. and MALAN, A.P.** 2019. Microbial control of phytophagous invertebrate pests in South Africa: Current status and future prospects. *Journal of Invertebrate Pathology* 165:54-66.
- HAZIR, S., KAYA, H.K., STOCK, S.P. and KESKÜ, N.** 2003. Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) for biological control of soil pests. *Turkish Journal of Biology* 27:181-202.
- HERNÁNDEZ-RUIZ, J. and ARNAO, M.B.** 2016. Phytomelatonin, an interesting tool for agricultural crops. *Focus on Science* 2(2):1-7.
- HOMINICK, W.M., REID, A.P., BOHAN, D.A. and ANDBRISCOE, B.R.** 1996. Entomopathogenic nematodes: Biodiversity, geographical distribution and the convention on biological diversity. *Biocontrol Science and Technology* 6:317-331.
- JAMES, M., MALAN, M.P. and ADDISON, P.** 2018. Surveying and screening South African entomopathogenic nematodes for the control of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). *Crop Protection* 105:41-48.
- JONES, R.K., STOREY, S., KNOETZE, R. and FOURIE, H.** 2017. Nematode pests of potato and other vegetable crops. pp. 231-260. In: *Nematology in South Africa: A view from the 21st Century*. (Eds. Fourie, H., Spauls, V.W., Jones, R.K., Daneel, M.S. and De Waele, D.) Springer International Publishing: Cham.
- KAMALI, S., KARIMI, J. and KOPPENHÖFER, A.M.** 2018. New insight into the management of the tomato leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae) with entomopathogenic nematodes. *Journal of Economic Entomology* 111(1):112-119.
- KATUMANYANE, A., MALAN, A.P., TIEDT, L.R., and HURLEY, B.P.** 2019. *Steinernema bertusi* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. *Nematology* 22(3):343-360.
- KAYA, H.K., AGUILLERA, M.M., ALUMAI, A., CHOO, H.Y., DE LA TORRE, M., FODOR, A., GANGULY, S., HAZÂR, S., LAKATOS, T., PYE, A., WILSON, M., YAMANAKA, S., YANG, H. and EHLERS, R.U.** 2006. Status of entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of the world. *Biological Control* 38:134-155.

- KAYA, H.K. and GAUGLER, R.** 1993. Entomopathogenic nematodes. *Annual Review of Entomology* 38:181-206.
- KELLEY, W.T. and BOYHAN, G.** 2006. History, significance, classification and growth. pp. 3. In: *Commercial tomato production handbook*. (Eds. Coolong, G. and Boyhan, T.E.). Bulletin 1312: University of Georgia
- KOGAN, M.** 1998. Integrated pest management: historical perspectives and contemporary developments. *Annual Review of Entomology* 43:243-270.
- LACEY, L.A., FRUTOS, R., KAYA, H.K. and VAIL, P.** 2001. Insect pathogens as biological control agents: Do they have a future? *Biological Control* 21:230-248.
- LACEY, L.A. and GEORGIS, R.** 2012 Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. *Journal of Nematology* 44(2):218-225.
- LAUGHLIN, R.** 1953. Absorption of water by the egg of the garden chafer, *Phyllopertha horticola* L. *Nature* 171(4352):577.
- LIETTI, M.M.M., BOTTO, E. and ALZOGARAY, R.A.** 2005. Insecticide resistance in Argentine populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotropical Entomology* 34(1):113-119.
- LONI, A., ROSSI, E. and VAN ACHTERBERG, K.** 2011. First report of *Agathis fuscipennis* in Europe as parasitoid of the tomato leafminer *Tuta absoluta*. *Bulletin of Insectology* 64(1):115-117.
- MAHMOUD, M.F.** 2016. Biology and use of entomopathogenic nematodes in insect pests biocontrol, a generic view. *Cercetări Agronomice în Moldova* 4(168):85-105.
- MALAN, A.P. and FERREIRA, T.** 2017. Entomopathogenic nematodes. pp. 459-480. In: *Nematology in South Africa: A review from the 21st Century*. (Eds. Fourie, H., Spaull, V.W., Jones, R.K., Daneel, M.S. and De Waele, D.). Springer International Publishing: Cham.
- MALAN, A.P., KNOETZE, R. and TIEDT, L.R.** 2014. *Heterorhabditis noenieputensis* n. sp. (Rhabditida: Heterorhabditidae), a new entomopathogenic nematode from South Africa. *Journal of Helminthology* 88:139-151.
- MALAN, A.P., KNOETZE, R. and TIEDT, L.R.** 2016a. *Steinernema jeffreyense* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. *Journal of Helminthology* 90:262-278.

- MALAN, A.P., KNOETZE, R. and TIEDT, L.R.** 2016b. *Steinernema nguyenii* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. *Nematology* 18:571-590.
- MEGIDO, R.C., HAUBRUGE, E. and VERHEGGEN, F.J.** 2012. First evidence of deuterotokous parthenogenesis in the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Journal of Pest Science* 85:409-412.
- MEGIDO, R.C., HAUBRUGE, E. and VERHEGGEN, F.J.** 2013. Pheromone-based management strategies to control the tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae). *Biotechnology, Agronomy, Society and Environment* 17(3):475-482.
- MEKKI, M.** 2007. Biology, distribution and impacts of silverleaf nightshade (*Solanum elaeagnifolium* Cav.). *Bulletin OEPP/EPPO Bulletin* 37:114-118.
- MIRANDA, M.M.M., PICANÇO, M.C., ZANUNCIO, J.C., LEANDRO BACCI, L. and DA SILVA, E.M.** 2005. Impact of integrated pest management on the population of leafminers, fruit borers, and natural enemies in tomato. *Ciencia Rural* 35(1):204-208.
- MOHAMED, E.S.I., MAHMOUD, M.E.E., ELHAJ, M.A.M., MOHAMED, S.A. and EKESI, S.** 2015. Host plants record for tomato leaf miner *Tuta absoluta* (Meyrick) in Sudan. *Bulletin OEPP/EPPO Bulletin* 45(1):108-111.
- NTHENGA, I., KNOETZE, R., BERRY, S., TIEDT, L.R. and MALAN, A.P.** 2014. *Steinernema sacchari* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. *Nematology* 16:475-494.
- OZORES-HAMPTON, M., OBREZA, T.A., and STOFFELLA, P.J.** 2001. Weed control in vegetable crops with composted organic mulches. pp. 275-286. In: *Compost utilization in horticultural cropping systems*. (Eds. Stoffella, P.J. and Kahn, B.A.). CRC Press LLC: Boca Raton.
- PARK, Y.H., WEST, M.A. and ST. CLAIR, D.A.** 2004. Evaluation of AFLPs for germplasm fingerprinting and assessment of genetic diversity in cultivars of tomato (*Lycopersicon esculentum* L.). *Genome* 47(3):510-518.
- PEREYRA, P.C. and SÁNCHEZ, N.E.** 2006. Efecto de dos solanáceas cultivadas sobre el desarrollo y los parámetros poblacionales de la polilla del tomate, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotropical Entomology* 35(5):671-676.
- PESTICIDE ACTION NETWORK INTERNATIONAL (PAN).** 2019. PAN international consolidated list of banned pesticides. PAN international: Asia.

- PLATT, T., STOKWE, N.F. and MALAN, A.P.** 2019a. Foliar application of *Steinernema yirgalemense* to control *Planococcus ficus*: assessing adjuvants to improve efficacy. *South African Journal of Entomology and Viticulture* 40(1):1-7.
- PLATT, T., STOKWE, N.F. and MALAN, A.P.** 2019b. Grapevine leaf application of *Steinernema yirgalemense* to control *Planococcus ficus* in semi-field conditions. *South African Journal of Enology Viticulture* 40(1):1-9.
- POINAR JR., G.O. and GREWAL, P.S.** 2012. History of entomopathogenic nematology. *Journal of Nematology* 44(2):153-161.
- POINAR JR., G.O., JACKSON, T.A. and BELL, N.L.** 2002. *Elaeolenchus parthenonema* n. g., n. sp. (Nematoda: Sphaerularioidea: Anandranematidae n. fam.) parasitic in the palm-pollinating weevil *Elaeidobius kamerunicus* Faust, with a phylogenetic synopsis of the Sphaerularioidea Lubbock, 1861. *Systematic Parasitology* 52(3):219-225.
- PŮŽA, V.** 2015. Control of insect pests by entomopathogenic nematodes. pp. 175-183. In: *Principles of plant-microbe interaction, microbes for sustainable agriculture* (Ed. Lugtenberg, B). Springer International Publishing: Cham.
- PŮŽA, V., ZDENĚK MRÁČEK, Z. and NERMUŤ, J.** 2016. Novelties in pest control by entomopathogenic and mollusc-parasitic nematodes. pp. 71-102. In: *Integrated Pest Management (IPM): Environmentally Sound Pest Management*. (Eds. Gill, H.K. and Goyal, G.). InTech: <http://dx.doi.org/10.5772/64578>
- RENAULT, D., LAPARIE, M., MCCAULEY, S.J. and BONTE, D.** 2018. Environmental adaptations, ecological filtering and dispersal central to insect invasion. *Annual Review of Entomology* 63:645-368.
- RODITAKIS, E., VASAKIS, E., GARCÍA-VIDAL, L., DEL ROSARIO MARTÍNEZ-AGUIRRE, M., RISON, J.L., HAXAIRE-LUTUN, M.O., NAUEN, R., TSAGKARAKOU, A. and BIELZA, P.** 2018. A four-year survey on insecticide resistance and likelihood of chemical control failure for tomato leaf miner *Tuta absoluta* in the European/Asian region. *Journal of Pest Science* 91(1):421-435.
- RUBERSON, J.R., NECHOLS, J.R. and TUABER, M.J.** 1999. Biological control of arthropod pests. pp. 417-448. In: *Handbook of Pest Management*. (Ed. Ruberson J.R.). Marcel Dekker, Inc: New York.
- SANNINO, L. and ESPINOSA, B.** 2010a. Morphological characteristics of *Tuta absoluta*. pp. 17-32 In: *Tuta absoluta. Biology Guide and integrated control approaches*. (Eds. Sannino, L. and Espinosa, B.). L'Informatore Agrario: Verona

SANNINO, L. and ESPINOSA, B. 2010b. Diffusion by commerce. pp. 11-16. In: *Tuta absoluta. Biology Guide and integrated control approaches*. (Eds. Sannino, L. and Espinosa, B.). L'Informatore Agrario: Verona.

SANNINO, L. and ESPINOSA, B. 2010c. Host plant and biology of *Tuta absoluta* leafminer moth. pp. 33-50. In: *Tuta absoluta. Biology Guide and integrated control approaches*. (Eds. Sannino, L. and Espinosa, B.). L'Informatore Agrario: Verona.

SANNINO, L. and ESPINOSA, B. 2010d. Host plant and biology of *Tuta absoluta* leafminer moth. pp. 51-70. In: *Tuta absoluta. Biology Guide and integrated control approaches*. (Eds. Sannino, L. and Espinosa, B.). L'Informatore Agrario: Verona.

SEID, A., FININSA, C., MEKETE, T., DECRAEMER, W. and WESEMAEL, W.M. 2015. Tomato (*Solanum lycopersicum*) and root-knot nematodes (*Meloidogyne* spp.): a century-old battle. *Nematology* 17(9):995-1009.

SHAPIRO-ILAN, D.I., GOUGE, D.H., PIGGOTT, S.J. and FIFE, J.P. 2006. Application technology and environmental considerations for use of entomopathogenic nematodes in biological control. *Biological Control* 38:124-133.

SIQUEIRA, H.Á.A., GUEDES, R.N.C. and PICANÇO, M.C. 2000. Insecticide resistance in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Agricultural and Forest Entomology* 2:147-153.

SPARKS, T.C. and NAUEN, R. 2015. IRAC: Mode of action classification and insecticide resistance management. *Pesticide Biochemistry and Physiology* 121:122-128.

STEYN, W.P., DANEEL, M.S. and MALAN, A.P. 2019. Field application of entomopathogenic nematodes against *Thaumatotibia leucotreta* in South Africa avocado, litchi and macadamia orchards. *Biocontrol* 64:401-411.

STEYN, W.P., KNOETZE, R., TIEDT, L.R. and MALAN, A.P. 2017. *Steinernema litchii* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa *Nematology* 19:1157-1177.

STEYN, W.P., MALAN, A.P., DANEEL, M.S. and SLABBERT, R.M. 2017. Entomopathogenic nematodes from north-eastern South Africa and their virulence against false codling moth, *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae). *Biocontrol Science and Technology* 27(11):1265-1278.

STOCK, S.P. 2015. Diversity, biology and evolutionary. pp. 3-28. In: *Nematode pathogenesis of insects and other pests, ecology and applied technologies for sustainable*

plant and crop protection (Ed. Campos-Herrera, R.). Springer International Publishing: Cham.

STOCK, S.P. and HUNT, D.J. 2005. Morphology and systematics of nematodes used in biocontrol. pp. 3-46. In: *Nematodes as Biocontrol Agents*. (Eds. Grewal, P.S., Ehlers, R. and Shapiro-Ilan, D.I.) CAB International: Wallingford.

TROPEA GARZIA, G., SISCARO, G., BIONDI, A. and ZAPPALÀ, L. 2012. *Tuta absoluta*, a South American pest of tomato now in the EPPO region: biology, distribution and damage. *Bulletin OEPP/EPPO Bulletin* 42(2):205-210.

UNITED STATES DEPARTMENT OF AGRICULTURE-ANIMAL AND PLANT HEALTH INSPECTION SERVICE (USDA-APHIS). 2011. New pest response guidelines: tomato leafminer (*Tuta absoluta*). Riverdale: USDA-APHIS-PPQ- EDP Emergency Management.

UNLU, L. 2012. Potato: a new host plant of *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae) in Turkey. *Pakistan Journal of Zoology* 44(4):1183-1184.

URBANEJA, A., GONZÁLEZ-CABRERA, J., ARNO, J. and GABARRA, R. 2012. Prospects for the biological control of *Tuta absoluta* in tomatoes of the Mediterranean basin. *Pest Management Science* 68:1215-1222.

VAN DAMME, V.M., BECK, B.K., BERCKMOES, E., MOERKENS, R., WITTEMANS, L., DE VIS, R., NUYTTENS, D., CASTEELS, H.F., MAES, M., TIRRY, L. and DE CLERCQ, P. 2016. Efficacy of entomopathogenic nematodes against larvae of *Tuta absoluta* in the laboratory. *Pest Management Science* 72:1702-1709.

VAN ZYL, C. and MALAN, A.P. 2014. The role of entomopathogenic nematodes as biological control agents of insect pests, with emphasis on the history of their mass culturing and *in vivo* production. *African Entomology* 22(2):235-249.

VARELA, M.C., REINOSO, H., LUNA, V. and CENZANO, A.M. 2018. Seasonal changes in morphophysiological traits of two native Patagonian shrubs from Argentina with different drought resistance strategies. *Plant Physiology and Biochemistry* 127:506-515.

VISSER, D. 2015. Tomato, brinjal and peppers. pp 66-73. In: *Insects of cultivated plants and natural pastures in Southern Africa*. (Eds. Prinsloo, G.L. and Uys, V.M.). Entomological: Society of Southern Africa: Hatfield.

VISSER, D., UYS, V.M., NIEUWENHUIS, R.J. and PIETERSE, W. 2017. First records of the tomato leaf miner *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) in South Africa. *BioInvasions Records* 6(4):301-305.

ZAPPALÀ, L., BIONDI, A., ALMA, A., AL-JBOORY, I.J., ARNO`, J., BAYRAM, A., CHAILLEUX, A., EL-ARNAOUTY, A., GERLING, D., GUENAOUI, Y., SHALTIEL-HARPAZ, L., SISCARO, G., STAVRINIDES, M., TAVELLA, L., AZNAR, R.V., URBANEJA, A. and DESNEUX, N. 2013. Natural enemies of the South American moth, *Tuta absoluta*, in Europe, North Africa and Middle East, and their potential use in pest control strategies. *Journal of Pest Science* 86:635-647.

ZOLFAGHARIAN, M., SAEEDIZADEH, A. and ABBASIPOUR, H. 2016. Efficacy of two entomopathogenic nematode species as potential biocontrol agents against the diamondback moth, *Plutella xylostella* (L.). *Journal of Biological Control* 30(2):78-83.

Chapter 2: Efficacy of indigenous entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) against *Tuta absoluta* (Lepidoptera: Gelechiidae) in laboratory bioassays

Abstract

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is a new invasive pest of tomato and other Solanaceous crops in South Africa. *Tuta absoluta* developed resistance to various insecticide groups in Asia, Europe, South and North America which emphasizes the need for an integrated pest management (IPM) approach to control this pest. Laboratory bioassays were conducted to determine the potential of entomopathogenic nematodes (EPNs) as a possible biological control agent for *T. absoluta*. The infective juveniles (IJs) of four indigenous nematode species were screened *in vitro* for their efficacy in killing fourth instar larvae and pupae of *T. absoluta*. *Steinernema jeffreyense* Malan, Knoetze and Tiedt, 2015, *Steinernema yirgalemense* Nguyen, Tesfamariam, Gözel, Gaugler and Adams, 2005, *Heterorhabditis baujardi* Phan, Subbotin, Nguyen and Moens, 2003 and *Heterorhabditis noenieputensis* Malan, Knoetze and Tiedt, 2014 were all highly effective and killed 100% of the *T. absoluta* larvae. The EPNs were, however, less effective in killing *T. absoluta* pupae. The highest mortality was caused by *S. yirgalemense* (41.7%), with less than 30% mortality caused by *S. jeffreyense*, *H. baujardi* and *H. noenieputensis*. The highest percentage infection was by *S. yirgalemense* (38%), while infection by all the other species was lower than 20%. The number of IJs that penetrated *T. absoluta* larvae and pupae was also low. All four EPN species tested have potential for use in an IPM system for to control of *T. absoluta* larvae.

Key words: biocontrol, EPNs, *Heterorhabditis* spp., laboratory bioassays, native, *Steinernema* spp., tomato leafminer

2.1. Introduction

Biological control is the most important component of an integrated pest management (IPM) program (Hoodle, 2006) and an alternative to chemical control of pests (De Bach and Rosen, 1991). The growing need to develop biological control as an alternative method to control insect pests coincided with the rate at which entomopathogenic nematode (EPN) species was discovered and described (Adams and Nguyen, 2002). Most of the EPN species originally used as biopesticides were described before 1990. One or a few isolates from these species, were initially collected from insects that were naturally parasitized (Bedding, 2006). Little research has been done on EPN species before 1960 when the use of insecticides was affordable and effective (Adams and Nguyen, 2002). The negative environmental effects of pesticides were recognized during 1960 (Adams and Nguyen, 2002). When the use of pesticides became more restricted and costly, and their efficacy decreased gradually,

alternative control methods became more attractive (Adams and Nguyen, 2002). Biological control received renewed attention from scientists with research on EPNs that rapidly expanded during the 1980's. The search for new species that could provide effective control and be commercialised was prioritised (Adams and Nguyen, 2002). The first investigations of the potential of EPNs as biological control agents against insect pests, specifically *Steinernema glaseri* Steiner, 1929 for control of the Japanese beetle, was done in the early 1930s (Poinar and Grewal, 2012). This EPN species effectively controlled the Japanese beetle in field experiments (Glaser *et al.*, 1935). Although *Steinernema kraussei* (Steiner, 1923) Travassos, 1927 was the first EPN to be discovered, *S. glaseri* was the first EPN species investigated for its potential as a biological control agent (Poinar and Grewal, 2012). *Heterorhabditis bacteriophora* Poinar, 1976 (Heterorhabditidae) was discovered and described in 1975 by Poinar (1975). It has since been used in various virulence testings (Chyzik *et al.*, 1996; Han and Ehlers, 2000; Ehlers *et al.*, 2003; Anbesse *et al.*, 2008, Batalla-Carrera *et al.*, 2010; Van Damme *et al.*, 2016; Kamali *et al.*, 2018). Entomopathogenic nematodes have thus been marketed and already proved to be effective bio-pesticides in the early years of discovery (Gaugler *et al.*, 1997). *Steinernema* spp., as well as *Heterorhabditis* spp. are now commercially available (Gözel and Gozel, 2016; Abate *et al.*, 2017) and commercially produced (Georgis *et al.*, 2006, Keshari *et al.*, 2019) for control of various insect pests worldwide.

Six *Steinernema* spp., viz. *Steinernema carpocapsae* (Weiser, 1955) Wouts, Mráček, Gerdin and Bedding, 1982; *Steinernema feltiae* (Filipjev, 1934) Wouts, Mráček, Gerdin and Bedding, 1982; *S. glaseri*; *Steinernema kraussei*, *Steinernema riobrave* Cabanillas, Poinar and Raulston, 1994; *Steinernema scapterisci* Nguyen and Smart, 1992; and two *Heterorhabditis* sp., viz. *H. bacteriophora* and *Heterorhabditis megidis* Poinar, Jackson and Klein, 1988 are the most commonly produced species. These nematode species are produced in a liquid culture and are successfully applied in the industry. Other species commercially available are *Steinernema kushidai* Mamiya, 1988; *Steinernema scarabaei* Stock and Koppenhöfer, 2003; *Heterorhabditis indica* Poinar, Karunakar and David, 1992; *Heterorhabditis marelata* Liu and Berry, 1996 and *Heterorhabditis downesi* Stock, Griffin and Brunell, 2002 (Kaya *et al.*, 2006; Georgis *et al.*, 2006; Lacey *et al.*, 2015; Abate *et al.*, 2017).

Entomopathogenic nematodes have a broad host spectrum and can be cultivated *in vivo* or *in vitro* on a large scale (Mahmoud, 2016). They do not pose a threat towards any plants, vertebrates and many invertebrates (Boemare *et al.*, 1996). The short life cycle, ability to kill hosts fast and effective mass culturing of EPNs contribute to their status as good potential biological control agents (Kaya and Gaugler, 1993; Griffin, 2012).

In the family Steinernematidae, at least 88 species have been identified in the genus *Steinernema* (Abate *et al.*, 2017) and only one species in the genus *Neosteinernema* Nguyen and Smart, 1994 (Hunt and Subbotin, 2016). The family Heterorhabditidae has only one genus, namely *Heterorhabditis* with 19 identified species (Abate *et al.*, 2017). *Steinernema carpocapsae* and *S. feltiae* occur worldwide as well as *H. indica* and *H. bacteriophora*, with *H. bacteriophora* being the most widespread EPN in the world (Hominick, 2002).

The application of EPNs in South Africa is not exempted from registration unlike most of the European countries, North America or the United Kingdom (Van Zyl and Malan, 2014). South Africa is subjected to regulations by the Department of Agriculture, Forestry and Fisheries (DAFF) for the import of EPNs, in terms of importation of exotic species for use in biological control (Van Zyl and Malan, 2014). This is enforced by the South African Agricultural Pests Act 36 of 1983, which prohibits importation of exotic organisms, including foreign EPNs, without a permit and a full-impact study provided by DAFF under the Agricultural Pest Act (DAFF, 2020; Klein *et al.*, 2011). This is needed since the introduction of exotic EPNs could reduce and possibly displace native nematodes, and it could also have unknown effects on non-target organisms (Malan *et al.*, 2011).

It is therefore important to search for local EPNs that could be used for possible biological control. Currently South Africa has 19 identified EPN species consisting of 12 *Steinernema* spp. and 7 *Heterorhabditis* spp. The *Steinernema* spp. are *Steinernema beitlechemi* Çimen, Puža, Nermuť, Hatting, Ramakuwela, Faktorová and Hazir, 2016 (Çimen, Puža, Nermuť, Hatting, Ramakuwela, Faktorová *et al.*, 2016), *Steinernema biddulphi* Çimen, Puža, Nermuť, Hatting, Ramakuwela and Hazir, 2016 (Çimen, Puža, Nermuť, Hatting, Ramakuwela and Hazir, 2016), *Steinernema citrae* Stokwe, Malan, Nguyen and Tiedt, 2011 (Malan *et al.*, 2014), *Steinernema fabii* Abate, Malan, Tiedt, Wingfield, Slippers and Hurley, 2016 (Abate *et al.*, 2016), *Steinernema innovationi* Çimen, Lee, Hatting, Hazir and Stock, 2014 (Çimen *et al.*, 2015), *Steinernema jeffreyense* Malan, Knoetze and Tiedt, 2015 (Malan *et al.*, 2016a), *Steinernema khoisanae* Nguyen, Malan and Gozel, 2006 (Malan *et al.*, 2014), *Steinernema litchii* Steyn, Knoetze, Tiedt and Malan, 2017 (Steyn, Knoetze *et al.*, 2017), *Steinernema nguyenii* Malan, Knoetze and Tiedt, 2016 (Malan *et al.*, 2016b), *Steinernema sacchari* Nthenga, Knoetze, Berry, Tiedt and Malan, 2014 (Nthenga *et al.*, 2014), *Steinernema tophus* Çimen, Lee, Hatting, Hazir and Stock, 2014 (Çimen *et al.*, 2014), *Steinernema yirgalemense* Nguyen, Tesfamariam, Gozel, Gaugler and Adams, 2005 (Malan *et al.*, 2014). The *Heterorhabditis* spp. include *H. bacteriophora* (Malan *et al.*, 2014), *Heterorhabditis baujardi* Phan, Subbotin, Nguyen and Moens, 2003 (Steyn, Malan *et al.*, 2017), *H. indica* (James *et al.*, 2018), *Heterorhabditis noenieputensis* Malan, Knoetze and Tiedt, 2014 (Malan *et al.*, 2014), *Heterorhabditis safricana* Malan, Nguyen, de Waal and Tiedt, 2008 (Hatting *et al.*, 2018),

Heterorhabditis taysaerae Shamseldean, Abou-El-Sooud, Ab-El-Gawad and Saleh, 1996 (Steyn, Malan *et al.*, 2017) and *Heterorhabditis zealandica* Poinar, 1990 (Steyn, Malan *et al.*, 2017).

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is an invasive insect pest which poses a threat to Solanaceous crop production in South Africa since 2016 (Visser *et al.*, 2017). This pest has also been reported to be resistant to insecticides from groups that have various modes of action from regions in Europe and South America (Guedes *et al.*, 2019). It caused increased tomato production costs and crop losses, in newly invaded areas (Sannino and Espinosa, 2010). It is therefore important to use alternative control methods for effective control of this pest and also to reduce selection pressure for insecticide resistant development (Roditakis *et al.*, 2018). Biological agents can be considered as an alternative control method for *T. absoluta* (Desneux *et al.*, 2010).

Entomopathogens such as fungi, bacteria and nematodes are used to control a variety of insect pests (Lacey *et al.*, 2001). Studies on the efficacy of EPNs against insect pests have been conducted with promising results, but it is yet to be implemented for commercial use in South Africa. These include studies conducted in South Africa with indigenous EPNs against key lepidopteran pests, such as *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae) (De Waal *et al.*, 2011), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) (Steyn, Malan *et al.*, 2017), *Helicoverpa armigera* (Lepidoptera: Noctuidae) (Jankielsohn and Hatting, 2005) and *Busseola fusca* (Lepidoptera: Noctuidae) (Ramakuwela *et al.*, 2011). A more recent study conducted on *Phlyctinus callosus* (Schönherr) (Coleoptera: Curculionidae), the banded fruit weevil also illustrated that various EPN species have the potential to control this pest, with *S. yirgalemense* and *H. indica* that provided effective control. These species were also highly effective in controlling the stages of the banded fruit weevil that occurred in the soil in field experiments (Dlamini *et al.*, 2019). *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) the vine mealybug, another key grapevine pest in South Africa, was also found to be successfully controlled by *S. yirgalemense*, which is highly pathogenic to the adult females (Le Vieux and Malan, 2013). Since one EPN species is pathogenic to more than one insect species, only one EPN species could therefore be applied in a field to target more than one important pest (Dlamini *et al.*, 2019).

Only one EPN species, *H. bacteriophora*, is currently registered for use in South Africa. It is available as Cryptonem® for control of the false codling moth, codling moth, weevils and gnats (Hatting *et al.*, 2018). According to the above-mentioned successful trial results, potential for more EPN species to be registered for control of various insect species in South Africa exists.

The objective of this study was to evaluate the efficacy of four native EPN species, viz. *S. jeffreyense*, *S. yirgalemense*, *H. baujardi* and *H. noenieputensis* against *T. absoluta* in South Africa.

2.2. Material and methods

Tuta absoluta stock colony

A rearing colony was established with *T. absoluta* larvae collected from commercially produced tomatoes at Tarlton (26°03'29.07"S 27°40'44.00"E; Gauteng Province), Ventersdorp (26°24'24.07"S 26°47'36.00"E; North-West Province) and Polokwane (23°46'48.03"S 29°28'00.02"E; Limpopo Province). The larvae were released onto potted tomato plants (cvs Money maker and Monica) that were placed in a 4 x 3 m netted enclosure at Potchefstroom (26°40'26.65"S 27°06'23.23"E; North-West Province) under environmental conditions. Uninfested plants were regularly introduced into the cage serving as food for the larvae. Larvae from this stock colony were used in all bioassays.

Entomopathogenic nematodes

Infective juveniles (IJs) of four EPN species, viz. *S. jeffreyense* (J194), *S. yirgalemense* (157-C), *H. baujardi* (MT17) and *H. noenieputensis* (SF669) were obtained from the Department of Conservation Ecology and Entomology, Stellenbosch University, Western Cape Province, South Africa. The nematodes were kept in vented culture flasks in 150 ml filtered water, in a dark incubator at 15±1 °C and the flasks were shaken weekly to improve aeration and nematode survival (Platt *et al.*, 2019).

Bioassay protocol

Six milliliters of the base EPN solution, containing IJs, reared *in vivo* for inoculation purposes were pipetted into a 50 ml capacity plastic tube containing two small magnetic stirrers. The tube was nested upright into a 200 ml capacity glass beaker, which was placed onto a magnetic stirrer. The content was stirred at 2 000 rpm for 2 min. Eight, 10 µL drops were then removed from the stirring nematode solution using a micropipette. Each drop was placed individually on a De Gisse nematode counting dish (De Gisse, 1963). The number of IJs in each drop was counted. This was repeated six times (8 drops of 10 µL each x 6 replicates) and the mean number of IJs was hence determined per 10 µL drop (number of IJs counted in the 10 x 8 µL drops / 8). This enabled calculation of the volume to be extracted, from the base solution, to obtain the required number of IJs (100) for inoculation on filter paper discs for the respective experiments. The following equation was used to determine how many microliters were needed to obtain 100 IJs. Firstly, the number of IJs present in the 6 mL (6 000 µL) extracted from the base solution were calculated as follows:

$$= \frac{\text{Volume extracted from base solution (6 000 } \mu\text{L)} \\ \text{in which IJs was suspended in}}{\text{volume of individual drops (10 } \mu\text{L) IJs were suspended in} \\ \text{for counting}} \times \text{mean number of IJs in 8 individual 10 } \mu\text{L drops (6 replicates)}$$

= number of IJs in base suspension in (6 000 μ L).

The next step entailed the calculation of the volume to be extracted from the 6 000 μ L base solution to obtain 100 IJs for inoculation purposes, which was done as follows:

$$= \frac{\text{number of IJs needed (namely 100IJ)}}{\text{total number of IJs suspended in the base suspension of 6 000 } \mu\text{L}} \times \text{volume of base solution, namely 6 000 } \mu\text{L}$$

= volume (μ L) needed to obtain 100IJs

Every second well of a 24 multi-well plate (Cellstar® Cat.-No. 662 102) was lined with a circular filter paper disc (13 mm diameter). The IJs were inoculated at a rate of 100 IJs/50 μ L water into each of these wells. Leaf disks (10 mm diameter) were cut from tomato leaves and inoculated with one, fourth instar *T. absoluta* larva per disk. One of these leaf disks containing a larva that had already mined into the disk, was transferred to each of the EPN inoculated wells (Fig. 2.1A).

Two-day old pupae were also collected from the stock colony and bioassays were conducted by placing one *T. absoluta* pupa on a paper disk in every second well (Fig 2.1B). For the respective experiments with either larvae or pupae, each plate, containing 12 individuals, served as a replicate and there were five replicates per experiment. The bioassay plates were sealed with lids, placed into plastic containers lined with moist paper towels and closed with a lid to maintain high humidity. The treatment and control multi-well plates were kept in separate containers. These containers were kept at 25 ± 1 °C in an incubator in darkness. Mortality of *T. absoluta* larvae or pupae (depending on the experiment) was determined by means of gentle prodding with a thin, soft brush 72 h after inoculation. Larvae and pupae from the respective experiments were frozen after 72 h and were later dissected. The number of nematodes inside each individual was counted to determine the penetration success.

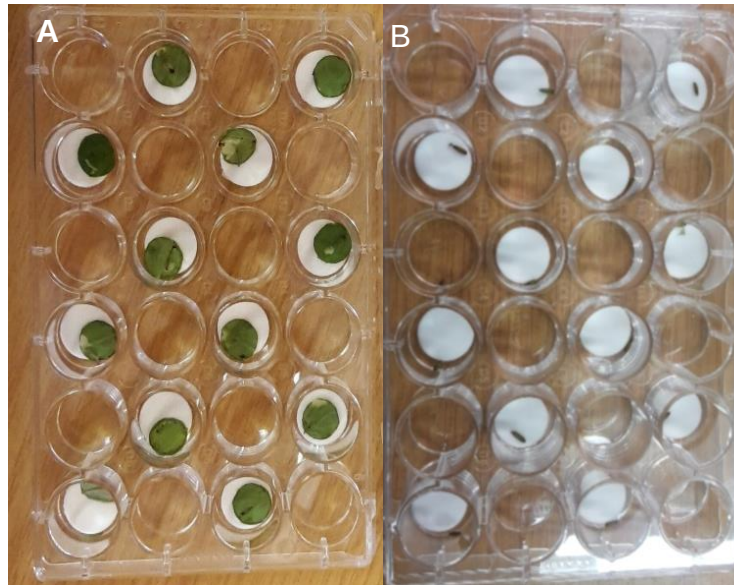


Figure 2.1: Multi-well plates containing, (A) filter paper disc and a leaf disc with one *Tuta absoluta* larva and (B) pupae placed on filter paper discs. Filter paper discs were inoculated with 100 infective juveniles (IJs) of entomopathogenic nematode species per 50 μ L stock solution before insect larvae or pupae were transferred (Photo's: Odette Coleman, NWU, Potchefstroom).

Data analysis

Larval and pupal mortality and infection caused by the IJs of the respective EPN species were compared against the control treatment by means of binomial distribution tests. Each experiment for each EPN species used, larvae and pupae, were repeated once. Data from both experiments were combined and one-way ANOVAs were used to compare the number of IJs of the respective EPN species that penetrated *T. absoluta* larvae and pupae, respectively. Means were separated by Tukey's HSD test ($P < 0.05$). All analyses were done with Statistica Version 13.3 (TIBCO Software Inc., 2017).

2.3. Results

Infective juvenile efficacy of four entomopathogenic nematode species against *Tuta absoluta* larvae and pupae

Infected juveniles of all four EPN species, viz. *S. jeffreyense*, *S. yirgalemense*, *H. baujardi* and *H. noenieputensis* successfully penetrated fourth instar *T. absoluta* larvae (Table 2.1). All *T. absoluta* larvae died after infestation with the respective EPN species in both bioassays conducted. Mortality of *T. absoluta* larvae in the control treatments of all bioassays was 13% or less, except in one bioassay with *S. jeffreyense* where 20% of larvae in the control treatment died (Table 2.1). Larval mortality caused by all four EPN species differed significantly from larval mortality in the respective control treatments (Table 2.1).

Table 2.1: Mean percentage mortality and infection of *Tuta absoluta* larvae with infective juveniles of *Steinernema jeffreyense*, *Steinernema yirgalemense*, *Heterorhabditis baujardi* and *Heterorhabditis noenieputensis*, respectively, after 72 hours exposure.

Parameter ($\pm$ SE)	Bioassay 1			Bioassay 2		
	Treatments		P-value	Treatments		P-value
	<i>Steinernema jeffreyense</i>	Control (water)		<i>Steinernema jeffreyense</i>	Control (water)	
Mortality (%)	100	11.67 $\pm$ 6.24	<0.001	100	20.00 $\pm$ 6.24	<0.001
Infected <i>T. absoluta</i> larvae (%)	100	0	<0.001	100	0	<0.001
	<i>Steinernema yirgalemense</i>	Control (water)		<i>Steinernema yirgalemense</i>	Control (water)	
Mortality (%)	100	0	<0.001	100	11.67 $\pm$ 3.33	<0.001
Infected <i>T. absoluta</i> larvae (%)	100	0	<0.001	96.67 $\pm$ 3.33	0	<0.001
	<i>Heterorhabditis baujardi</i>	Control (water)		<i>Heterorhabditis baujardi</i>	Control (water)	
Mortality (%)	100	8.33 $\pm$ 2.64	<0.001	100	10.00 $\pm$ 6.24	<0.001
Infected <i>T. absoluta</i> larvae (%)	100	0	<0.001	100	0	<0.001
	<i>Heterorhabditis noenieputensis</i>	Control (water)		<i>Heterorhabditis noenieputensis</i>	Control (water)	
Mortality (%)	100	13.00 $\pm$ 5.00	<0.001	100	11.67 $\pm$ 6.24	<0.001
Infected <i>T. absoluta</i> larva (%)	98.33 $\pm$ 1.67	0	<0.001	100	0	<0.001

P-value determined with the binomial distribution test; Standard Error (SE).

Although a significantly higher percentage pupae was infected by *S. jeffreyense* IJs in the first bioassay compared to the pupae not exposed to the EPN in the control ($P<0.001$), only 23% was infected. There was, however, no significant difference in the percentage pupae infected between the treated and control pupae that were not exposed to any IJs ($P>0.05$) (Table 2.2). Percentage *T. absoluta* pupae penetrated by *S. yirgalemense* was low (38% and 8% in the two bioassays, respectively), despite significantly more *T. absoluta* pupae penetrated by IJs compared to untreated control pupae ($P<0.001$ and $P<0.02$). The percentage penetration of *T. absoluta* larvae with *H. baujardi* and *H. noenieputensis* differed significantly from the

uninfected pupae in the respective control treatments of one of the two bioassays. The percentage penetration was, however, below 20% in both these bioassays (Table 2.2).

Table 2.2: Mean percentage mortality and infection of *Tuta absoluta* pupae with infective juveniles of *Steinernema jeffreyense*, *Steinernema yirgalemense*, *Heterorhabditis baujardi* and *Heterorhabditis noenieputensis*, respectively, after 72 hours exposure.

Parameter (±SE)	Bioassay 1			Bioassay 2		
	Treatments		P-value	Treatments		P-value
	<i>Steinernema jeffreyense</i>	Control (water)		<i>Steinernema jeffreyense</i>	Control (water)	
Mortality (%)	23.33±6.12	8.33±2.64	0.027	6.67±3.12	3.33±2.04	0.402
Infected <i>Tuta absoluta</i> pupae (%)	36.67±8.96	0	<0.001	1.67±1.67	0	0.315
	<i>Steinernema yirgalemense</i>	Control (water)		<i>Steinernema yirgalemense</i>	Control (water)	
Mortality (%)	41.67±3.73	11.67±2.04	0.002	18.33±4.86	3.33±2.04	0.008
Infected <i>Tuta absoluta</i> pupae (%)	38.33±4.25	0	<0.001	8.33±4.56	0	0.022
	<i>Heterorhabditis baujardi</i>	Control (water)		<i>Heterorhabditis baujardi</i>	Control (water)	
Mortality (%)	16.67±3.73	13.33±4.25	0.540	10±4.08	6.67±1.67	0.509
Infected <i>Tuta absoluta</i> pupae (%)	13.33±3.73	0	0.004	1.67±1.67	0	0.315
	<i>Heterorhabditis noenieputensis</i>	Control (water)		<i>Heterorhabditis noenieputensis</i>	Control (water)	
Mortality (%)	28.33±3.33	5.00±3.33	0.006	16.67±2.64	15.00±6.12	0.803
Infected <i>Tuta absoluta</i> pupae (%)	18.33±5.53	0	0.005	0	0	1.000

P-value determined with the binomial distribution test.

The number of IJs that penetrated *T. absoluta* larvae or pupae in both bioassays conducted with the respective EPN species, was combined and is provided as the mean number IJ per *T. absoluta* larva or pupa per EPN species (Table 2.3). There were significant differences in the number of IJs that penetrated larvae of the respective species ($F_{3, 476} = 31.62$), but not in the number that penetrated pupae of the respective species ($F_{3, 47} = 0.72$). Significantly fewer *S. yirgalemense* IJs penetrated *T. absoluta* larvae compared to all three other species. The number of *H. noenieputensis* IJs that penetrated *T. absoluta* was also significantly lower

compared to *S. jeffreyense*, but did not differ significantly from *H. baujardi* (Table 2.3). *Heterorhabditis baujardi* and *S. jeffreyense* had the highest number of IJs that penetrated *T. absoluta* larvae. The number of IJs that penetrated *T. absoluta* pupae, were however low, with no significant difference in the mean number of IJs among the four species (Table 2.3).

Table 2.3: Mean number of *Steinernema jeffreyense*, *Steinernema yirgalemense*, *Heterorhabditis baujardi* and *Heterorhabditis noenieputensis* infective juveniles that penetrated *Tuta absoluta* larvae and pupae.

EPN species	Mean IJ/larva	Mean IJ/pupa
<i>Steinernema jeffreyense</i>	15.44±1.05c	6.50±3.50a
<i>Steinernema yirgalemense</i>	6.71±0.53a	6.45±0.88a
<i>Heterorhabditis baujardi</i>	12.00±0.63bc	5.00±0.93a
<i>Heterorhabditis noenieputensis</i>	9.84±0.60b	4.55±0.87a

Means within a column followed by the same lowercase letter are not significantly different at $P<0.05$ (Tukey).

2.4. Discussion

The potential for control of *T. absoluta* with EPNs has been investigated extensively in Europe (Batalla-Carrera *et al.*, 2010; Garcia-del-Pino *et al.*, 2013; Van Damme *et al.*, 2016). The present study was, however, according to our knowledge, the first one conducted in South Africa where native EPN species was evaluated for control of *T. absoluta* larvae and pupae. All four EPN species evaluated, viz. *S. jeffreyense*, *S. yirgalemense*, *H. baujardi* and *H. noenieputensis* were effective, with 100% control of fourth instar *T. absoluta* larvae under controlled laboratory conditions. Although fewer *S. yirgalemense* IJs infected fourth instar *T. absoluta* larvae compared to the other three species, 100% mortality was still achieved. Similar results with high levels of mortality at a dosage rate of 25 IJs/cm² caused by IJs of *S. feltiae* (100%), *S. carpocapsae* (85.7%) and *H. bacteriophora* (78.6%) were reported by Batalla-Carrera *et al.* (2010) in Spain. The potential of *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* to control *T. absoluta* larvae inside tomato leaf mines, was also confirmed by Van Damme *et al.* (2016) under laboratory conditions. High efficacy of *H. bacteriophora* and *S. carpocapsae* for control of *T. absoluta* larvae was recently also reported by Kamali *et al.* (2018) in Iran, with *H. bacteriophora* that caused $99.1 \pm 0.03\%$ mortality of last instar *T. absoluta* larvae at 20 and 50 IJs/cm².

The mean number of IJs that penetrated fourth instar *T. absoluta* larvae in the current study was below 20 although inoculation was done with 100 IJs per larva or pupa. Invasion of larvae does, however, decrease with a decrease in insect size as well as with an increase in

nematode species size (Bastidas *et al.*, 2014). Smaller EPN species control smaller insect species more effectively compared to bigger EPNs (Steyn *et al.*, 2019). Size of the EPN species applied to small insect pests can therefore jeopardize good control and persistence of EPNs (Bastidas *et al.*, 2014; Steyn *et al.*, 2019).

Steyn *et al.* (2019) determined the efficacy of seven EPN species collected in South Africa, viz. *H. bacteriophora* (SF351), *H. baujardi* (MT19), *H. indica* (SGS), *H. zealandica* (MJ2C), *H. noenieputensis* (SF669), *S. jeffreyense* (J194) and *S. yirgalemense* (157C) against *Holocacista capensis* Van Nieukerken and Geertsema (Lepidoptera: Heliozelidae). High larval mortality (>83%) was recorded for *H. indica*, *H. noenieputensis* and *H. baujardi* which was ascribed to their foraging ability to locate and penetrate leaf mining galleries (Steyn *et al.*, 2019). He found a low percentage larval mortality (26% - 50%) for the other species evaluated.

The *T. absoluta* pupal infection rate by *S. jeffreyense*, *H. baujardi* and *H. noenieputensis* in the first bioassay of this study, was higher than what was reported by Batalla-Carrera *et al.* (2010) for *S. feltiae*, *S. carpocapsae* and *H. bacteriophora*, while results from the second experiment corresponded with results from these authors. The EPN species in the study of Batalla-Carrera *et al.* (2010) as well as those in the present study, were not effective in controlling the pupae. The only EPN species found to significantly infect and kill *T. absoluta* pupae, when compared to pupae of the untreated control, was *S. yirgalemense*. The number of *S. yirgalemense* IJs that infected *T. absoluta* pupae were, however, similar to IJ infection numbers of *S. jeffreyense*, *H. baujardi* and *H. noenieputensis*. Despite this finding, and according to the low infection rate of all EPN species evaluated in this study, none will be able to successfully control *T. absoluta* pupae.

Pupae from different insect species can vary in susceptibility in terms of penetration by IJs of EPN species. Henneberry *et al.* (1995) ascribed the inability of EPNs to infect pink bollworm, *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae) pupae, to a lack of punctures in the integument. Yan *et al.* (2019) tested 15 EPN isolates and reported the same tendency with less penetration and lower efficacy of EPNs against *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) pupae, and as a result, lower mortality, compared to efficacy of control of the larval stage. A possible reason for the low infection rate of *T. absoluta* pupae in this study may also be a lack of entry route since two-day-old *T. absoluta* pupae of which the cuticle has already hardened, were used in this study. The age of pupae affects their susceptibility, but it may vary between species (Kaya and Hara, 1981). Almost no penetration by EPNs was reported for three-day old, and older, *S. litura* pupae, while the pre-pupa and soft one-day-old pupae were infected when exposed to IJs (Kondo and Ishibashi, 1986). Should pupae survive after unsuccessful penetration by EPNs, it may still hold benefits in

terms of control, since Kondo and Ishibashi (1986) reported that moths from such pupae were infected upon emergence and were killed a short while afterwards.

Foraging behaviours had no definitive effect in this study. *Steinernema jeffreyense* is an ambusher (Dlamini *et al.*, 2020) that wait for hosts (Campbell and Gaugler, 1997). Ambush foragers scan for cues while they wait and are more effective in finding resources with higher mobility (Lewis *et al.*, 2006). It is therefore doubtful that *S. jeffreyense* IJs will be successful in finding and infecting *T. absoluta* pupae. The low *T. absoluta* pupal infestation by *S. yirgalemense* recorded in this study could, however, not be explained by its foraging behaviour as an intermediate forager. Intermediate foragers act as both ambushers and as cruisers (Dlamini *et al.*, 2020). It is therefore expected that the cruiser behaviour of *S. yirgalemense* should assist this species in its ability to find host pupae also.

Repelling cues of a host could also inhibit further penetration and prevent over population of resources, as demonstrated with *Steinernema* spp. (*S. carpocapae*, *S. riobrave* and *S. feltiae*) by Glazer (1997). More nematodes invade susceptible hosts in which they can develop into the adult stage, reproduce and produce offspring (Bastidas *et al.*, 2014). A suitable host does not only provide food, but also a safe environment for mating and where competition can be avoided (Lewis *et al.*, 2006). A small host is a limited food source and can therefore sustain a lower number of individuals in contrast to larger hosts that can sustain a higher number of nematodes (Bastidas *et al.*, 2014). The competition in smaller hosts is higher and a limited number of individuals could enter these hosts. The number of IJs that would penetrate a host, should be sufficient to overcome its defences, but the risk of overcrowding prevents overpopulating a host (Lewis *et al.*, 2006).

In conclusion, this study showed the potential of *S. jeffreyense*, *S. yirgalemense*, *H. baujardi* and *H. noenieputensis* as good candidates for control of *T. absoluta* larvae. Tomato are commercially produced in greenhouses as well as under field conditions. It is known that environmental factors such as temperature extremes and low humidity levels have detrimental effects on the success of foliar applied EPNs as biocontrol agents. These conditions are controlled in greenhouses compared to open fields. The efficacy of control of *T. absoluta* larvae with these EPNs under greenhouse conditions, therefore valid further evaluation.

2.5. References

ABATE, B.A., MALAN, A.P., TIEDT, L.R., WINGFIELD, M.J., SLIPPERS, B. and HURLEY, B.P. 2016. *Steinernema fabii* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. *Nematology* 18:235-255.

ABATE, B.A., WINGFIELD, M.J., SLIPPERS, B. and HURLEY, B.P. 2017.

Commercialisation of entomopathogenic nematodes: should import regulations be revised? *Biocontrol Science and Technology* 27(2):149-168.

ADAMS, B.J. and NGUYEN, K.B. 2002. Taxonomy and systematics. pp. 1-33. In:

Entomopathogenic nematology. 1st edition. (Ed. Gaugler, R.). CAB International: Wallingford.

ANBESSE, S., GEBRU, W. and ADGE, B. 2008. Laboratory screening for virulent entomopathogenic nematodes (*Heterorhabditis bacteriophora* and *Steinernema yirgalemense*) and fungi (*Metarhizium anisopliae* and *Beauveria bassiana*) and assessment of possible synergistic effects of combined use against grubs of the barley chafer *Coptognathus curtipennis*. *Nematology* 10(5):701-709.

BASTIDAS, B., PORTILLO, E. and SAN-BLAS, E. 2014. Size does matter: The life cycle of *Steinernema* spp. in micro-insect hosts. *Journal of Invertebrate Pathology* 121:46-55.

BATALLA-CARRERA, L., MORTON, A. and GARCIA-DEL-PINO, F. 2010. Efficacy of entomopathogenic nematodes against the tomato leafminer *Tuta absoluta* in laboratory and greenhouse conditions. *BioControl* 55:523-530.

BEDDING, R.A. 2006. Entomopathogenic nematodes from discovery to application. *Biopesticides International* 2:87-119.

BOEMARE, N., LAUMOND, C. and MAULEON, H. 1996. The entomopathogenic nematode-bacterium complex: biology, life cycle and vertebrate safety. *Biocontrol Science and Technology* 6(3):333-346.

CAMPBELL, J.F. and GAUGLER, R. 1997. Inter-specific variation in entomopathogenic nematode foraging strategy: dichotomy or variation along a continuum? *Fundamental and Applied Nematology* 20(4):393-398.

CHYZIK, R., GLAZER, I. and KLEIN, M. 1996. Virulence and efficacy of different entomopathogenic nematode species against western flower thrips (*Frankliniella occidentalis*). *Phytoparasitica* 24(2):103-110.

ÇIMEN, H., LEE, M.M., HATTING, J., HAZI, S. AND STOCK, S.P. 2014. *Steinernema tophus* sp. n. (Nematoda: Steinernematidae), a new entomopathogenic nematode from South Africa. *Zootaxa*: <https://doi.org/10.11646/zootaxa.0000.0.0>

ÇIMEN, H., LEE, M.M., HATTING, J., HAZI, S. and STOCK, S.P. 2015. *Steinernema innovationi* n. sp. (Panagrolaimomorpha: Steinernematidae), a new entomopathogenic nematode species from South Africa. *Journal of Helminthology* 89:415-427.

ÇIMEN, H., PUŽA, V., NERMUŤ, J., HATTING, J., RAMAKUWELA, T., FAKTOROVÁ, L. and HAZIR, S. 2016. *Steinernema beitlechemi* n. sp., a new entomopathogenic nematode (Nematoda: Steinernematidae) from South Africa. *Nematology* 18:439-453.

ÇIMEN, H., PUŽA, V., NERMUŤ, J., HATTING, J., RAMAKUWELA, T. and HAZIR, S. 2016. *Steinernema biddulphi* n. sp., a new entomopathogenic nematode (Nematoda: Steinernematidae) from South Africa. *Journal of Nematology* 48(3):148-158.

DE BACH, P. and ROSEN, D. 1991. *Biological control by natural enemies*. Cambridge University Press: Cambridge.

DE GRISSE, A. 1963. A counting dish for nematodes excluding border effect. *Nematropica* 9:162.

DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES (DAFF). 2020. Pesticide management policy. <https://www.daff.gov.za/daffweb3/Branches/Agricultural-Production-Health-Food-Safety/Agriculture-Inputs-Control/Policies-Legislation>

DESNEUX, N., WAJNBERG, E., WYCKHUYS, K.A.G., BURGIO, G., ARPAIA, S., NARVÁEZ-VASQUEZ, C.A., GONZÁLEZ-CABRERA, J., RUESCAS, C.D., TABONE, E., FRANDON, J., PIZZOL, J., PONCET, C., CABELLO, T. and URBANEJA, A. 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. *Journal of Pest Science* 83:197-215.

DE WAAL, J.Y., MALAN, A.P. and ADDISON, M.F. 2011. Efficacy of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) against codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae) in temperate regions. *Biocontrol Science and Technology* 21(10):1161-1176.

DLAMINI, B.E., DLAMINI, N., MASARIRAMBI, M.T. and NXUMALO, K.A. 2020. Control of tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Celychiidae) larvae in laboratory using entomopathogenic nematodes from subtropical environment. *Journal of Nematology* 52:1-8.

DLAMINI, B.E., MALAN, A.P. and ADDISON, P. 2019. Control of the banded fruit weevil *Phlyctinus callosus* (Coleoptera: Curculionidae) using entomopathogenic nematodes. *Australasian Entomology* 58:687-695.

EHLERS, R.U., PREMACHANDRA, D., BERNDT, O., POEHLING, H.M. and BORGEMEISTER, C. 2003. Laboratory bioassays of virulence of entomopathogenic nematodes against soil-inhabiting stages of *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae). *Nematology* 5(4):539-547.

- GARCIA-DEL-PINO, F., ALABERN, X. and MORTON, A.** 2013. Efficacy of soil treatments of entomopathogenic nematodes against the larvae, pupae and adults of *Tuta absoluta* and their interaction with the insecticides used against this insect. *BioControl* 58:723-731.
- GAUGLER, R., LEWIS, E. and STUART, R.J.** 1997. Ecology in the service of biological control: the case of entomopathogenic nematodes. *Oecologia* 109(4):483-489.
- GEORGIS, R., KOPPENHOFER, A.M., LACEY, L.A., BELAIR, G., DUNCAN, L.W., GREWAL, P.S., SAMISH, M., TAN, L., TORR, P. and VAN TOL, R.W.H.M.** 2006. Successes and failures in the use of parasitic nematodes for pest control. *Biological Control* 38:103-123.
- GLASER, R.W., FARRELL, C.C. and GOWEN, J.W.** 1935. Field experiments with the Japanese beetle and its nematode parasite. *Journal of the New York Entomological Society* 43(3):345-371.
- GLAZER, I.** 1997. Effects of infected insects on secondary invasion of steinernematid entomopathogenic nematodes. *Parasitology* 114:597-604.
- GÖZEL, U. and GOZEL, C.** 2016. Entomopathogenic Nematodes in Pest Management. pp 55-69. In: *Integrated Pest Management (IPM): Environmentally Sound Pest Management*. (Eds. Gill, H.K. and Goyal, G.). InTech: <http://dx.doi.org/10.5772/63894>
- GRIFFIN, C.T.** 2012. Perspectives on the behavior of entomopathogenic nematodes from dispersal to reproduction: traits contributing to nematode fitness and biocontrol efficacy. *Journal of Nematology* 44(2):177-184.
- GUEDES, R.N.C., RODITAKIS, E., CAMPOS, M.R., HADDI, K., BIELZA, P., SIQUEIRA, H.A.A., TSAGKARAKOU, A., VONTAS, J. and NAUEN, R.** 2019. Insecticide resistance in the tomato pinworm *Tuta absoluta*: patterns, spread, mechanisms, management and outlook. *Journal of Pest Science* 92:1329-1342.
- HAN, R. and EHLERS, R.U.** 2000. Pathogenicity, development, and reproduction of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* under axenic in vivo conditions. *Journal of Invertebrate Pathology* 75(1):55-58.
- HATTING, J.L., MOORE, S.D. and MALAN, A.P.** 2018. Microbial control of phytophagous invertebrate pests in South Africa: Current status and future prospects. *Journal of Invertebrate Pathology* 165:54-66.
- HENNEBERRY, T.J., FORLOW, J.L. and BURKE, R.A.** 1995. Pink bollworm (Lepidoptera: Gelechiidae), cabbage looper, and beet armyworm (Lepidoptera: Noctuidae) pupal

susceptibility to steinernematid nematodes (Rhabditida: Steinernematidae). *Journal of Economic Entomology* 88:835-839.

HODDLE, M.S. 2006. Challenges to IPM advancement: pesticides, biocontrol, genetic engineering, and invasive species. *New Zealand Entomologist* 29(1):77-88.

HOMINICK, W.M. 2002. Biogeography. pp 115-144. In: *Entomopathogenic nematology*. (Ed. Gaugler, R.). CAB International: Wallingford.

HUNT, D.J. and SUBBOTIN, S.A. 2016. Taxonomy and systematics. pp 13-58. In: *Advances in entomopathogenic nematode taxonomy and phylogeny. Nematode monograph and perspectives*, Vol 12. (Eds. Hunt, D.J. and Nguyen, K.B.). Brill Academic Pub: Leiden.

JAMES, M., MALAN, M.P. and ADDISON, P. 2018. Surveying and screening South African entomopathogenic nematodes for the control of the Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann). *Crop Protection* 105:41-48.

JANKIELSOHN, A. and HATTING, J.L. 2005. Efficacy of entomopathogenic nematodes, applied in an insect cadaver, as biological control agent against soil-dwelling stages of bollworm (*Helicoverpa armigera* Hübner). Proceedings of the 38th Annual Meeting of the Society for Invertebrate Pathology. 7-11 August. Anchorage, Alaska.

KAMALI, S., KARIMI, J. and KOPPENHÖFER, A.M. 2018. New insight into the management of the tomato leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae) with entomopathogenic nematodes. *Journal of Economic Entomology* 111(1):112-119.

KAYA, H.K., AGUILLERA, M.M., ALUMAI, A., CHOO, H.Y., DE LA TORRE, M., FODOR, A., GANGULY, S., HAZIR, S., LAKATOS, T., PYE, A., WILSON, M., YAMANAKA, S., YANG, H. and EHLERS, R.-U. 2006. Status of entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of the world. *Biological Control* 38:134-155.

KAYA, H.K. and GAUGLER, R. 1993. Entomopathogenic nematodes. *Annual Review of Entomology* 38:181-206.

KAYA, H.K. and HARA, A.H. 1981. Susceptibility of various species of lepidopterous pupae to the entomogenous nematode *Neoplectana carpocapsae*. *Journal of Invertebrate Pathology* 13:291-294.

KESHARI, A.K., HARI, B.K.C., BHAT, A.H. and SHAH, M.M. 2019. Prospects and present status of Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in Nepal. *Journal of Applied and Advanced Research* 4(1):30-35.

KLEIN, H., HILL, M.P., ZACHARIADES, C. and ZIMMERMANN, H.G. 2011. Regulation and risk assessment for importations and releases of biological control agents against invasive alien plants in South Africa. *African Entomology* 19:488-497.

KONDO, E. and ISHIBASHI, N. 1986. Infection efficiency of *Steinernema feltiae* (DD-136) to the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae), on the soil. *Applied Entomology and Zoology* 21:561-571.

LACEY, L.A., FRUTOS, R., KAYA, H.K. and VAIL, P. 2001. Insect pathogens as biological control agents: Do they have a future? *Biological Control* 21:230-248.

LACEY, L.A., GRZYWACZ, D., SHAPIRO-ILAN, D.I., FRUTOS, R., BROWNBRIDGE, M. and GOETTEL, M.S. 2015. Insect pathogens as biological control agents: Back to the future. *Journal of Invertebrate Pathology* 132:1-41.

LE VIEUX, P.D. and MALAN, A.P. 2013. The potential use of entomopathogenic nematodes to control *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae). *South African Journal of Enology and Viticulture* 34:296-306.

LEWIS, E.E., CAMPBELL, J., GRIFFIN, C., KAYA, H. and PETERS, A. 2006. Behavioural ecology of entomopathogenic nematodes. *Biological Control* 38(1):66-79.

MAHMOUD, M.F. 2016. Biology and use of entomopathogenic nematodes in insect pests biocontrol, a generic view. *Cercetări Agronomice în Moldova* 4(168):85-105.

MALAN, A.P., KNOETZE, R. and MOORE, S.D. 2011. Isolation and identification of entomopathogenic nematodes from citrus orchards in South Africa and their biocontrol potential against false codling moth. *Journal of Invertebrate Pathology* 108:115-125.

MALAN, A.P., KNOETZE, R. and TIEDT, L.R. 2014. *Heterorhabditis noenieputensis* n. sp. (Rhabditida: Heterorhabditidae), a new entomopathogenic nematode from South Africa. *Journal of Helminthology* 88:139-151.

MALAN, A.P., KNOETZE, R. and TIEDT, L.R. 2016a. *Steinernema jeffreyense* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. *Journal of Helminthology* 90:262-278.

MALAN, A.P., KNOETZE, R. and TIEDT, L.R. 2016b. *Steinernema nguyenii* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. *Nematology* 18:571-590.

NTHENGA, I., KNOETZE, R., BERRY, S., TIEDT, L.R. and NTHENGA, I., KNOETZE, R., BERRY, S., TIEDT, L.R. and MALAN, A.P. 2014. *Steinernema sacchari* n. sp. (Rhabditida:

Steinernematidae), a new entomopathogenic nematode from South Africa. *Nematology* 16:475-494.

PLATT, T., STOKWE, N.F. and MALAN, A.P. 2019. Foliar application of *Steinernema yirgalemense* to control *Planococcus ficus*: assessing adjuvants to improve efficacy. *South African Journal of Entomology and Viticulture* 40(1):1-7.

POINAR, G.O. 1975. Description and biology of new insect parasitic rhabditoid, *Heterorhabditis bacteriophora* n. gen., n. sp. (Rhabditida; Heterorhabditidae n. fam.). *Nematologica* 21(4):463-470.

POINAR JR, G.O. and GREWAL, P.S. 2012. History of entomopathogenic nematology. *Journal of Nematology* 44(2):153.

RAMAKUWELA, T., ERASMUS, A. and HATTING, J. 2011. Maize stalkborer, *Busseola fusca*, as potential target for control with entomopathogenic nematodes. *South African Journal of Plant and Soil* 28(4):255-256.

RODITAKIS, E., VASAKIS, E., GARCÍA-VIDAL, L., DEL ROSARIO MARTÍNEZ-AGUIRRE, M., RISON, J.L., HAXAIRE-LUTUN, M.O., NAUEN, R., TSAGKARAKOU, A. and BIELZA, P. 2018. A four-year survey on insecticide resistance and likelihood of chemical control failure for tomato leaf miner *Tuta absoluta* in the European/Asian region. *Journal of Pest Science* 91(1):421-435.

SANNINO, L. and ESPINOSA, B. 2010. Diffusion by commerce. pp 11-16 In: *Tuta absoluta. Biology guide and integrated control approaches*. (Eds. Sannino, L. and Espinosa, B.). L'Informatore Agrario: Verona.

STEYN, L.A.I., ADDISON, P. and MALAN, A.P. 2019. Potential of South African entomopathogenic nematodes to control the leaf miner, *Holocacista capensis* (Lepidoptera: Heliozelidae). *South African Journal for Enology and Viticulture*: 40(2): <http://dx.doi.org/10.21548/40-2-3420>

STEYN, W.P., KNOETZE, R., TIEDT, L.R. and MALAN, A.P. 2017. *Steinernema litchii* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. *Nematology* 19:1157-1177.

STEYN, W.P., MALAN, A.P., DANEEL, M.S. and SLABBERT, R.M. 2017. Entomopathogenic nematodes from north-eastern South Africa and their virulence against false codling moth, *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae). *Biocontrol Science and Technology* 27(11):1265-1278.

TIBCO SOFTWARE INC. 2017. STATISTICA (data analysis software system), version 13.3
www.tibco.com.

VAN DAMME, V.M., BECK, B.K., BERCKMOES, E., MOERKENS, R., WITTEMANS, L., DE VIS, R., NUYTENS, D., CASTEELS, H.F., MAES, M., TIRRY, L. and DE CLERCQ, P. 2016. Efficacy of entomopathogenic nematodes against larvae of *Tuta absoluta* in the laboratory. *Pest Management Science* 72:1702-1709.

VAN ZYL, C. and MALAN, A.P. 2014. The role of entomopathogenic nematodes as biological control agents of insect pests, with emphasis on the history of their mass culturing and in vivo production. *African Entomology* 22(2):235-249.

VISSER, D., UYS, V.M., NIEUWENHUIS, R.J. and PIETERSE, W. 2017. First records of the tomato leaf miner *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) in South Africa. *BioInvasions Records* 6(4):301-305.

YAN, X., ARAIN, M.S., LIN, Y., GU, X., ZHANG, L., LI, J. and HAN, R. 2019. Efficacy of entomopathogenic nematodes against the tobacco cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 113(1):64-72.

Chapter 3: Efficacy of *Steinernema yirgalemense* and *Steinernema jeffreyense* applied as foliar applications for control of *Tuta absoluta* on tomato under greenhouse conditions in South Africa

Abstract

Entomopathogenic nematodes (EPNs) were mainly used as biological control agents of soil-inhabiting insect pests, but interest to use it in above-ground applications increased with restrictions on the use of insecticides and increased availability of products containing EPNs. Greenhouse bioassays were conducted to determine the efficacy of two indigenous nematode species, *Steinernema jeffreyense* Malan, Knoetze and Tiedt, 2015 and *Steinernema yirgalemense* Nguyen, Tesfamariam, Gözel, Gaugler and Adams, 2005 against third to fourth instar *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) larvae on tomato seedlings. The nematodes were applied at four concentrations, viz. 250, 500, 1 000 and 2 000 IJs mL⁻¹ distilled water to *T. absoluta* infested tomato seedlings. Leaves with *T. absoluta* larvae were collected 24 h after application of the nematodes and placed in petri dishes on moist filter paper, and kept in a growth chamber at 25±1 °C. Mortality of *T. absoluta* larvae were recorded after a further 48 h. The larvae were dissected and the number of IJs that penetrated each larva was recorded. Despite a low number of IJs of both species found in the host larvae, high mortality rates of more than 80% were achieved at high application rates of 1 000 and 2 000 IJs mL⁻¹ water. *Steinernema jeffreyense* and *S. yirgalemense* are therefore promising biocontrol agents of *T. absoluta* larvae infesting tomato cultivated under greenhouse conditions in South Africa.

Key words: EPNs, foliar application, *Steinernema yirgalemense*, *Steinernema jeffreyense*, greenhouse, tomato leafminer.

3.1. Introduction

Entomopathogenic nematodes (EPNs) are generally applied as an inundative biological control strategy (Beck *et al.*, 2013). The early uses of EPNs as biological control agents were mainly aimed to control soil-inhabiting insect pests, primarily in high valued crops (Kaya *et al.*, 2006). The interest was partially due to a lack of other means to effectively control soil-inhabiting pests in an environmentally friendly manner (Klein, 1990). The application of EPNs to foliage is limited due to the sensitivity of these nematodes to diverse biotic and abiotic factors (Smits, 1996; Noosidum *et al.*, 2016). The main limiting environmental factors are insufficient moisture (desiccation), exposure to extreme temperatures and ultraviolet radiation (Kaya and Gaugler, 1993; Laznik and Trdan, 2012). Entomopathogenic nematodes are soil inhabiting organisms and are therefore protected from adverse environmental conditions (Kaya and Gaugler, 1993). Above-ground applications expose EPNs to environmental

conditions, which reduces their efficacy (Kaya and Gaugler, 1993). Most nematode species do not infect hosts when temperatures exceed 32 °C (Grewal, 2002), and infective juveniles (IJs) exposed to dry conditions desiccate and die quickly (Moore, 1965). The tolerance levels of IJs are therefore taken to the extremes with above-ground application and desiccation is imminent during warm days. Infective juveniles can survive on leaf surfaces during the evening for up to 12 h (Moore, 1965). Application of EPNs should therefore be done from dusk to evenings (Lello *et al.*, 1996), or during dawn and cloudy days (Smith-Fiola *et al.*, 1996).

Restrictions on the use of insecticides and increased availability of products containing EPNs renewed the interest in use in above-ground applications (Arthurs *et al.*, 2004). Protection from environmental conditions is provided to IJs by cryptic habitats. Entomopathogenic nematodes have therefore been tested in these habitats, which include holes and galleries in stems or wood, leaf mines, curled leaves, reproductive plant structures and on leaf surfaces (Arthurs *et al.*, 2004).

For EPNs to be effective biopesticides, application should enable the IJs to find and infect a host (Shapiro-Ilan *et al.*, 2006). The application of nematodes in both glasshouses and fields can be done by means of conventional liquid application (Grewal, 2002). An aqueous nematode mix can be applied with general agrochemical or horticultural ground application equipment including hand-held pressurized sprayers, mist blowers, electrostatic or spinning disc systems, aircraft-mounted atomizer sprayers and irrigation systems such as trickle, centre-pivot or furrow irrigation (Georgis, 1990; Arthurs *et al.*, 2004). The same methods are used for soil as well as foliar applications (Grewal, 2002).

A constraint for application of liquid formulations of nematodes is sedimentation in the sprayer tanks (Grewal, 2002; Schroer *et al.*, 2005; Beck *et al.*, 2013). Conventional hydraulic nozzles, viz. standard fan and full cone provide a wide range of droplet sizes. These droplets are, however, too small to carry IJs (Lello *et al.*, 1996). Higher output hydraulic nozzles deposit nematodes onto foliar parts more effectively and, up to 98% mortality of *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) on Chinese cabbage was achieved when using these nozzles for application (Lello *et al.*, 1996). With the spraying of nematodes, droplets can bounce or roll from leaves. The larger and coarser the droplets, the higher the risk becomes for bouncing and rolling from leaves (Crease *et al.*, 1991), which increases the loss of EPNs. The size of the EPN species determines if the droplet should be larger or whether it can be smaller (Beck *et al.*, 2013).

Surfactants can alter the surface tension of the droplet preventing spray droplets from bouncing and rolling of leaf surfaces which can increase the deposit of EPNs (Beck *et al.*, 2013). The addition of an adjuvant increases the deposit of IJs on foliage, but can also have

an anti-desiccation action (Mason *et al.*, 1998; Laznik and Trdan, 2012). Nematode application at a pressure lower than 2070 kPa is recommended, since nematodes can be damaged with high pressure and extensive recycling through pumping systems (Grewal, 2002). Hydraulic pumps that develop high internal pressure, can shred nematodes. Most pumping systems do, however, make use of membrane or roller pumps and high pressure or force that could damage the IJs, is not generated by these pumps (Grewal, 2002). Oxygen deprivation in tanks and hoses can also inactivate nematodes. It is aggravated when equipment is exposed to direct sunlight which increases the temperature and oxygen demand (Grewal, 2002).

Application of nematodes should be avoided when the temperature inside tanks, hoses or nozzles of application equipment, exceeds 30 °C (Grewal, 2002). Cool temperatures reduce activity and infectivity while warmer temperatures reduce survival (Grewal, 2002). The equipment used should always be adjusted to enhance motility, survival and deliverance of IJs during spray applications (Brusselman *et al.*, 2012).

Steinernema jeffreyense Nguyen, Tesfamariam, Gozel, Gaugler and Adams, 2005 (J194) and *Steinernema yirgalemense* Malan, Knoetze and Tiedt, 2015 (157-C) (Rhabditida: Steinernematidae) were highly effective in controlling *T. absoluta* larvae in laboratory bioassays (Chapter 2). Both these species were readily available from the Department of Conservation Ecology and Entomology, Stellenbosch University and were therefore used in this greenhouse study. The objective of this study was to evaluate the efficacy of *S. jeffreyense* and *S. yirgalemense* applied as foliar sprays for the control of *T. absoluta* larvae in tomato plants under controlled greenhouse conditions.

3.2. Materials and Methods

Entomopathogenic nematodes

Steinernema jeffreyense and *S. yirgalemense* IJs were obtained from the Department of Conservation Ecology and Entomology, Stellenbosch University, Western Cape Province, South Africa to test their efficacy against third and fourth instar *T. absoluta* larvae in tomato plants, in a greenhouse trial. The nematodes were kept in vented culture flasks in 150 ml filtered water, in a dark incubator at 15 ± 1 °C. The flasks were shaken weekly to improve aeration and nematode survival following Platt *et al.* (2019).

Bioassay protocol

Two 4-week-old tomato seedlings, cv. Monica were planted in 4-L capacity pots in sandy loam soil consisting of percentages of sand:silt:clay = 90.8:2.2:7.0, pH = 6.83 and organic matter = 1.78 % by weight. Third and fourth instar *T. absoluta* larvae were inoculated onto leaves of seedlings. Each seedling was inoculated with five larvae that tunneled into the leaves and

developed well-formed tunnels. The infested plants were kept in a greenhouse with temperature and relative humidity (RH) set at 24 ± 0.5 °C and $80 \pm 2\%$, respectively. Temperature and RH were recorded at 60-min intervals using iButtons® from ColdChain Thermo Dynamics (Fairbridge Technologies). Only pots containing seedlings with five larvae that tunnelled into the leaves and formed tunnels (10 larvae per pot) were used. Seedlings were sprayed with water prior to the application of nematode treatments. The treatments consisted of *S. jeffreyense* and *S. yirgalemense* IJs applied at four concentrations, viz. 250, 500, 1 000, and 2 000 IJs mL⁻¹ distilled water. Calculation of the respective concentrations was done according to the method described in chapter 2 (See bioassay protocol). For application of the respective nematodes at each concentration, a 200 mL spray mixture was prepared, which consisted of the EPNs in distilled water and 0.05 mL Nu-Film-P adjuvant (Miller Chemical & Fertilizer CO, Hygrotech). There were eight replicates (pots) for each treatment per EPN species. The untreated control also consisted of eight pots sprayed with a water and Nu-Film adjuvant mixture only. The respective treatments were applied, one day after *T. absoluta* inoculation, with a spray bottle onto the leaves of the potted tomato seedlings until run off.

Leaves with *T. absoluta* larvae were collected 24 h after application of the nematodes and placed in petri dishes on moist filter paper. The petri dishes were kept in a growth chamber at 25 ± 1 °C for a further 48 h where after the larvae were lightly probed to elicit a response and noted as dead when no movement was detected. The *T. absoluta* larvae were dissected to confirm infection by nematodes and the number of IJs that penetrated each larvae was recorded. The experiment was repeated twice.

Data analysis

Results from each experiment conducted with *S. jeffreyense* and *S. yirgalemense* on different test dates, were analysed separately. Abbott's formula was used to correct the data for control mortality (Abbott, 1925). One way ANOVAs were used to compare the corrected percentage mortality, followed by Tukey's HSD test ($P < 0.05$). The mean number of *S. jeffreyense* and *S. yirgalemense* IJs per *T. absoluta* larva after exposure to the two nematode species at different concentrations, were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). These assumptions were not met. The respective data sets were therefore analysed by means of Kruskal–Wallis test followed by Dunn's multiple comparison post hoc test. All statistical analyses were performed using TIBCO Statistica™ 13.3 (TIBCO Software, Inc., 2017).

3.3. Results

Mortality of *T. absoluta* larvae was high in the control treatment of one experiment where *S. jeffreyense* was applied. This experiment was, therefore omitted from further analyses. The corrected percentage mortality for *T. absoluta* larvae exposed to *S. yirgalemense* varied from 52% to 88% (Exp. 1) and 59 to 87% (Exp. 2) at the respective dosage rates applied and differed significantly between application rates ($F_{3,28} = 6.75$; $P < 0.01$; Exp. 1) and ($F_{3,28} = 4.30$; $P < 0.05$; Exp. 2). Significantly more *T. absoluta* larvae died at an application rate of 1 000 *S. yirgalemense* IJs mL⁻¹ when compared to the other application rates in experiment 1 (Fig. 3.1). Mortality recorded after IJ application at 250, 500 and 2 000 IJs mL⁻¹, did, however, not differ significantly. However, in experiment 2, significantly more larvae were killed by *S. yirgalemense* at an application rate of 2 000 IJs mL⁻¹ compared to application rates of 250 and 500 IJs mL⁻¹. Control of *T. absoluta* larvae at application rates of 1 000 and 2 000 IJs mL⁻¹ did not differ significantly.

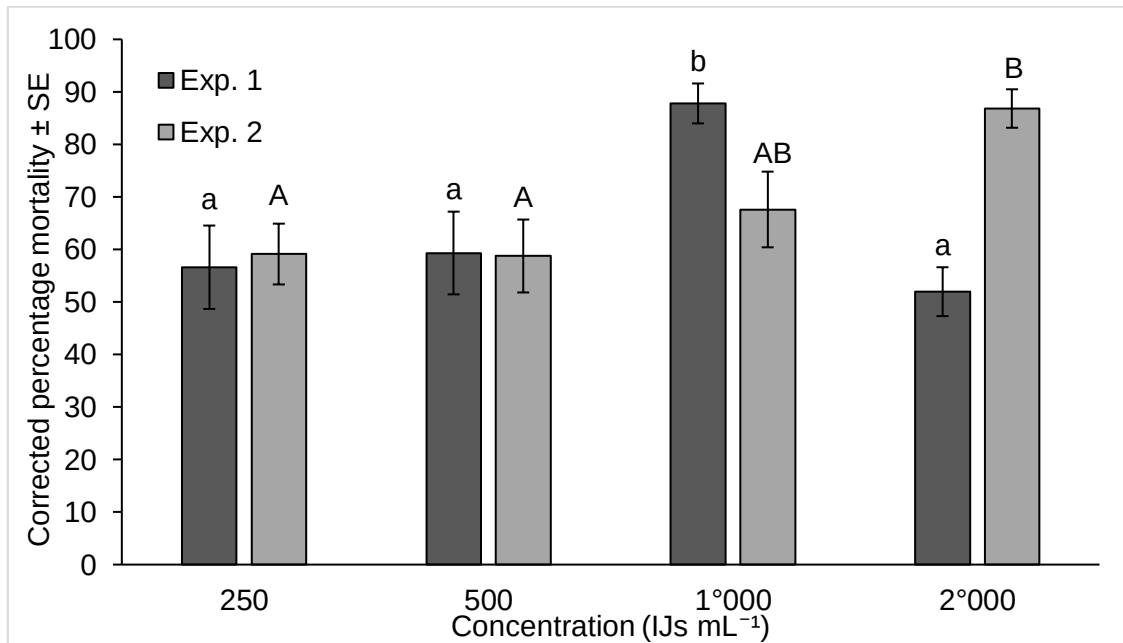


Figure 3.1: Mean corrected percentage mortality $\pm$ SE of *Tuta absoluta* larvae on tomato seedlings caused by *Steinernema yirgalemense* applied at concentrations of 250 IJs, 500 IJs, 1 000 IJs and 2 000 IJs mL⁻¹ in a (water + 0.05% Nu-Film-P) spray solution. Bars capped with the same lower or upper case letters are not significantly different at $P < 0.05$ (Tukey).

The dosage rate at which *S. jeffreyense* was applied to tomato foliage had a significant effect on the corrected percentage mortality of *T. absoluta* larvae in galleries ($F_{3,28} = 6.16$; $P < 0.01$). The highest mortality was recorded with application rates of 1 000 and 2 000 IJs mL⁻¹ (Fig. 3.2).

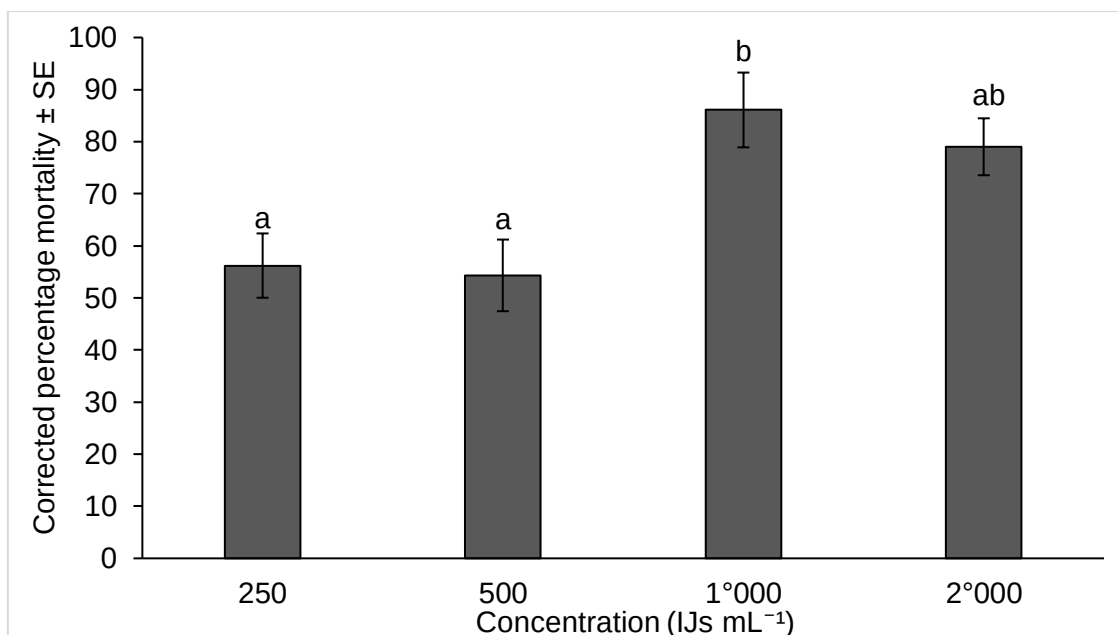


Figure 3.2: Mean corrected percentage mortality $\pm$ SE of *Tuta absoluta* larvae on tomato seedlings caused by *Steinernema jeffreyense* applied at concentrations of 250 IJs, 500 IJs, 1 000 IJs and 2 000 IJs mL⁻¹ in a (water + 0.05% Nu-Film-P) spray solution. Bars capped with the same letter are not significantly different at $P < 0.05$ (Tukey).

Mean number of IJs that infected *T. absoluta* larvae was less than three per larva (Table 3.1). Significantly fewer *S. yirgalemense* IJs, applied at 500 IJs mL⁻¹, penetrated *T. absoluta* larvae compared to the number that penetrated after application at 2 000 IJs mL⁻¹ ((H (3, N = 186) = 18.47; $P < 0.05$). The number of IJs that penetrated after application at concentrations of 250, 1 000 and 2 000 IJs mL⁻¹ did not differ significantly. The number of *S. jeffreyense* IJs that penetrated *T. absoluta* larvae did not differ significantly regardless of the application rates of 250, 500, 1 000 and 2 000 IJs mL⁻¹ ((H (3, N = 221) = 9.24; $P = 0.06$).

Table 3.1: Mean number of infected *Steinernema jeffreyense* and *Steinernema yirgalemense* juveniles ($\pm$ SE) per *Tuta absoluta* larva at the respective concentrations applied to tomato seedlings in a greenhouse.

Concentrations (IJs mL ⁻¹)	<i>Steinernema jeffreyense</i>	<i>Steinernema yirgalemense</i>
	Mean IJs $\pm$ SE	Mean IJs $\pm$ SE
250	1.72 $\pm$ 0.16a	1.84 $\pm$ 0.18ab
500	1.58 $\pm$ 0.11a	1.33 $\pm$ 0.10a
1 000	1.66 $\pm$ 0.11a	1.74 $\pm$ 0.13ab
2 000	2.25 $\pm$ 0.18a	2.51 $\pm$ 0.23b

Means within a column followed by the same lowercase letter are not significantly different at $P < 0.05$ (Kruskal-Wallis).

3.4. Discussion

Chemical control of pests and weeds affects non-target organisms, which are exposed to the toxins (Sanchez-Bayo, 2011). It is also detrimental to human health causing acute and chronic problems (Paoletti and Pimentel, 2000). Although pesticides benefit producers economically (Pimentel and Ali, 1998), health risks and the development of resistance to insecticides necessitate a change in management strategies to better control insect pests (Ehlers, 1996). The use of EPNs as a biological control practice minimizes the impact on human and environmental health (Ehlers, 1996).

Tuta absoluta larvae feed on parenchyma cells inside leaf galleries (Sannino and Espinosa, 2010) and foliar applications of EPNs will therefore be needed for its control. Foliar application does, however, expose EPNs to the harsh environmental conditions and limits the efficacy of EPNs applied against foliar pests (Lacey and Georgis, 2012). The effective control of pests on foliar parts as well as in stems and trunks with application of EPNs under field conditions was, however, reported by Tomalak *et al.* (2005). Another important consideration is the susceptibility of the target insect species to the EPN species under consideration. This was proven by Belair *et al.* (2003) who reported that *S. carpocapsae* applied as a foliar application, was not effective in controlling the cabbageworm, *Artogeia rapae* (Linneaus) (Lepidoptera: Pieridae), despite its efficacy against a wide range of insect pests.

In this study, both *S. jeffreyense* and *S. yirgalemense* controlled *T. absoluta* larvae effectively after foliar application to infested tomato seedlings in a greenhouse. High mortality rates of more than 80% were achieved at high application rates of 1 000 and 2 000 IJs mL⁻¹. These were lower than the mortality rates of the two EPNs in laboratory bioassays (Chapter 2). It was, however, in agreement with the results reported by Batalla-Carrera *et al.* (2010) who reported high efficacy of control of *T. absoluta* larvae (87 – 95%) with *S. carpocapsae*, *Steinernema feltiae* (Filipjev, 1934) Wouts, Mráček, Gerdin and Bedding, 1982 and *Heterorhabditis bacteriophora* Poinar, 1976 applied at 1 000 IJs mL⁻¹ to tomato seedlings in a greenhouse experiment. Kamali *et al.* (2018) reported 50% efficacy of *T. absoluta* larvae of various instars by *S. carpocapsae* and *H. bacteriophora* at a dosage rate of 50 IJs cm⁻² applied without an adjuvant in a greenhouse experiment. This difference in efficacy reported between the two studies could be explained by the difference in larval instars exposed as well as the addition of an adjuvant in the foliar application of Batalla-Carrera *et al.* (2010). Adjuvants increase the efficacy of control by EPNs (Kamali *et al.*, 2018; Platt *et al.*, 2019).

For a 95% control of the leafminer, *Liriomyza bryoniae* (Kaltenbach) (Diptera: Agromizidae) with *S. feltia*, a high dosage rate of 10 000 IJs mL⁻¹ was reported (Williams and Walters, 2000).

This difference in dosage rates necessary were ascribed to the differences in insect behaviour as well as the types of leaf mines created by respective insect species (Batalla-Carrera *et al.*, 2010). *Liriomyza bryoniae* creates only one entry route during oviposition with no other entry made by the larva during its development inside the leaf mine. *Tuta absoluta* larvae create big tunnels which provides for entry by EPNs as well as protection against harsh environmental conditions. Insect larvae of this species also move between leaves which give EPNs effortless access to their host (Batalla-Carrera *et al.*, 2010).

A high percentage larval mortality, although infected with only a few nematodes was found in this study. It could be explained by the bacterial release by the IJs (Gaugler *et al.*, 1994). Bacteria are released before the host immune response encapsulate and melanise invading IJs (Wang *et al.*, 1994). Lethal bacteria are also carried on the nematode surface or enter insect host through wound that nematodes created on point of entry and could kill hosts on invasion (Poinar and Kaul, 1981). Insect mortality should be recorded to determine the insecticidal effect of EPNs, and penetration rate of insects by IJs can be used to compare infectivity of entomopathogenic nematode strains, but a lack of correlation between pathogenicity and levels of host invasion can still occur (Caroli *et al.*, 1996). The low number of IJs of both species found in the host larvae, could possibly be explained by the size of the *T. absoluta* larvae and the size of the nematodes. A small size host affects the number of nematodes that infects the host (Bastidas *et al.*, 2014). A higher number of IJs recorded in some larvae, did, however, indicate that development and reproduction of nematodes occurred after invasion also.

The foraging traits of EPNs are important to take into account when different species are considered for foliar applications. The foraging behaviour of the two species did also not explain the low number of IJs that penetrated *T. absoluta* larvae. *Steinernema yirgalemense* is an intermediate forager, which acts as an ambusher (wait for its prey) as well as a cruiser that search for prey (Dlamini *et al.*, 2020). *Steinernema jeffreyense* acts as an ambusher (Dlamini *et al.*, 2020) that waits for hosts (Campbell and Gaugler, 1997). A similar number of *S. yirgalemense* and *S. jeffreyense* IJs penetrated *T. absoluta* larvae in these greenhouse experiments. Host seeking activity as explanation for their efficacy, was therefore excluded.

The success in control of *T. absoluta* larvae by *S. jeffreyense* and *S. yirgalemense* determined in these greenhouse experiments, indicated the potential of two EPN species as biocontrol agents for *T. absoluta* larvae under greenhouse conditions in South Africa. It is recommended that additional studies be conducted to verify the optimal concentration for application.

3.5. References

- ABBOTT, W.W.** 1925. A method for computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18(2):265-267.
- ARTHURS, S., HEINZ, K.M. and PRASIFKA, J.R.** 2004. An analysis of using entomopathogenic nematodes against above-ground pests. *Bulletin of Entomological Research* 94:297-306.
- BASTIDAS, B., PORTILLO, E. and SAN-BLAS, E.** 2014. Size does matter: The life cycle of *Steinernema* spp. in micro-insect hosts. *Journal of Invertebrate Pathology* 121:46-55.
- BATALLA-CARRERA, L., MORTON, A. and GARCIA-DEL-PINO, F.** 2010. Efficacy of entomopathogenic nematodes against the tomato leafminer *Tuta absoluta* in laboratory and greenhouse conditions. *BioControl* 55:523-530.
- BECK, B., BRUSSELMAN, E., NUYTTENS, D., MOENS, M., POLLET, S., TEMMERMAN, F. and SPANOGHE, P.** 2013. Improving foliar applications of entomopathogenic nematodes by selecting adjuvants and spray nozzles. *Biocontrol Science and Technology* 23:507-520.
- BELAIR, G., FOURNIER, Y. and DAUPHINAIS, N.** 2003. Efficacy of steinernematid nematodes against three insect pests of crucifers in Quebec. *Journal of Nematology* 35(3):259-265.
- BRUSSELMAN, E., BECK, B., POLLET, S., TEMMERMAN, F., SPANOGHE, P., MOENS, M. and NUYTTENS, D.** 2012. Effect of the spray application technique on the deposition of entomopathogenic nematodes in vegetables. *Pest Management Science* 68:444-453.
- CAMPBELL, J.F. and GAUGLER, R.** 1997. Inter-specific variation in entomopathogenic nematode foraging strategy: dichotomy or variation along a continuum? *Fundamental and Applied Nematology* 20(4):393-398.
- CAROLI, L., GLAZER, I. and GAUGLER, R.** 1996. Entomopathogenic nematode infectivity assay: comparison of penetration rate into different hosts. *Biocontrol Science and Technology* 6(2):227-234.
- CREASE, G.J., HALL, F.R. and THACKER, J.R.M.** 1991. Reflection of agricultural sprays from leaf surfaces. *Journal of Environmental Science and Health* 26(4):383-407.
- DLAMINI, B.E., DLAMINI, N., MASARIRAMBI, M.T. and NXUMALO, K.A.** 2020. Control of tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Ctenopidae) larvae in laboratory using entomopathogenic nematodes from subtropical environment. *Journal of Nematology* 52:1-8.

- EHLERS, R.-U.** 1996. Current and future use of nematodes in biocontrol practice and commercial aspects with regard to regulatory policy issues. *Biocontrol Science and Technology* 6:303-316.
- GAUGLER, R., WANG, Y. and CAMPBELL, J.F.** 1994. Aggressive and evasive behaviors in *Popillia japonica* (Coleoptera: Scarabaeidae) larvae: defences against entomopathogenic nematode attack. *Journal of Invertebrate Pathology* 64:193-199.
- GEORGIS, R.** 1990. Formulation and application technology. pp. 173-191. In: *Entomopathogenic nematodes in biological control*. (Eds. Gaugler, R. and Kaya, H.K.). CRC Press: Boca Raton.
- GREWAL, P.S.** 2002. Formulation and application technology. pp. 265-287. In: *Entomopathogenic nematology* (Ed. Gaugler, R.). CAB International: Wallingford.
- KAMALI, S., KAMRIMI, J. and KOPPENHOFER, A.M.** 2018. New insight into the management of the tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae) with entomopathogenic nematodes. *Journal of Economic Entomology* 111(1):112-119.
- KAYA, H.K., AGUILLERA, M.M., ALUMAI, A., CHOO, H.Y., DE LA TORRE, M., FODOR, A., GANGULY, S., HAZÂR, S., LAKATOS, T., PYE, A., WILSON, M., YAMANAKA, S., YANG, H. and EHLERS, R.-U.** 2006. Status of entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of the world. *Biological Control* 38:134-155.
- KAYA, H.K. and GAUGLER, R.** 1993. Entomopathogenic nematodes. *Annual Review of Entomology* 38:181-206.
- KLEIN, M.G.** 1990. Efficacy against soil-inhabiting insect pests. pp. 195–214. In: *Entomopathogenic Nematodes in Biological Control*. (Eds. Gaugler, R. and Kaya, H.K.). CRC Press: Boca Raton.
- LACEY, L.A. and GEORGIS, R.** 2012. Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. *Journal of Nematology* 44(2):218-225.
- LAZNIK, Z. and TRDAN, S.** 2012. Entomopathogenic nematodes (Nematoda: Rhabditida) in Slovenia: From Tabula Rasa to implementation into crop production systems. pp. 627-656 In: *Insecticides - advances in integrated pest management*. (Ed. Perveen, F.). InTech: London.

- LELLO, E.R., PATEL, M.N., MATTHEWS, G.A. and WRIGHT, D.J.** 1996. Application technology for entomopathogenic nematodes against foliar pests. *Crop Protection* 13(6):567-574.
- MASON, J.M., MATHEWS, G.A. and WRIGHT, D.J.** 1998. Screening and selection of adjuvants for the spray application of entomopathogenic nematodes against a foliar pest. *Crop Protection* 17:461-470.
- MOORE, G.E.** 1965. The bionomics of an insect-parasitic nematode. *Journal of the Kansas Entomology Society* 38(2):101-105.
- NOOSIDUM, A., SATWONG, P., CHANDRAPATYA, A. and LEWIS, E.E.** 2016. Efficacy of *Steinernema* spp. plus anti-desiccants to control two serious foliage pests of vegetable crops, *Spodoptera litura* F. and *Plutella xylostella* L. *Biological Control* 97:48-56.
- PAOLETTI, M.G. and PIMENTEL, D.** 2000. Environmental risks of pesticides versus genetic engineering for agricultural pest control. *Journal of Agricultural and Environmental Ethics* 12(3):279-303.
- PIMENTEL, D. and ALI, S.** 1998. An economic and environmental assessment of herbicide-resistant and insect/pest-resistant crops. pp 241-253. In: *Cost-benefit analysis: environmental & ecological perspective*. (Ed. Puttaswasmaiah, K.). Transaction publishers: New Brunswick.
- PLATT, T., STOKWE, N.F. and MALAN, A.P.** 2019. Foliar application of *Steinernema yirgalemense* to control *Planococcus ficus*: assessing adjuvants to improve efficacy. *South African Journal of Entomology and Viticulture* 40(1):1-7.
- POINAR JR, G.O. and KAUL, H.N.** 1981. Parasitism of the mosquito *Culex pipiens* by the nematode *Heterorhabditis bacteriophora*. *Journal of Invertebrate Pathology* 39:382-387.
- SANCHEZ-BAYO, F.** 2011. Impacts of agricultural pesticides on terrestrial ecosystems. pp 63-87. In: *Ecological impacts of toxic chemicals* (Eds. Sanchez-Bayo, F., Van den Brink, P.J. and Mann, R.M.). Bentham Science Publishers Ltd:
<https://www.researchgate.net/publication/235903011>
- SANNINO, L. and ESPINOSA, B.** 2010. Morphological characteristics of *Tuta absoluta*. pp. 17-32 In: *Tuta absoluta. Biology guide and integrated control approaches*. (Eds. Sannino, L. and Espinosa, B.). L'Informatore Agrario: Verona.
- SCHROER, S., ZIERMANN, D. and EHLERS, R.-U.** 2005. Mode of action of a surfactant-polymer formulation to support performance of the entomopathogenic nematode

Steinernema carpocapsae for control of diamondback moth larvae (*Plutella xylostella*). *Biocontrol Science and Technology* 15:601-613.

SHAPIRO-ILAN, D.I., GOUGE, D.H., PIGGOTT, S.J. and FIFE, J.P. 2006. Application technology and environmental considerations for use of entomopathogenic nematodes in biological control. *Biological Control* 38(1):124-133.

SMITH-FIOLA, D.C., GILL, S.A. and WAY, R.G. 1996. Evaluation of entomopathogenic nematodes as biological control against the banded ash clearwing borer. *Journal of Environmental Horticulture* 14(2):67-71.

SMITS, P.H. 1996. Post-application persistence of entomopathogenic nematodes. *Biocontrol Science and Technology* 6(3):379-388.

TIBCO SOFTWARE INC. 2017. Statistica (data analysis software system), version 13.3. www.tibco.com.

TOMALAK, M., PIGGOTT, S. and JAGDALE, G.B. 2005. Glasshouse applications. pp 147-166. In: *Nematodes as biocontrol agents*. (Eds. Grewal, P.S., Ehlers, R-U. and Shapiro-Ilan, D.I.). CAB International: Oxon.

WANG, Y., GAUGLER, R. and CUI, L. 1994. Variations in immune response of *Popillia japonica* and *Acheta domesticus* to *Heterorhabditis bacteriophora* and *Steinernema* species. *Journal of Nematology* 26(1):11-18.

WILLIAMS, E.C. and WALTERS, K.F.A. 2000. Foliar application of the entomopathogenic nematode *Steinernema feltiae* against leafminers on vegetables. *Biocontrol Science and Technology* 10(1):61-70.

Chapter 4: Conclusion and recommendations

The South American tomato pinworm, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) invaded Africa in 2008, and continued to spread rapidly. It is now a key, devastating insect pest in the tomato-producing areas in Northern and sub-Saharan Africa (Mansour *et al.*, 2018). It also became a threat to South African producers after its detection in the country in 2016 (Visser *et al.*, 2017).

Current management tactics for *T. absoluta* are mainly based on monitoring with sex pheromone traps and application of synthetic insecticides (Biondi *et al.*, 2018; Mansour *et al.*, 2018). Although application of insecticides was considered the most effective control method for *T. absoluta* (Desneux *et al.*, 2010), the importance of using an integrated pest management (IPM) strategy for its control and to slow down insecticide resistant development, was earlier emphasized by Georgiou (1983). Resistance to insecticides did, however, develop (Siqueira *et al.*, 2000; Lietti *et al.*, 2005; Silva *et al.*, 2011; Haddi *et al.*, 2012; Roditakis *et al.*, 2013; 2015; 2018). *Tuta absoluta* populations from Greece, Italy, Israel, Spain and UK in the Euro-Asian region, showed resistance to emamectin benzoate, indoxacarb and spinosad. Except for *T. absoluta* populations in Spain, resistance was also reported to chlorantraniliprole (Roditakis *et al.*, 2018). The authors attributed this finding to an IPM program which included non-chemical measures and the rotation of different mode of action classes when insecticides were applied.

Host plant resistance has been investigated with susceptibility testing of tomato cultivars to *T. absoluta*. Larval survival, development time, fecundity and fertility of the pest were used as parameters (Gharekhani and Salek-Ebrahimi, 2014a,b; Ghaderi *et al.*, 2017; Krechemer and Foerster, 2017; Azadi *et al.*, 2018). Results reported by these authors were similar to that of Oliveira *et al.* (2009) indicating that some tomato cultivars are less susceptible to *T. absoluta*, but no cultivar is entirely resistant to this pest. Although tomato cultivars containing *Bt Cry* proteins have been developed and proven to be successful in controlling *T. absoluta*, it has not been released for commercial use (Selale *et al.*, 2017).

The failure to control *T. absoluta* may have serious economic impacts, and strategies to control it with natural enemies, is therefore important (Bajonero *et al.*, 2008). Over the years, many parasitoids in various families were reported in the area where *T. absoluta* originated from, as well as in Asia and Europe (Faria *et al.*, 2008; Desneux *et al.*, 2010; Doğanlar and Yiğit, 2011; Loni *et al.*, 2011; Luna *et al.*, 2012; 2015; Al-Jboory *et al.*, 2012; Cabello *et al.*, 2012; Ferracini *et al.*, 2012; Zappalà *et al.*, 2012; Biondi *et al.*, 2013; Chailleux *et al.*, 2013; El-Arnaouty *et al.*, 2014; Gabarra *et al.*, 2014; Bayram *et al.*, 2016). To date, parasitoids from only four families have been reported in Africa, namely Braconidae (Boualem *et al.*, 2012; Abbes *et al.*, 2014;

Idriss *et al.*, 2018), Eulophidae (Boualem *et al.*, 2012; Abbes *et al.*, 2014), Ichneumonidae (Boualem *et al.*, 2012) and Pteromalidae (Idriss, 2019). Pesticides affects natural enemies and will consequently reduce biological control of this pest (Luna *et al.*, 2007).

It is therefore important to recommend approaches consisting of reduced-risk insecticide compounds, such as plant extracts or microbial-based selective insecticides, and/or biorational alternatives for effective control of *T. absoluta* in Africa and worldwide (Mansour *et al.*, 2018). The correct use of insect-proof nets (impregnated with insecticides or not), the use of pheromone-based tools (especially for mass trapping) and the conservation and augmentation of indigenous biological control agents have also recommended (Mansour *et al.*, 2018).

Mirids, especially *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) used in IPM programs also provide effective control of *T. absoluta* (Mollá *et al.*, 2011). A variety of insect pests are controlled with entomopathogens such as fungi, bacteria and nematodes (Lacey *et al.*, 2001). Control of *T. absoluta* with *Metarhizium anisopliae* var. *anisopliae* (Metsch.) Soroki, *Beauveria bassiana* (Balsamo) Vuillemin (Ínanli *et al.*, 2013) and *Bacillus thuringiensis* (Berliner), has also been reported (González-Cabrera *et al.*, 2011).

The increased rate of insecticide resistance development prompted the search for new, effective, environmentally safe control methods and increased the interest in entomopathogenic nematodes (EPNs) since their discovery (Abate *et al.*, 2017; Guedes *et al.*, 2019). Entomopathogenic nematodes are produced commercially to control insect pests globally (Abate *et al.*, 2017). The EPNs, *Heterorhabditis bacteriophora* Poinar, 1976 *Steinernema feltiae* (Filipjev, 1934) Wouts, Mráček, Gerdin and Bedding, 1982 and *Steinernema carpocapsae* (Weiser, 1955) Wouts, Mráček, Gerdin and Bedding, 1982, were evaluated and found to effectively control *T. absoluta* (Batalla-Carrera *et al.*, 2010; Van Damme *et al.*, 2016; Kamali *et al.*, 2018).

Importation of exotic species without a permit and a full impact study is, however, not allowed in South Africa due to the regulations of the Department of Agriculture, Forestry and Fisheries (DAFF), under the Agricultural pest act 36 (Klein *et al.*, 2011; DAFF, 2020). With these restrictions in place research for control of *T. absoluta* with native biological control agents is therefore warranted.

In this study, four native EPN species viz. *Steinernema jeffreyense* Malan, Knoetze, Tiedt, 2015, *Steinernema yirgalemense* Nguyen, Tesfamariam, Gozel, Gaugler and Adams, 2005. *Heterorhabditis baujardi* Phan, Subbotin, Nguyen and Moens, 2003 and *Heterorhabditis noenieputensis* Malan, Knoetze and Tiedt, 2014, were found to be highly effective in killing fourth instar *T. absoluta* larvae, with 100% larvae killed in laboratory bioassays. The efficacy

of these EPNs was, however, lower against *T. absoluta* pupae. The highest pupal mortality was caused by *S. yirgalemense* (41.7%), and less than 30% mortality was recorded for *S. jeffreyense*, *H. baujardi* and *H. noenieputensis* (Chapter 2). The number of IJs that penetrated *T. absoluta* larvae and pupae in the laboratory bioassays was also low. Fewer *S. yirgalemense* IJs penetrated *T. absoluta* larvae compared to IJs of the other species used in this study. The number of IJs that penetrated *T. absoluta* pupae, was also fewer compared to the number of IJs that penetrated larvae. On the basis of these laboratory bioassays, all four EPN species tested have potential for use in an IPM system for control of *T. absoluta* larvae (Chapter 2). Subsequent greenhouse trials were conducted to investigate the potential of *S. yirgalemense* and *S. jeffreyense* for control of *T. absoluta* larvae in tomato cultivated in greenhouses (Chapter 3).

High *T. absoluta* larval mortality rates were recorded with *S. yirgalemense* and *S. jeffreyense* applied at 1 000 IJs mL⁻¹ to tomato foliage in a greenhouse infested with third and fourth instar larvae. It is in agreement with high efficacy of control of *T. absoluta* larvae reported with *S. carpocapsae*, *Steinernema feltiae* and *Heterohabditis bacteriophora*, also applied at 1 000 IJs mL⁻¹ to tomato seedlings in a greenhouse (Batalla-Carrera *et al.*, 2010). These studies indicated the efficacy of various EPN species to kill *T. absoluta* larvae and their potential to act as successful biological control agents of this pest. The importance of high dosage rates of 1 000 IJs mL⁻¹ and higher, under moderate temperature and high humidity conditions was also confirmed in the current study.

The size of the *T. absoluta* larvae to which EPNs are applied, is also important for successful control (Van Damme *et al.*, 2016). Third and fourth instar larvae were reported to be the most susceptible. Control was lower when EPNs were applied to smaller (first and second instar) larvae (Bastidas *et al.*, 2014). A decrease in host size and an increase in nematode size decrease invasion of the host by EPNs (Bastidas *et al.*, 2014; Van Damme *et al.*, 2016; Kamali *et al.*, 2018). Third and fourth instar *T. absoluta* larvae should therefore be the stage to target when applying EPNs for control of this pest.

Both *H. baujardi* and *H. noenieputensis* were also highly effective in killing *T. absoluta* larvae in laboratory bioassays of this study. It justifies evaluation of these species for control of *T. absoluta* in tomato under controlled greenhouse conditions. Future research could also include the evaluation of other EPN species isolated in South Africa for control of *T. absoluta* larvae.

The susceptibility of *T. absoluta* larvae from different instars to all native EPNs found to be effective in preliminary studies, could also be investigated in future studies. It will be an added advantage to an IPM program if a species could be identified that is effective against the

smaller instar larvae. Furthermore, when effective control of *T. absoluta* in controlled greenhouse conditions could be confirmed for native EPNs, research on the efficacy of EPN field applications, or improvement of control with the addition of adjuvants in foliar applications, could be conducted. It is necessary to determine the species most suited for control of *T. absoluta* under local environmental conditions, which allows for better tolerance to desiccation and a prolonged lifetime taking into consideration exposure to high temperatures and ultraviolet radiation, also. It will not only benefit tomato producers, but can be advantageous to producers of other Solanaceous crops, which host *T. absoluta*.

4.1. References

ABATE, B.A., WINGFIELD, M.J., SLIPPERS, B. and HURLEY, B.P. 2017.

Commercialisation of entomopathogenic nematodes: should import regulations be revised? *Biocontrol Science and Technology* 27(2):149-168.

ABBES, K., BIONDI, A., ZAPPALÀ, L. and CHERMITI, B. 2014. Fortuitous parasitoids of the invasive tomato leafminer *Tuta absoluta* in Tunisia. *Phytoparasitica* 42:85-92.

AL-JBOORY, I.J., KATBEH-BADER, A. and AL-ZAIDI, S. 2012. First observation and identification of some natural enemies collected from heavily infested tomato by *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Jordan. *Middle East Journal of Science Research* 11(6):787-790.

AZADI, F., RAJABPOUR, A., ABADI, A.L.J. and MAHJOUN, M. 2018. Resistance of tomato cultivars to *Tuta absoluta* (Lepidoptera: Gelechiidae) under field conditions. *Journal of Crop Protection* 7(1):87-92.

BAJONERO, J., CORDOBA, N., CANTOR, F., RODRIGUEZ, D. and CURE, J.R. 2008. Biology and life cycle of *Apanteles gelechiidivoris* (Hymenoptera: Braconidae) parasitoid of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Agronomía Colombiana* 26(3):417-426.

BASTIDAS, B., PORTILLO, E. and SAN-BLAS, E. 2014. Size does matter: The life cycle of *Steinernema* spp. in micro-insect hosts. *Journal of Invertebrate Pathology* 121:46-55.

BATALLA-CARRERA, L., MORTON, A. and GARCIA-DEL-PINO, F. 2010. Efficacy of entomopathogenic nematodes against the tomato leafminer *Tuta absoluta* in laboratory and greenhouse conditions. *Biocontrol* 55:523-530.

BAYRAM, Y., GÜLER, Y., FURSOV, V., KODAN, M. and ÖĞRETEN, A. 2016. First record of *Necremnus cosmopterix* Ribeset Bernardo, 2015 (Hymenoptera: Eulophidae), as a larval parasitoid of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Turkey. *Egyptian Journal of Biological Pest Control* 26(4):853-854.

- BIONDI, A., DESNEUX, N., AMIENS-DESNEUX, E., SISCARO, G. and ZAPPALÀ, L.** 2013. Biology and developmental strategies of the Palaearctic parasitoid *Bracon nigricans* (Hymenoptera: Braconidae) on the Neotropical moth *Tuta absoluta* (Lepidoptera: Gelechiidae). *Journal of Economic Entomology* 106(4):1638-1647.
- BIONDI, A., GUEDES, R.N.C., WAN, F.-H. and DESNEUX, N.** 2018. Ecology, worldwide spread, and management of the invasive South American tomato pinworm, *Tuta absoluta*: past, present and future. *Annual Review of Entomology* 63:293-258.
- BOUALEM, M., ALLAOUI, H., HAMADI, R. and MEDJAHED, M.** 2012. Biologie et complexe des ennemis naturels de *Tuta absoluta* à Mostaganem (Algérie). *Bulletin OEPP/EPPO Bulletin* 42(2):268-274.
- CABELLO, T., GALLEG0, J.R., FERNANDEZ, F.J., GAMEZ, M., VILA, E., DEL PINO, M. and HERNANDEZ, E.** 2012. Biological control strategies for the South American tomato moth (Lepidoptera: Gelechiidae) in greenhouse tomatoes. *Journal of Economic Entomology* 105(6):2085-2096.
- CHAILLEUX, A., BIONDI, A., HAN, P., TABONE, E. and DESNEUX, N.** 2013. Suitability of the pest–plant system *Tuta absoluta* (Lepidoptera: Gelechiidae) –tomato for *Trichogramma* (Hymenoptera: Trichogrammatidae) parasitoids and insights for biological control. *Journal of Economic Entomology* 106(6):2310-2321.
- DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES (DAFF).** 2020. Pesticide management policy. <https://www.daff.gov.za/daffweb3/Branches/Agricultural-Production-Health-Food-Safety/Agriculture-Inputs-Control/Policies-Legislation>
- DESNEUX, N., WAJNBERG, E., WYCKHUYS, K.A.G., BURGIO, G., ARPAIA, S., NARVÁEZ-VASQUEZ, C.A., GONZÁLEZ-CABRERA, J., RUESCAS, C.D., TABONE, E., FRANDON, J., PIZZOL, J., PONCET, C., CABELLO, T. and URBANEJA, A.** 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. *Journal of Pest Science* 83:197-215.
- DOĞANLAR, M. and YİĞİT, A.** 2011. Parasitoid complex of the tomato leaf miner, *Tuta absoluta* (Meyrick 1917), (Lepidoptera: Gelechiidae) in Hatay, Turkey. *KSU Journal of Natural Sciences* 14(4):28-37.
- EL-ARNAOUTY, S.A., PIZZOL, J., GALA, H.H., KORTAM, M.N., AFIFI, A.I., BEYSSAT, V., DESNEUX, N., BIONDI, A. and HEIKAL, I.H.** 2014. Assessment of two *Trichogramma* species for the control of *Tuta absoluta* in North African tomato greenhouses. *African Entomology* 22(4):801-809.

- FARIA, C.A., TORRES, J.B., FERNANDES, A.M.V. and FARIAS, A.M.I.** 2008. Parasitism of *Tuta absoluta* in tomato plants by *Trichogramma pretiosum* Riley in response to host density and plant structures. *Ciencia Rural* 38(6):1504-1509.
- FERRACINI, C., INGEGNO, B.L., NAVONE, P., FERRARI, E., MOSTI, M., TAVELLA, L. and ALMA, A.** 2012. Adaptation of indigenous larval parasitoids to *Tuta absoluta* (Lepidoptera: Gelechiidae) in Italy. *Journal of Economic Entomology* 105(4):1311-1319.
- GABARRA, R., ARNÓ, J., LARA, L., VERDÚ, M.J., RIBES, A., BEITIA, F., URBANEJA, A., TÉLLEZ, M.M., MOLLÁ, O. and RIUDAVETS, J.** 2014. Native parasitoids associated with *Tuta absoluta* in the tomato production areas of the Spanish mediterranean coast. *BioControl*, 59:45-54.
- GEORGHIOU, G.P.** 1983. Management of resistance in arthropods. pp. 769-792. In: *Pest resistance to pesticides*. (Eds. Georgiou, G.P. and Saito, T.). Springer: New York.
- GHADERI, S., FATHIPOUR, Y. and ASGARI, S.** 2017. Susceptibility of seven selected tomato cultivars to *Tuta absoluta* (Lepidoptera: Gelechiidae): Implications for its Management. *Journal of Economic Entomology* 110(2):421-429.
- GHAREKHANI, G.H. and SALEK-EBRAHIMI, H.** 2014a. Evaluating the damage of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on some cultivars of tomato under greenhouse condition. *Archives of Phytopathology and Plant Protection* 47(4):429-436.
- GHAREKHANI, G.H. and SALEK-EBRAHIMI, H.** 2014b. Life table parameters of *Tuta absoluta* (Lepidoptera: Gelechiidae) on different varieties of tomato. *Journal of Economic Entomology* 107(5):1765-1770.
- GONZÁLEZ-CABRERA, J., MOLLÁ, O., MONTÓN, H. and URBANEJA, A.** 2011. Efficacy of *Bacillus thuringiensis* (Berliner) in controlling the tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *BioControl* 56:71-80.
- GUEDES, R.N.C., RODITAKIS, E., CAMPOS, M.R., HADDI, K., BIELZA, P., SIQUEIRA, H.A.A., TSAGKARAKOU, A., VONTAS, J. and NAUEN, R.** 2019. Insecticide resistance in the tomato pinworm *Tuta absoluta*: patterns, spread, mechanisms, management and outlook. *Journal of Pest Science* 92:1329-1942.
- HADDI, K., BERGER, M., BIELZA, P., CIFUENTES, D., FIELD, L.M., GORMAN, K., RAPISARDA, C., WILLIAMSON, M.S. and BASS, C.** 2012. Identification of mutations associated with pyrethroid resistance in the voltage-gate sodium channel of the tomato leaf miner (*Tuta absoluta*). *Insect Biochemistry and Molecular Biology* 42:506-513.

- IDRISS, G.E.A.** 2019. *Bio-ecological studies of Tuta absoluta in Sudan*. PhD thesis, North-West University: Potchefstroom campus.
- IDRISS, G.E.A., MOHAMED, S.A., KHAMIS, F., DU PLESSIS, H. and EKESI, S.** 2018. Biology and performance of two indigenous larval parasitoids on *Tuta absoluta* (Lepidoptera: Gelechiidae) in Sudan. *Biocontrol Science and Technology* 28(6):614-628.
- İNANLI, C., YOLDAŞ, Z. and BİRGÜCÜ, A.K.** 2012. Entomopatojen funguslar *Beauveria bassiana* (Bals.) ve *Metarhizium anisopliae* (Metsch.)'nin *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)'nin yumurta ve larva dönemlerine etkisi. *Ege Üniversitesi Ziraat Fakültesi Dergisi* 49(3):239-242.
- KAMALI, S., KARIMI, J. and KOPPENHÖFER, A.M.** 2018. New insight into the management of the tomato leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae) with entomopathogenic nematodes. *Journal of Economic Entomology* 111(1):112-119.
- KLEIN, H., HILL, M.P., ZACHARIADES, C. and ZIMMERMANN, H.G.** 2011. Regulation and risk assessment for importations and releases of biological control agents against invasive alien plants in South Africa. *African Entomology* 19:488-497.
- KRECHEMER, F.S. and FOERSTER, L.A.** 2017. Development, reproduction, survival, and demographic patterns of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on different commercial tomato cultivars. *Neotropical Entomology* 46(6):694-700.
- LACEY, L.A., FRUTOS, R., KAYA, H.K. and VAIL, P.** 2001. Insect pathogens as biological control agents: Do they have a future? *Biological Control* 21:230-248.
- LIETTI, M.M.M., BOTTO, E. and ALZOGARAY, R.A.** 2005. Insecticide resistance in argentine populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotropical Entomology* 34(1):113-119.
- LONI, A., ROSSI, E. and VAN ACHTERBERG, C.** 2011. First report of *Agathis fuscipennis* in Europe as parasitoid of the tomato leafminer, *Tuta absoluta*. *Bulletin of Insectology* 64(1):115-117.
- LUNA, M.G., PEREYRAL, P.C., COVIELLA, C.E., NIEVES, E., SAVINO, V., GERVASSIO, N.G.S., LUFT, E., VIRLA, E. and SÁNCHEZ, N.E.** 2015. Potential of biological control agents against *Tuta absoluta* (Lepidoptera: Gelechiidae): current knowledge in Argentina. *Florida Entomologist* 98(2):489-494.
- LUNA, M.G., SÁNCHEZ, N.E. and PEREYRA, P.C.** 2007. Parasitism of *Tuta absoluta* (Lepidoptera, Gelechiidae) by *Pseudapanteles dignus* (Hymenoptera, Braconidae) under laboratory conditions. *Journal of Environmental Entomology* 36(4):887-893.

- LUNA, M.G., SÁNCHEZ, N.E., PEREYRA, P.C., NIEVES, E., SAVINO, V., LUFT, E., VIRLA, E. and SPERANZA, S.** 2012. Biological control of *Tuta absoluta* in Argentina and Italy: evaluation of indigenous insects as natural enemies. *Bulletin OEPP/EPPO Bulletin* 42(2):260-267.
- MANSOUR, R., BREVAULT, T., CHAILLEUX, A., CHERIF, A., GRISSA-LEBDI, K., HADDI, K., MOHAMED, S.A., NOFEMELA, R.S., OKE, A., SYLLA, S., TONNANG, H.E.Z., ZAPPALA, L., KENIS, M., DESNEUX, N. and BIONDI, A.** 2018. Occurrence, biology, natural enemies and management of *Tuta absoluta* in Africa. *Entomologia Generalis* 38(2):83-112.
- MOLLÁ, O., GONZÁLEZ-CABRERA, J. and URBANEJA, A.** 2011. The combined use of *Bacillus thuringiensis* and *Nesidiocoris tenuis* against the tomato borer *Tuta absoluta*. *BioControl* 56:883-891.
- OLIVEIRA, F.A., SILVA, D.J.H., LEITE, G.L.D., JHAM, G.N. and PICANCO, M.** 2009. Resistance of 57 greenhouse grown accessions of *Lycopersicon esculentum* and three cultivars to *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Scientia Horticulturae* 119:182-187.
- RODITAKIS, E., SKARMOUTSOU, C. and STAURAKAKI, M.** 2013. Toxicity of insecticides to populations of tomato borer *Tuta absoluta* (Meyrick) from Greece. *Pest Management Science* 69:834-840.
- RODITAKIS, E., VASAKIS, E., GARCIA-VIDAL, L., MARTINEZ-AGUIRRE, M.R., RISON, J.L., HAXAIRE-LUTUN, M.O., NAUEN, R., TSAGKARAKOU, A. and BIELZA, P.** 2018. A four-year survey on insecticide resistance and likelihood of chemical control failure of tomato leaf miner *Tuta absoluta* in the European/Asian region. *Journal of Pest Science* 91:421-435.
- RODITAKIS, E., VASAKIS, E., GRISPOU, M., STAVRAKAKI, M., NAUEN, R., GRAVOUIL, M. and BASSI, A.** 2015. First report of *Tuta absoluta* resistance to diamide insecticides. *Journal of Pest Science* 88:9-16.
- SELALE, H., DAĞLI, F., MUTLU, N., DOĞANLAR, S. and FRARY, A.** 2017. Cry1Ac-mediated resistance to tomato leaf miner (*Tuta absoluta*) in tomato. *Plant Cell, Tissue and Organ Culture* 131(1):65-73.
- SILVA, G.A., PICANCO, M.C., BACCI, L., CRESPO, A.L.B., ROSADO, J.F. and GEUDES, R.N.C.** 2011. Control failure likelihood and spatial dependence of insecticide resistance in the tomato pinworm, *Tuta absoluta*. *Pest Management Science* 67:913-920.

SIQUEIRA, H.A.A., GEUDES, R.N.C. and PICANCO, M.C. 2000. Insecticide resistance in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Agricultural and Forest Entomology* 2:147-153.

VAN DAMME, V.M., BECK, B.K., BERCKMOES, E., MOERKENS, R., WITTEMANS, L., DE VIS, R., NUYTENS, D., CASTEELS, H.F., MAES, M., TIRRY, L. and DE CLERCQ, P. 2016. Efficacy of entomopathogenic nematodes against larvae of *Tuta absoluta* in the laboratory. *Pest Management Science* 72:1702-1709.

VISSER, D., UYS, V.M., NIEUWENHUIS, R.J. and PIETERSE, W. 2017. First records of the tomato leaf miner *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) in South Africa. *BioInvasions Records* 6(4):301-305.

ZAPPALÀ, L., BERNARDO, U., BIONDI, A., COCCO, A., DELIPRRI, S., DELRIO, G., GIORGINI, M., PEDATA, P.C., RAPISARDA, C., TROPEA GARZIA, G. and SISCARO, G. 2012. Recruitment of native parasitoids by the exotic pest *Tuta absoluta* in Southern Italy. *Bulletin of Insectology* 65(1):51-61.

Appendix A

Declaration of language editing

Language editing statement

To whom this may concern,

I, Prof. Hannalene du Plessis, hereby declare that the thesis titled: "Efficacy of entomopathogenic nematodes for control of *Tuta absoluta* in South Africa" by Odette Coleman has been edited for language correctness and spelling by the supervisors. No changes were made to the academic content or structure of this work.

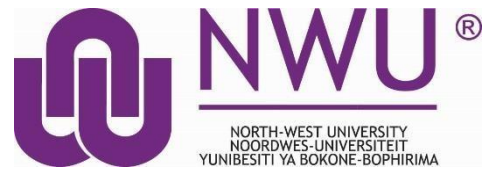


Prof. Hannalene du Plessis

16 May 2020

Date

Appendix B



FNAS Ethics Committee

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Date: 22-May- 2019

Dear Researcher,

Re: Ethics weaver

This letter serves as confirmation that based on the scientific committee assessment of the research proposal concluded that:

Student: Ms O Coleman (24093807)

Title: Efficacy of entomopathogenic nematodes for control of *Tuta absoluta* in South Africa.

Does not require ethical clearance as the study has no/low risk.

Supervisor (s)

Hannalene Du Plessis

Signature: Chairperson of FNAS Ethics Committee

Prof Lesetja Motadi

Appendix C

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