Host plant suitability and baseline susceptibility of *Tuta absoluta* (Lepidoptera: Gelechiidae) to insecticides in South Africa

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DECLARATION BY THE CANDIDATE

I, JP Hefer, 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.

Signature  Date 18 November 2020

DECLARATION AND APPROVAL BY SUPERVISORS

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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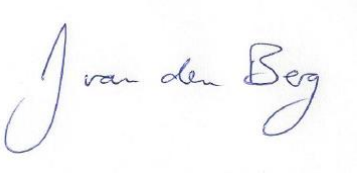
Signature  Date 18 November 2020

Abstract

The devastating tomato pest, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (tomato leafminer/tomato pinworm), originates from South America. It invaded South Africa in 2016. It not only causes severe damage to tomato, but also to other cultivated crops, which mainly belong to the Solanaceous family. Knowledge on the expansion of the host range of *T. absoluta* is important, since movement between crops and weeds can have an influence on the management of this pest. In this study, the aims were to evaluate selected weed species to determine which of these can act as hosts, and to determine baseline toxicity of selected insecticides to *T. absoluta*. Laboratory choice tests were conducted to determine the suitability of five weed species, from four plant families, for reproduction and development of *T. absoluta.* These weeds were *Amaranthus hybridus* L. (Cape pigweed) (Amaranthaceae)*, Solanum scabrum* (Garden huckleberry/African nightshade) (Solanaceae) and *S. retroflexum* (Wonderberry) (Solanaceae), *Malva parviflora* (Malvaceae) and *Galinsoga parviflora* (Gallant soldier) (Asteraceae). *Amaranthus hybridus*, *M. parviflora* and *G. parviflora* did not sustain larval development, and will therefore not contribute to the establishment of *T. absoluta* in invaded areas. In contrast, the two solanaceous weeds, *S. scabrum* and *S*. *reteroflexum,* are potentially as suitable as *S. lycopersicum* L. (tomato) for reproduction and development of *T. absoluta*, and may therefore contribute to the establishment of *T. absoluta*. A number of insecticides are registered in South Africa for control of *T. absoluta*, but its susceptibility to these insecticides is unknown, and as a result, no baseline toxicity data exist in South Africa. The leaf dip bioassay, method 022, approved by the international Insecticide Resistance Action Committee was used to estimate baseline toxicity of indoxacarb, emamectin benzoate, spinetoram and lufenuron for *T. absoluta* populations from Mareetsane (North-West province), Polokwane (Limpopo province) and Swartwater (Limpopo province), in South Africa. The probability for control failure of insecticides was also determined. All three *T. absoluta* populations were highly susceptible to indoxacarb, emamectin benzoate and spinetoram, resulting in low variability in the responses of *T. absoluta* from all three localities, to these active ingredients. The differences in LC_{50} values were below 2.5 fold. No control failure to these active ingredients is therefore currently expected. The *T. absoluta* population from Polokwane was significantly less susceptible to lufenuron, compared to the populations from Swartwater and Mareetsane. All three populations were, however, susceptible to lufenuron despite the Polokwane population being 47 times and 18 times less susceptible than the populations from Swartwater and Mareetsane, respectively. Although not registered for control of *T. absoluta* in South Africa, effective control will be achieved when lufenuron is applied for control of potato tuber moth, at the registered dosage rate.

Key words: Bioassay, choice tests, development, oviposition, resistance, susceptibility, *Tuta absoluta.*

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Chapter 1: Introduction and literature review

1.1 General introduction

Vegetable crops are considered important elements in achieving sustainable development since they contribute to food security and dietary balance (FAO, 2012; Brévault *et al.*, 2014). Tomato is, second to potato, the most important vegetable crop globally (Desneux *et al.*, 2011). The global tomato production in 2019 was estimated at 181M tons, and the South African production, 6M tons (FAOSTAT, 2020). South Africa is the main tomato producing country in the Southern African Development Cooperation (SADC) region (Malherbe and Marais, 2015).

Insects are the most important crop pests in terms of yield loss. These also include invasive or alien species. Invasive species can be described as any organism that is not native to a specific ecosystem (Blackburn *et al.*, 2014). Invasive species are successful in their new environment since there is only a few or no natural enemies or other constrains to control the population numbers. In general, high reproductive rates coupled with high mobility, enable pests to rapidly colonize their new habitat and respond to changing environments (Kennedy and Storer, 2000). The increase in globalization over the past decade resulted in a drastic increase in the diversity as well as a magnitude of invasions by especially exotic invertebrates into new areas (Roques *et al.*, 2008; Hulme, 2009; Biondi *et al.*, 2018). The economy can be directly affected by the decrease in crop yields resulting from damage caused by these invaders, or indirectly through limitations on trade or variations in the value of produce (Kenis and Branco, 2010).

The South American tomato pinworm or tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a widespread invasive pest of economically importance solanaceous crops (Desneux *et al*., 2010). Its invasion is a major concern for tomato producers globally (Guedes *et al*., 2019). It is a major pest of tomato, in both open field and greenhouse crops (Cocco *et al*., 2013). The pest was initially described in 1917 as *Phthorimaea absoluta* by Meyrick from specimens collected in Peru (Desneux *et al.*, 2010). There was a sequence of taxonomic revisions from the mid-1960s until the mid-1990s (Guedes *et al.*, 2019), until it was described by Povolny in 1994 under the genus *Tuta,* as *Tuta absoluta* (Barrientos *et al*., 1998; Desneux *et al.*, 2010).

1.2 *Tuta absoluta*

1.2.1 Biology

The moths are grey in colour with dark markings on the wing surfaces (Torres *et al.*, 2001) (Fig. 1.1a). Plant volatiles are used by the females to find a suitable host plant for oviposition, but contact with plant surfaces are critical to induce oviposition (Proffit *et al.*, 2011). The eggs are very small and yellow and laid individually on the upper surface of young leaves (Proffit *et al.*, 2011) (Fig. 1.1b), and to a lesser extent on stems, flowers and fruit (Shiberu and Getu, 2017). A female can lay approximately 250 eggs (Cuthbertson *et al.*, 2013). Larvae hatch 5 - 7 days after oviposition at 25 - 30°C and 60-75% RH, and complete four instars in approximately 20 days (Torres *et al.*, 2001). The larvae tunnel into leaves and feed on the mesophyll, producing mines (Fig. 1.2a, b). When larvae are present in high numbers, they also tunnel into young stems and tomato fruit below the sepals (Desneux *et al*., 2010). Fourth-instar larvae, considered as mature larvae, drop to the soil, spin silk cocoons and transform into pre-pupae and then pupae (Biondi *et al*., 2018). Pupation can also take place within a leaf (Desneux *et al.*, 2010). The duration of the pupal stage is 6 - 10 days (Torres *et al.*, 2001).

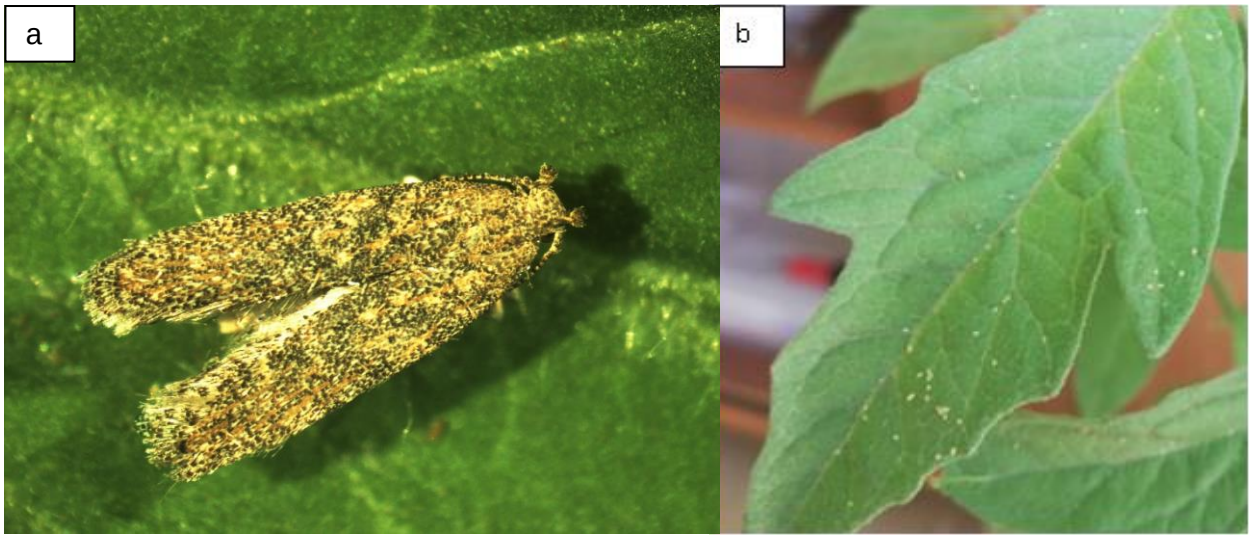

Figure 1.1: *Tuta absoluta* (a) moth and (b) eggs laid on the leaf of a tomato plant.

1.2.2 Damage symptoms

Since its migration out of its indigenous area, *T. absoluta,* became a serious threat to tomato production in Europe, Africa and the Middle East (Desneux *et al.*, 2010; 2011).

Tomato plants are attacked by *T. absoluta* during all developmental stages, with damage inflicted to leaves, stems, fruit and flowers (Desneux *et al*., 2010; Bawin *et al*., 2015). Tunnelling into leaves by the larvae, affects the photosynthetic capability of plants (Campos *et al*., 2017). Tunnelling into the stems of tomato plants, kills young plants. The yield is also affected, and feeding in fruit result in escalated post-harvest costs (Galdino *et al*., 2015; Campos *et al*., 2017). Secondary infections can cause damage indirectly through pathogens that develop in infested plants and fruit tissue (Tropea Garzia *et al*., 2012; Campos *et al*., 2017).

Damage by larvae can cause severe yield loss, which can reach 100% if no control measures are implemented (Desneux *et al.*, 2010). As a result, more applications of insecticides are being done, which contributes to an increase in price and bans on trading of tomato, including seedlings (Abbes *et al.*, 2016). Early infestations of *T. absoluta* are difficult to detect due to the feeding habit of this pest (Biondi *et al.*, 2018). As a result, severe damage to younger plants and fruit occur, and the visual appearance of harvested tomato fruits is also affected (De Castro *et al.*, 2013; Biondi *et al.*, 2018).

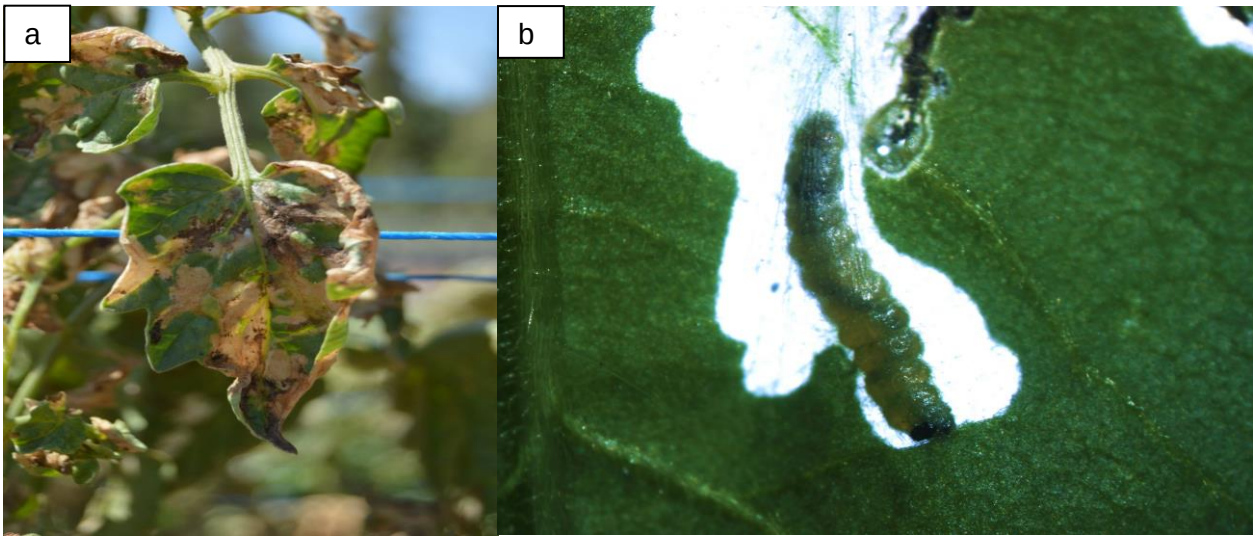

Figure 1.2: a) Tomato leaves damaged by *Tuta absoluta* larvae. The extended mines created by larval feeding is visible as desiccated, brown areas, b) *Tuta absoluta* larva feeding inside a mine in a tomato leaf.

1.2.3 Distribution

The origin of *T. absoluta* was initially believed to be in Central America, however a recent hypothesis indicates that it is native to the Peruvian central highlands from where it migrated into Latin American countries during the 1960s (Desneux *et al*., 2011; Campos *et al*., 2017). For over 50 years, *T. absoluta* was a well-established key pest of tomato only in South America, before it was detected in Spain in 2006 (Desneux *et al*. 2010). The pest has spread across the Mediterranean coastal tomato-producing areas (Desneux *et al*., 2010; 2011), both eastward as well as southward at an approximate speed of 800 km per year (Desneux *et al*., 2011; Campos *et al*., 2017). It also spread across Asia and was first detected in China in 2017 (Zhang *et al*., 2020). *Tuta absoluta* was found in Africa, in Morocco in 2008, and invaded almost the entire African continent over eight years, with the first detection of *T. absoluta* in South Africa in August 2016 (Visser *et al.*, 2017). It quickly reached pest status on tomato in the invaded areas (Desneux *et al*., 2011; Campos *et al*., 2017).

1.2.4 Host plants

The host plant range of *T. absoluta* mainly comprises of Solanaceae, with tomato *(Solanum lycopersicum* L.*)* as the main crop host plant (Tonnang *et al.*, 2015). However, the leafminer also feed on other cultivated Solanaceae for example potato (*Solanum tuberosum* L.), eggplant (*S. melongena* L.), sweet pepper (*S. muricatum* L.) and tobacco (*Nicotiana tabacum* L.) (Desneux *et al.*, 2010), Cape gooseberry (*Physalis peruviana* L.) and goji berry (*Lycium* sp.) (EPPO, 2009; Abdul-Rassoul, 2014). Alternative host plants were also reported in plant families other than the Solanaceae, *viz*. in the Fabaceae, common beans (*Phaseolus vulgaris*), broad bean (*Vicia faba*), cowpea (*Vigna unguiculata*), and alfalfa (*Medicago sativa*). In the Brassicaceae family, wild radish (*Raphanus raphanistrrum*) also serves as a host (EPPO, 2009; Abdul-Rassoul, 2014).

Certain weeds are also regarded as hosts (Abbes *et al*., 2012). These include non-cultivated solanaceous plants, *viz*. *Solanum nigrum* L*, S. elaeagnifolium* L*, Datura stramonium* L *and D. ferox* L (Desneux *et al.*, 2010; Mohamed *et al.*, 2015). New host plants reported in Sudan for the first time by Mohamed *et al*. (2015) are watermelon, *Citrullus lanatus* (Cucurbitaceae), physic nut, *Jatropha curcas* (Euphorbiaceae), spiny amaranth (*Amaranthus spinosus*) (Amaranthaceae), ramtouk (*Xanthium brasilicum*) (Asteraceae) and *Solanum dubium* (Solanaceae).

Although Mohamed *et al*. (2015) reported *T. absoluta* to have also shifted to hosts belonging to plant families other than Solanaceae in Sudan, these observations could not be confirmed in a follow-up study by Idriss *et al*. (2020).

Alternative host plants allow *T. absoluta* to survive in areas where tomato are absent or unavailable (Abbes *et al.*, 2016). The host plants used by herbivorous insects are related to the physiology, morphology, chemical- and also physical defences of the plants (Singer *et al.*, 2004; Pereyra and Sánchez, 2006).

Previous larval experience of a host plant that has been retained to the adult stage, can affect the oviposition preference or reproductive behaviour of *T. absoluta* female moths (Idriss *et al.*, 2020). Knowledge on its effective host range could therefore provide insight into, and contribute to development of strategies to limit the distribution of *T. absoluta* (Bawin *et al.*, 2015).

1.3 Locating host plants

Insects use cues, including odour, colour, and shape, to locate and recognize their preferred hosts and mates (Yv *et al*., 2015). Olfaction plays an important role in insects, and are not only used for activities such as host- and mate finding, but also to avoid enemies (Yuvaraj *et al*., 2018). The olfactory system of adult insects consists of two pairs of main olfactory appendages on the head, i.e. the antennae and palps (Yuvaraj *et al*., 2018). The olfactory sensilla of insects contain specialized structures that detect the odour molecules (Schneider, 1964).

Volatile signals that relate to differences in genotype, phenological stage and environmental conditions are used by insects to determine suitable hosts (van Loon, 1996; Bengtsson *et al.*, 2001; Vallat and Dorn, 2005; Karlsson *et al*., 2009; Proffit *et al.*, 2011). Volatile organic compounds (VOC's) are therefore important to insects, providing them with information on the status of a plant. The VOC's released by plants therefore have multiple roles in their interaction with animals and other plants (Dudareva *et al*., 2004; Knudsen *et al*., 2006; Pichersky *et al*., 2006; Proffit *et al.*, 2011). For example, herbivorous insects use these compounds to locate their host plants from a distance and for finding mates (Berlocher and Feder, 2002; Linn *et al*., 2003; Bengtsson *et al*., 2006; Tasin *et al*., 2006; Pinero and Dorn, 2009; Cha *et al*., 2008; Schmidt-Busser *et al*., 2009; Sole *et al*., 2010; Proffit *et al.*, 2011).

Volatile organic compounds released by plants can either promote or discourage females from laying eggs (Cook *et al*., 2007; Witzgall *et al*., 2008; Karlsson *et al.*, 2009). Toxic substances, lack of feeding stimulants and morphological characteristics of the host plant can also affect the survival of larvae (Gupta and Thorsteinson, 1960; Eigenbrode and Shelton, 1992, Hough-Goldstein and Hahn, 1992; Idris and Grafius, 1996). The attraction and oviposition of *T. absoluta* females are also known to be affected by the volatile signature of their host plant (Bawin *et al.*, 2015). Volatile organic compounds released by leaves provide important information to the female about the suitability of the plant for the larvae (Bawin *et al.*, 2015). Therefore, oviposition by females and the occurrence of young larvae may mainly be on plant parts with higher nutritional values (Torres *et al.*, 2001).

It is advantageous to herbivores, since host plant nutritional value affects survivorship and fecundity (Pereyra and Sánchez, 2006).

Attraction of *T. absoluta* females to other *Solanum* species, *viz*. potato and black nightshade are expected, since they are related to tomato (Lambinon *et al*., 2004; Bawin *et al.*, 2015). Wind tunnel and oviposition choice experiments performed by Proffit *et al.* (2011) using different tomato varieties, indicated that *T. absoluta* females favoured *S. lycopersicum* over the wild variety, *S. habrochaites,* which is resistant to larval feeding. Male preference is dictated by the female sex pheromone and males rarely respond to host plant volatiles (Ramaswamy, 1988; Bawin *et al.*, 2015).

Plant volatiles are produced by metabolic pathways, with many that derive from the isoprenoid or terpenoid pathways (Sacchettini and Poulter, 1997; Degenhardt *et al*., 2009; Proffit *et al.*, 2011). Plant volatiles are not only used by the herbivores to locate plants, solanaceous plants also produce many terpenes which is used for defence against herbivores (Snyder *et al*., 1993; Kennedy, 2003; Bleeker *et al*., 2009; Kang *et al*., 2010; Proffit *et al.*, 2011). Some terpenoids function in indirect plant defence by attracting arthropods that prey upon or parasitize herbivores (Kessler and Baldwin, 2001; Sharma *et al.*, 2017). Terpenoids are also produced in response to oviposition and are involved in the attraction of egg-parasitizing insects (Conti *et al.*, 2008; Hilker and Fatouros, 2015; Sharma *et al*., 2017).

1.4 Economic impact

The economy of developing countries relies on agriculture for sustaining economic growth and to reduce poverty (Zuberi *et al*., 2014). Biological invasions and climate change are, however, the greatest threats to biodiversity, agriculture, health and the global economy (Pimentel *et al*., 2001).

The global invasion of crop pests have great economic- and environmental impacts (Desneux *et al.*, 2011). There was a significant increase in insecticide applications after the first year of *T. absoluta* invasion in the Mediterranean Basin (Desneux *et al.*, 2011). The introduction of *T. absoluta* in countries bordering South America, has also led to an increase in the use of insecticides in tomato fields (Picanço *et al*., 1995; Siqueira *et al*., 2000a; Guedes and Picanço 2012; Zlof and Suffert, 2012; Gontijo *et al*., 2013; Campos *et al*., 2017). This has a direct effect on the economy due to increased control costs (Retta and Berhe, 2015). Besides environmental and human safety concerns pertained to insecticides, the cost related to tomato production more than tripled with the detection of *T. absoluta* (Campos *et al*., 2017).

There is, therefore, increased emphasis on the development and use of integrated pest management (IPM) strategies with the increasing awareness of environmental-, economic- and other problems caused by the over-reliance on chemical pesticides (Cuthbertson, 2020).

1.5 Integrated Pest Management

Integrated pest management is a combination of pest management evaluation, risk decision making and use of various control strategies (Cuthbertson, 2020). It includes identification and monitoring of pests, setting of action thresholds, prevention of a pest species from becoming a problem and if preventative methods are no longer effective or available, application of control options. The IPM approach therefore replaces the concept of pest control, to a balanced approach aimed at managing a pest population to levels that do not cause economic damage (Cuthbertson, 2020). The various control methods incorporated into IPM, include cultural-, biological- and chemical control, semiochemicals, host plant resistance and genetically modified plants (Dent 2000; Sparks and Nauen, 2015). Although pesticide use forms part of key IPM approaches, attempts have been made in pursuit of compounds with a lower impact on beneficial organisms and non-target arthropods (De Castro *et al.*, 2013). Control strategies for *T. absoluta* have therefore been established which is also environmentally friendly (Chhetri, 2018).

1.5.1 Cultural control

Mansour *et al*. (2018) suggested that an IPM program for *T. absoluta* should integrate prophylactic and cultural control methods to avoid major *T. absoluta* infestations. Cultural methods practiced for *T. absoluta* control, include crop rotation, destruction and selective removal of infested plant material (Chhetri, 2018), mass trapping (Lobos *et al*., 2013) and mating disruption (Cocco *et al*., 2013).

Early detection of *T. absoluta* in tomato crops can be done by means of a synthetic female sex pheromone in traps (Lobos *et al*., 2013). Male and female *T. absoluta* moths can be mass trapped simultaneously if light sources and water traps (for females) are used together with pheromone dispensers that contain synthetic female sex pheromone to trap males (Hassan and Al-Zaidi, 2010; Caparros Megido *et al*., 2013). Delta traps can also be used for mass trapping in association with sticky traps and a pheromone (Caparros Megido *et al*., 2013) (Fig 1.3).

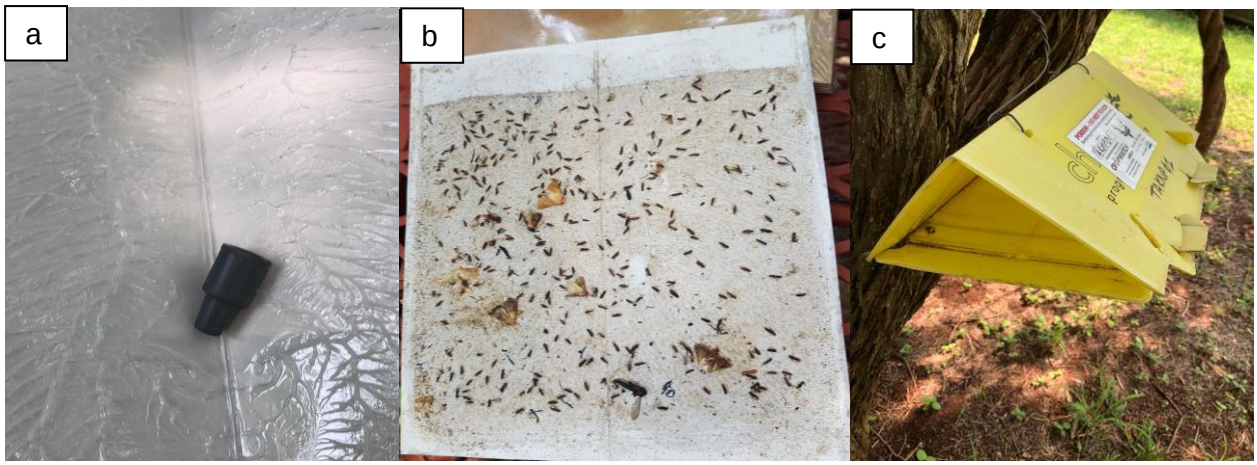

Figure 1.3: a) A pheromone lure to attract male moths of *Tuta absoluta*, b) sticky card used in delta traps, and c) a delta trap containing the pheromone lure and sticky card.

The first step in effective mating disruption of this pest in tomatoes cultivated under nets or in greenhouses is prevention of fertile *T. absoluta* females from gaining access to these structures (Biondi *et al*., 2018). This can be achieved through alternative control methods such as male annihilation or mating disruption by means of synthetic pheromones (Cocco *et al*., 2013). For mating disruption, the synthetic pheromone should saturate the atmosphere whereby the ability of males to find females will be affected, resulting in a reduction in population density (Cocco *et al*., 2013). Pheromones of *T. absoluta* are commonly used for mating disruption in greenhouses. At a rate of 30-60 g pheromones per hectare, the moth populations can be controlled and the damage to tomato in high-containment greenhouses, reduced (Vacas *et al.*, 2011; Cocco *et al*., 2013). However, *T. absoluta* females are able to reproduce by means of parthenogenesis, and also through multiple mating (Imenes, 1990; Lee *et al*., 2014). It is therefore important to prevent fecund females from entering greenhouses for mating disruption to be effective (Biondi *et al*., 2018).

Other means of cultural control include non-planting during peak-periods of pest infestation and good sanitation practices. For example, in terms of *T. absoluta* infestations, the late dry season is the most hazardous period for tomato cultivation in Senegal, resulting in farmers not producing tomato during this period anymore (Mansour *et al*., 2018). Alternative hosts, such as eggplant (*Solanum melongena* L.), may contain residual host populations of *T. absoluta* (Mansour *et al*., 2018). Management of populations on these reservoirs through cultural control methods such as removal of old eggplant fields, can reduce populations early in the dry season (Mansour *et al*., 2018; Sylla *et al*., 2018).

1.5.2 Biological control

Biological control of *T. absoluta* is practiced by means of parasitoids, predators and entomopathogens (Desneux *et al*., 2010; Chhetri, 2018).

Natural enemies in recently invaded regions need time to adapt to the exotic species by altering their behaviour and/or physiology to develop successfully on their exotic host or prey, to control it (Zappalà *et al.*, 2013). However, in Europe and North-Africa, various predators and parasitoids were found to spontaneously attack *T. absoluta* in tomato crops (Zappalà *et al.*, 2013). The majority of predators of *T. absoluta* recorded in the Western Palaearctic countries are hemipterans, which largely belong to the Miridae, Anthocoridae and Nabidae families (Zappalà *et al.*, 2013).

Overall, parasitoids play a vital role in biological control of pests, which can be attributed to their capability to control populations of several phytophagous insects (Arnó *et al*., 2018). The availability of a sufficient food supply for many of these parasitoids, will assist them to reach their maximum biological control potential (Arnó *et al*., 2018). Various species of parasitoids of *T. absoluta* larvae have been identified in the Mediterranean region, most of them belonging to s Eulophidae, Braconidae, and Ichneumonidae (Arnó *et al*., 2018). The parasitoids of *T. absoluta* reported in Africa, belong to four families, namely Braconidae (Boualem *et al.,* 2012; Abbes *et al*., 2014; Idriss, 2019), Eulophidae (Boualem *et al*., 2012; Abbes *et al*., 2014), Ichneumonidae (Boualem *et al*., 2012) and Pteromalidae (Idriss, 2019). Larval parasitoids may be beneficial to compliment the general activities of predators of this pest (Arnó *et al*., 2018).

Microbial control generally relies on the commercial strains of *Bacillus thuringiensis* (Bt) var. *kurstaki* and *aizawaii* which will kill the larvae when consumed (González-Cabrera *et al*., 2011; Biondi *et al*., 2018).

Additional *Bacillus* spp. along with the fungi *Beauveria bassiana* and *Metarhizium anisopliae* have also been studied (Contreras *et al*., 2014; Borgi *et al*., 2016; Biondi *et al*., 2018), but no commercial products explicitly intended for *T. absoluta* have been produced (Biondi *et al*., 2018).

1.5.3 Chemical control

The intake pathway of active ingredients from insecticides into the body of insects, can generally follow two routes. Firstly, through the cuticle, with the active ingredient attaching to the outer body surface of the insect and permeating into the body through the cuticle layer, known as contact toxicity (Vassilakos *et al.*, 2012)**.** Secondly, through dietary toxicity where the active ingredient is ingested when the insect feeds on a crop that has been treated with insecticides (Dripps *et al*., 2011; Vassilakos *et al.*, 2012).

Insecticides were intensively used against *T. absoluta* in invaded areas in South America (Guedes and Picanço, 2012), Europe and North America (Desneux *et al*., 2011 Campos *et al*., 2017). These were usually broad-spectrum insecticides (Campos *et al.*, 2015). Chemical control still remains one of the most common used methods for controlling *T. absoluta* populations (Siqueira *et al*., 2000a; Silva *et al*., 2011; Guedes and Picanço 2012; Roditakis *et al.*, 2013; Roditakis *et al.,* 2018). However, insecticides do not easily reach the larvae which feed on the mesophyll in the leaves, in the apical stem or in the fruit of tomato plants (Guedes *et al*., 1994; Picanço *et al*., 1995; Tomé *et al*., 2012; Guedes and Picanço, 2012).

Insecticides registered over the years against *T. absoluta* include organophospates, pyrethroids (Siqueira *et al*., 2000a), cartap (nereistoxin analogue), abamectin (avermectin) (Siqueira *et al*., 2000b; Guedes and Siqueira, 2012), indoxacarb (oxadiazine), chitin biosynthesis inhibitors (Silva *et al*., 2011; Guedes and Picanço, 2012), pyroroles, spinosyns and diamides (Silva *et al*., 2011; 2016a,b; Guedes *et al*., 2019).

Insecticides that received emergency registration for control of *T. absoluta* in South Africa, are chlorantraniliprole, flubendiamide, lambda-cyhalothrin (part of a mixture), cypermethrin, spinosad, spinetoram, indoxacarb, emamectin benzoate and lufenuron (DAFF, 2017).

1.5.3.1 Insecticide groups for *Tuta absoluta* control in South Africa

1.5.3.1.1 Group 28: Ryanodine receptor modulators

The diamides are in group 28 of the mode of action classification of the Insecticide Resistance Action Committee (IRAC).

Insecticides in this group are known as the ryanodine receptor modulators (IRAC, 2012). The diamides are phthalic acid (flubendiamide) and the anthranilic diamides (chlorantraniliprole and cyantraniliprole) (Campos *et al.,* 2015). The release of intracellular calcium stores is regulated by the ryanodine receptor, which is a non-voltage-gated calcium channel, vital for muscle contraction (Campos *et al.,* 2015). Insecticides in this group act by binding to ryanodine receptors as a selective agonist. It activates the uncontrolled release of calcium stores, resulting in feeding cessation, lethargy, contractile paralysis and finally death (Cordova *et al.,* 2006; Lahm *et al.,* 2009; Campos *et al.,* 2015).

1.5.3.1.2 Group 3: Sodium channel modulators

Voltage-gated sodium channels are transmembrane proteins responsible for action potential initiation and propagation in excitable cells (Silver *et al.,* 2010; Zhang *et al.,* 2019). Subsequent to membrane depolarization, sodium channels are opened allowing sodium ions to pass through into the cells, initiating membrane depolarization (Zhang *et al.,* 2019).

1.5.3.1.3 Group 5: Nicotinic acetylcholine receptor (Allosteric modulators)

Spinosyns include both spinosad and spinetoram (Bacci *et al*., 2016) and belong to the IRAC group 5 (IRAC, 2009). These insecticides mainly activate the nicotinic acetylcholine receptors by targeting a distinctive site (Salgado, 1998; Salgado *et al*., 1998; Sial and Brunner, 2010). Spinosyns are a family of broad spectrum insecticides with activity against numerous pest species such as whiteflies, leafrollers, thrips and leafminer flies (Sparks *et al*., 1998; Bacci *et al.*, 2016). It can therefore control multiple pest species that infest a crop simultaneously (Shimokawatoko *et al*., 2012).

1.5.3.1.4 Group 22: Voltage dependent sodium channel blockers

Indoxacarb act by blocking the sodium channel of neurons (McCann *et al*., 2001). These sodium channels are the most vulnerable at depolarized potentials, so that tonic sensory receptors and pacemaker neurons are highly delicate, generating a kind of paralysis where the nervous system exhibit little to no impulsive motion. The affected insect therefore appears paralyzed, however, the insect can be encouraged to convulse through phasic sensory receptor activation (Salgado and Hayashi, 2007). These convulsions terminate after a few seconds as a result of more sodium channels becoming blocked, after wich the insect laps back into a pseudo-paralyzed state (Salgado, 1990; Salgado and Hayashi, 2007).

1.5.3.1.5 Group 6: Chloride channel activators

Emamectin benzoate is a descendant from the avermectin family (Ishaaya *et al*., 2002). It affects the nervous system of arthropods by increasing chloride ion flux at the neuromuscular junction, resulting in cessation of feeding and irreversible paralysis. Avermectins are natural occurring macrocyclic lactones isolated from the fermentation products of a soil micro-organism (*Streptomyces avermitilis* MS and DMA-4680 strain) (Ishaaya *et al*., 2002). Emamectin benzoate acts on a range of lepidopteran pests (Ishaaya *et al*., 2002). Avermectins bind to sites in the head and muscle neuronal membranes of insects (Deng and Casida 1992, Rohrer *et al*., 1995; Sial and Brunner, 2010), and act as agonists for GABA-gated chloride channels (Mellin *et al*. 1983, Albrecht and Sherman, 1987; Sial and Brunner, 2010). Emamectin benzoate causes irreversible activation of the chloride channels of the nervous system of insects, preventing muscle contraction and causing cessation of feeding and death (Sial and Brunner, 2010).

1.5.3.1.6 Group 15-18: Insect growth regulators

Growth and develop of living organisms takes place according to hormone-guided processes (Casida, 2009).

Compounds that disrupt these processes serve as insect growth regulators (IGRs), plant growth regulators (PGRs), as well as fungal disease development regulators or host plant defence inducers (Casida, 2009). The development of insects is controlled by an amount of juvenile hormone to stay young, as well as a growth and differentiation hormone or ecdysone to develop, moult and become an adult (Casida, 2009). Juvenile hormones mimic and analogues are effective and selective but control with these insecticides is slow (Casida, 2009). Moulting disruptors such as ecdysone receptor agonists, act at the ecdysone binding site (Casida, 2009). Chitin biosynthesis inhibitors used against Lepidoptera (benzoylphenyl ureas), such as lufenuron classified under group 15, block chitin synthesis in vivo, but chitin synthase is not blocked in vitro, rendering the mechanism unsolved (Casida, 2009). Lufenuron is an effective insecticide against a wide range of insect pests since chitin is produced in great quantity by invertebrates, particularly arthropods such as insects (Cohen, 2001).

1.6 Insecticide resistance

Until the 1990s, the insecticides primarily applied against *T. absoluta* in South America were the organophosphate, methamidophos, along with cartap, abamectin and the pyrethroids, deltamethrin and permethrin (Lietti *et al*., 2005; Siqueira *et al*., 2000a; Guedes and Picanço, 2012).

Farmers in Brazil applied insecticides 10-12 times per cultivation cycle, after the pest was first introduced (Guedes *et al*., 2019), but after a few years, increased the numbers of applications to more than 30 per cropping cycle (Guedes and Siqueira, 2012; Guedes *et al*., 2019). This misuse of insecticides has selected for pest resistance to many classes of insecticide in Brazil (Campos *et al.*, 2015). Resistance to organophosphates and pyrethroids were reported from Chile (Salazar and Araya, 1997; 2001 cited in Silva *et al*., 2011), and to abamectin, cartap and permethrin in Brazil (Siqueira *et al*., 2000a). These were followed by further reports of Argentinean *T. absoluta* populations resistant to insecticides (Lietti *et al*., 2005; Guedes and Picanço, 2012).

Insecticides in other chemical groups were later registered for control of *T. absoluta* including pyrroles (chlorfenapyr), diamides (flubendiamide and chlorantraniliprole) and the spinosyns (spinetoram and spinosad) (Silva *et al*., 2011; Gontijo *et al*., 2013; Silva *et al*., 2016a,b; Guedes *et al*., 2019). The use of chitin synthesis inhibitors surpassed that of permethrin (Silva *et al*., 2011) as well as abamectin and cartap (Silva *et al.,* 2016b) for control of *T. absoluta* in Brazil. As a result, high levels of resistance to chitin synthesis inhibitors evolved in some regions (Silva *et al*., 2011; Guedes and Picanço, 2012).

Moderate levels of resistance to indoxacarb and low levels of resistance to spinosad were also reported (Silva *et al*., 2011; Guedes and Picanço, 2012). Resistance to emamectin benzoate, spinosad, indoxacarb and chlorantraniliprole was also reported in the European/Asian region by Roditakis *et al*. (2017). Spinosad, azadirachtin and *Bacillus thuringiensis* toxins (Bt toxins) are relied on for control of tis pest in organic tomato production systems (Silva *et al*., 2011; Biondi *et al*., 2018; Guedes *et al*., 2019).

1.7 Types of resistance

Resistance to insecticides evolves primarily by means of two mechanisms, *viz*. through enhanced production of metabolic enzymes, which sequester or detoxify the insecticide, and/or mutations of the target proteins, which decrease their sensitivity to the insecticide (Panini *et al*., 2016). There are also various subordinate physiological mechanisms which contribute to reduce insecticidal effects, for example lower penetration of chemicals or increased excretion (Panini *et al*., 2016).

Levels of resistance are often increased by the interaction of more than one mechanism that are present simultaneously (Panini *et al*., 2016). Combinations of different resistant mechanisms may be present in insect populations, but also in single individuals within a population (Panini *et al*., 2016).

“Multiple-resistance” occurs with the co-existence of different resistance mechanisms, which confer resistance to different insecticides to which the organism has been exposed (Oppenoorth and Welling, 1976; Yu, 2008; Panini *et al*., 2014; Panini *et al*., 2016).

“Cross-resistance” occurs when a single defence mechanism against one insecticide also causes resistance to other insecticides, even with no exposure of the insect to the other insecticides. It is not restricted to a specific chemical class, but it can also include insecticides with different modes of action (Panini *et al*., 2016). The presence of both multiple-resistance and cross-resistance is of significance, since they increase the difficulty to control the pest (Panini *et al*., 2016).

1.7.1 Metabolic resistance

Enzymes are used by insects to protect them against naturally occurring plant toxins such as alkaloids, terpenes and phenols, to overcome the potential toxicity of the plants they feed on (Gatehouse, 2002; Després *et al*., 2007; Heidel-Fischer and Vogel, 2015; Rane *et al*., 2016; Panini *et al*., 2016). Metabolic resistance against insecticides is based on these same enzymatic systems to detoxify/sequester insecticide molecules (Panini *et al.*, 2016).

Resistant insects have higher quantities of these enzymes as a result of improved transcription or gene amplification or their enzymes evolved higher catalytic rates to metabolise insecticides faster (Panini *et al.*, 2016). Rapid development of metabolic resistance against a broad spectrum of insecticides could be explained by the same enzymes being used to protect them against naturally occurring plant toxins (Isman, 2006; Panini *et al.*, 2016). When xenobiotics are detoxified by enzymes, they are turned into non-toxic compounds and/or into a more appropriate form for fast removal from the body (Panini *et al*., 2016). These detoxification strategies therefore rely on enzymatic cleavage and elimination (Panini *et al*., 2016). An additional defence mechanism is sequestration which a number of insects use to tolerate xenobiotics (Panini *et al*., 2016). Detoxification of xenobiotics in living organisms is facilitated by enzymes which is transcribed by members of large multigene families of esterases, oxidases, and GSTs (Panini *et al*., 2016).

1.7.2 Target-site resistance

Target site resistance occurs with point mutations which confer insensitivity to insecticides (Panini *et al*., 2016). It is not a common mechanism in insects for insensitivity to host plant chemicals (Després, 2007; Panini *et al*., 2016). Although mutations to the target protein of an insecticide can cause high levels of insensitivity in insects, it would likely be specific for a certain chemical class (Panini *et al*., 2016).

However, detoxification mechanisms have the ability to confer cross-resistance between plant toxins and insecticides due to these mechanisms having the potential to act against a broad range of molecules (Panini *et al*., 2016).

1.7.3 Penetration resistance

An insecticide has to penetrate the cuticle of an insect to reach its target site (Panini *et al*., 2016). Physico-chemical changes in cuticle structure, and resulting slower absorption of an insecticide or a reduced amount of the insecticide passing through the physical barriers of the insects' cuticle is known as penetration resistance (Panini *et al*., 2016). This mechanism protects insects from many insecticides, but provide low levels of resistance on its own (Panini *et al*., 2016). It usually occurs with other forms of resistance, enhancing their effects (Panini *et al*., 2016).

1.7.4 Behavioural resistance

Behavioural resistance can be defined as a change in insect behaviour to avoid an insecticide (Panini *et al.*, 2016). Resistant insects stop feeding or leave a treated area when they detect the insecticide (Gatton *et al*., 2013; Panini *et al.*, 2016).

They can either fly away from the treated area, travel deeper into the crop canopy or simply move to the bottom of the leaf where no or little amounts of insecticides are present (Gatton *et al*., 2013; Panini *et al.*, 2016).

1.8 Insecticide Resistance Management (IRM)

Resistance can practically be defined as "a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species" (Nauen *et al*., 2019). Insects are known to evolve resistance to insecticides through the misuse and overuse of an insecticide against an insect pest species (Nauen *et al*., 2019). If insecticides are frequently applied during the cropping season, the risk for resistance development increases, and an appropriate resistance monitoring scheme is required (Roditakis *et al.,* 2013).

The aim of insecticide resistance management (IRM) is to prevent or delay resistance to insecticides or to recover susceptibility in insect pest populations in which resistance has already evolved (Sparks and Nauen, 2015; Nauen *et al*., 2019). Insect resistance management is an important aspect of sustaining the efficacy of insecticides (Nauen *et al*., 2019). It is easier to proactively limit and delay resistance evolution than to reactively regain susceptibility (Nauen *et al*., 2019).

Valuable guidance for the design of effective IRM strategies is provided by the mode of action (MoA) classification of IRAC (Nauen *et al*., 2019). In terms of IRM, Roush (1989) recommended alteration of insecticides across pest generations, the use of non-persistent formulations and pesticides that confer low magnitudes of resistance. Nauen *et al*. (2019) elaborated on this approach towards IRM and described it as the alteration/rotation, sequences or even combinations of compounds from MoA groups.

A rotation approach frequently uses a “window” or block strategy which can be defined as the duration of a pest generation or crop growth stages (Nauen *et al*., 2012; Sparks and Nauen, 2015). This approach is based on the rotation of insecticides belonging to different MoAs in windows or blocks during the growing season to reduce selection for cross-resistance (Sparks and Nauen, 2015). For *T. absoluta* control, a treatment window can be defined as a period of 30 consecutive days which is the minimum duration of a single *T. absoluta* generation (Guedes *et al*., 2019). When the first application window is completed and additional insecticide applications are needed, an insecticide from a different MoA group than the previous insecticide should be used for the next 30 days (Guedes *et al*., 2019).

To delay the evolution of resistance by *T. absoluta* against insecticides, the basic rule to apply for adequate rotation is to avoid consecutive generations from being treated with insecticides belonging to the same MoA group (Guedes *et al*., 2019). Multiple applications of the same MoA are allowed within a treatment window (Fig. 1.4). However, a treatment window should be followed by application of a different MoA in the next window (30 days), and if possible, a different MoA should be applied in a third MoA treatment window (Guedes *et al*., 2019). A minimum of three insecticide MoA groups are required for this method to be effective, but ideally more MoA groups should be included. An example of an insecticide treatment windows approach by Guedes *et al*. (2019) for control of *T. absoluta* with four different MoAs is shown in Figure 1.4.

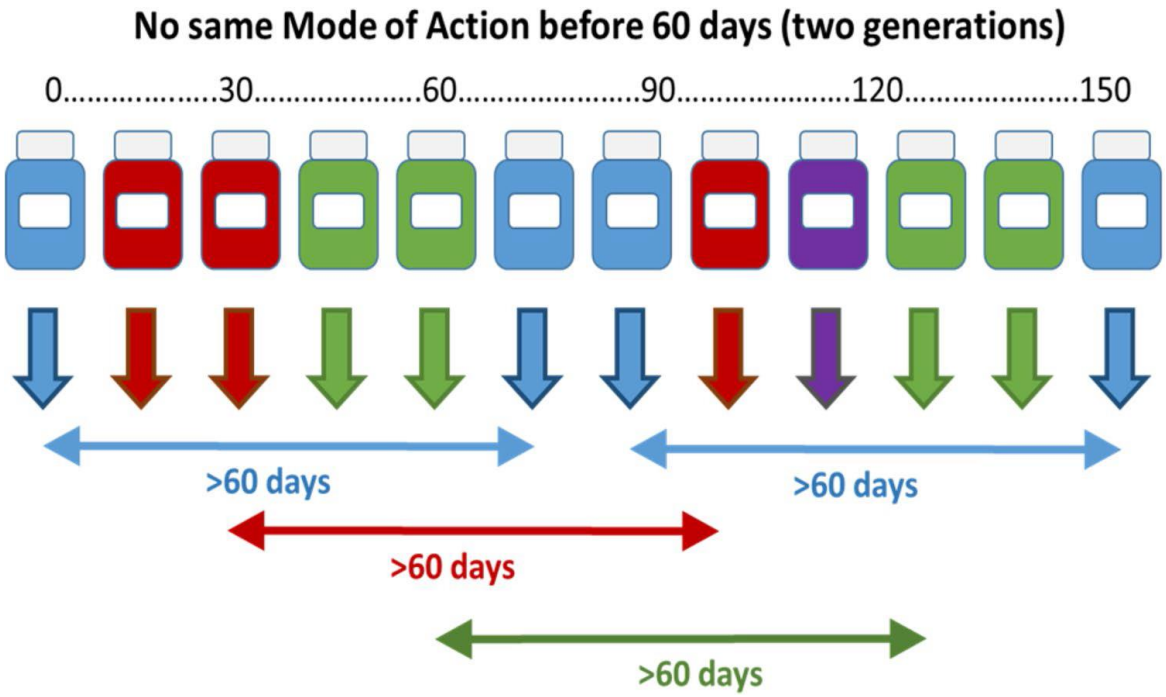

Figure 1.4: Insecticide treatment window for control of *Tuta absoluta* based on the modes of action of insecticides and the minimum duration of a single generation (30 days). Each colour represents a different mode of action. (From Guedes *et al*., 2019).

Frequent applications of the same insecticide will select for those individuals in a population that are able to survive the recommended rates of compounds as a result of their genetically fixed difference (Nauen, 2007). Continued application will therefore lead to a proportional increase of resistant compared to the susceptible individuals in the population (Nauen, 2007; Panini *et al*., 2016). If this selection pressure is strong, the resistant individuals will become the predominant part of the population (Panini *et al*., 2016).

However, key to addressing insecticide resistance issues is the ability to reliably detect the presence of insecticide-resistant strains of pest insects (Sparks and Nauen, 2015). In this regard, IRAC evaluated, validated and recommended a range of bioassay techniques to monitor insecticide and acaricide susceptibility for selected pest species of economic importance (Nauen *et al*., 2019)

1.9 Problem statement

Tuta absoluta arrived in South Africa in 2016 as an invasive agricultural pest (Visser *et al*., 2017). Knowledge on its natural host plant range in South Africa, is currently lacking. This pest is already resistant to various insecticide groups in South America (Silva *et al*., 2011; Guedes and Picanço, 2012, Guedes *et al*., 2019) as well as in the Euro-Asian region (Roditakis *et al*., 2018).

The insecticide susceptibility status of the populations that invaded South Africa, and those present inside the country since then, is unknown. *Tuta absoluta* is currently controlled by means of intensive chemical control programs. With its history of rapid insecticide resistance evolution, the potential for insecticide resistance evolution also exists in South Africa, with devastating consequences for both tomato and potato producers. It is therefore essential to study the relationship between the pest and its host plants to better understand its distribution behaviour and to develop IPM strategies. Reliable baseline susceptibility data for *T. absoluta* to insecticides in South Africa is therefore needed for resistance management of this pest.

1.10 Objectives

1.10.1 Main objective

The main objectives of this study were to evaluate the suitability of wild host plant species for oviposition and larval development of *T. absoluta* and to estimate its susceptibility to selected insecticides in South Africa**.**

1.10.2 Specific objectives

i) to determine the feeding and oviposition preference and larval development of *T. absoluta* on wild host plants.

ii) to estimate the susceptibility of *T. absoluta* to indoxacarb, lufenuron, emamectin benzoate and spinetoram in South Africa.

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Chapter 2: Suitability of five alternative host plants for development and oviposition of *Tuta absoluta*

Abstract

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), which invaded numerous countries including South Africa, is an important pest of *Solanum lycopersicum* (tomato). The main host plant for *T. absoluta* is *S. lycopersicum*, but other crops belonging to the family Solanaceae and various weed species may function as alternative hosts. The aim of this study was to determine the feeding and oviposition preference and larval development of *T. absoluta* on wild host plants. The suitability of five weed species for reproduction and development of *T. absoluta* was therefore investigated. *Amaranthus hybridus*, *Malva parviflora* and *Galinsoga parviflora* were not suitable and did not sustain larval development. These species will therefore not act as host plants and will not contribute to the establishment of *T. absoluta* in the course of an invasion. Two solanaceous weeds, *Solanum scabrum* and *Solanum reteroflexum* are, however, suitable reproductive and developmental hosts for *T. absoluta*, and may contribute to survival and establishment of this pest in invaded areas. Cognisance should be taken of the presence of these weed species inside solanaceous crop fields and in areas adjacent to these crops, in management strategies for this pest.

2.1 Introduction

The emergence of an invasive phytophagous insect such as *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) within a certain geographical range, is determined by its capability to successfully confine and develop on new host plants (Bawin *et al.*, 2016). The host range is inhibited by the behavioural and physiological characteristics of the insect (Suckling *et al.*, 2014; Bawin *et al.,* 2016).

Prediction of the host range of an alien invasive species is a key step in a risk assessment process to determine where a particular species will establish, if it could be eradicated or should be managed as a new pest species(Suckling *et al.*, 2014; Bawin *et al.*, 2015).

Because *T. absoluta* has various alternative hosts, control of this pest can become challenging (Barros *et al.*, 2010; Sylla *et al.*, 2019). For example, since host plants have different phenologies there may be hosts available for extended periods of the year. Different host plants may also be cultivated during different seasons or in close proximity to one another (Barros *et al.*, 2010; Sylla *et al.*, 2019).

The presence of several host plant species in a particular environment enables movement of the pest between crops and expose a variety of crops to the pest, which can result in the selection of new host plants owing to availability of resources (Sylla *et al.*, 2019). These shifts in host plants can also involve changes in life history traits for instance oviposition behaviour, larval feeding behaviour and/ or digestive physiology (Dethier, 1954; Sylla *et al.*, 2019). This can be seen in conjunction with the 'trade-off' hypothesis which is related to 'preference–performance', indicating that female moths will oviposit on host plants most suited for their offspring, especially when they are not very mobile (Gripenberg *et al.*, 2010; Sylla *et al.*, 2019).

As derived from the common names for *T. absoluta, viz.* tomato leaf-miner, tomato pinworm or tomato borer, it is clear that the primary host plant of this pest is tomato. While tomato (*Solanum lycopersicum* L.) is the preferred host, *T. absoluta* can also develop on other cultivated and non-cultivated Solanaceae species such as potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), and black nightshade (*Solanum nigrum* L.). These host plant species can therefore also be attacked by this pest (Portakaldali *et al.*, 2013).

Alternative host plant families are not confined to Solanaceae, but also include the Convolvulaceae and Chenopodiaceae (Portakaldali *et al.*, 2013). This pest can therefore also use other plant species as alternative hosts when tomato plants are not available (Jaenike, 1978; Portakaldali *et al*., 2013; Sylla *et al.*, 2019).

These secondary host plants allow the pest to survive in an unfavorable habitat until tomato plants are in abundance again (Portakaldali *et al*., 2013). This is important because it directly affects the strategies implemented to manage a pest population (Portakaldali *et al.,* 2013). Common control strategies with insecticides have already been challenged by pesticide resistant insects (Lietti *et al*., 2005; Bawin *et al.*, 2016), as well as the feeding behaviour of larvae, which is inside the mesophyll between the outer layers of the leaf (Cocco *et al*., 2013; Bawin *et al.*, 2016).

Reservoir host plants can play an important role in the preservation and spread of invasive agricultural insect pests beyond their geographical range (Sylla *et al.*, 2019). This is especially applicable when the pest is polyphagous and when it is ecologically plastic and exhibits high reproductive fitness (Abbes *et al.*, 2016). This was demonstrated by Portakaldali *et al.* (2013), who reported that *T. absoluta* was able to survive on cultivated as well as non-cultivated plants located kilometers away from any tomato crops.

Nutritional value of host plants affects the survivorship, fecundity and growth of insects. A change in diet to cultivated host plants, as opposed to wild plants, generally results in a positive effect on herbivore growth due to the nutrient rich and concentrated food source (Pereyra and Sánchez, 2006). The reproductive fitness of *T. absoluta* reared on the weed species *S. nigrum* (black nightshade) and potato was also reported to be similar (Abbes *et al.*, 2016). However, Caparros Megido *et al.* (2014) reported the pre-imaginal development period (egg to pupa) to differ for *T. absoluta* reared on tomato and potato, although the survival rates were similar.

Weeds, especially those belonging to the Solanaceae, have the potential to act as reservoir hosts for *T. absoluta.* It can carry over populations when tomato and potato crops are not available (Abbes *et al.*, 2016). This was also the finding of Loni *et al*. (2011), who sampled *T. absoluta* from the wild host, *S. nigram* that grew near tomato green houses. *Tuta absoluta* is more likely to infest alternative host plants which occur adjacent to tomato fields (Bawin *et al.*, 2016).

Most of the reported non-cultivated host plants of *T. absoluta*, can, however, be found in several environments for instance wastelands, croplands and urban areas (Lambinon *et al*. 2004 cited in Bawin *et al.*, 2015). The aim of this study was to determine the feeding and oviposition preference and larval development of *T. absoluta* on wild host plants.

2.2 Material and Methods

Tuta absoluta rearing

Tuta absoluta were sampled from infested fields at Swartwater (-22.8592, 28.2072), in the Limpopo province. The population was maintained on tomato plants (var. Moneymaker) in rearing cages (0.8(H) × 0.6(L) × 0.5(W) m) under ambient temperature and humidity conditions at the North-West University, Potchefstroom.

To get suitable numbers of male and female moths for use in experiments, pupae were removed from the rearing cages and sexed according to the methods described for lepidopteran pupae (Clausen, 1931; Anton and Garrido, 1996; Genç, 2016). A tomato leafminer has 10 visible segments on the ventral side of the abdomen, with the cremaster visible on the 10th segment where the anal opening is situated (Genç, 2016). Female pupae have a longitudinal suture or slit in the middle of the 8th abdominal segment in the middle of two small tubercles. Male pupae have a slit in the middle of the 9th abdominal segment (Figure 2.1) (Genç, 2016). After sexing, the males and females were kept in separate containers.

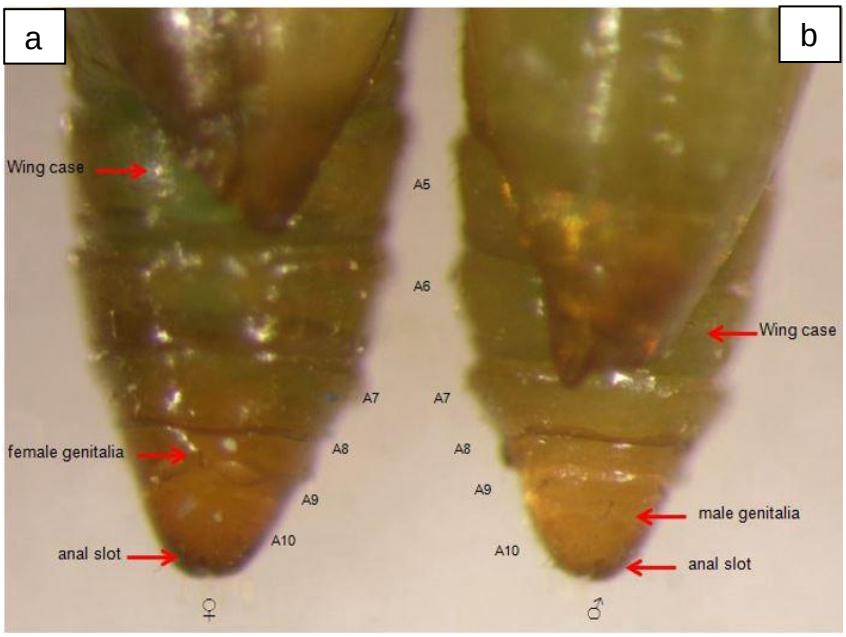

Figure 2.1: Characteristics of *Tuta absoluta* pupae used to distinguish between a) female and b) male pupae. Numbering indicate the abdominal segments, e.g. A10 = 10th abdominal segment (From Genç, 2016).

Host plants

Weed commonly occurring in South African tomato fields that was not studied before, were included in this study. Seed from *Amaranthus hybridus* (Cape pigweed) (Amaranthaceae), *Solanum scabrum* (Garden huckleberry/African nightshade) (Solanaceae) and *S. retroflexum* (Wonderberry) (Solanaceae) were obtained from the Plant Genetic Resources Unit, Agricultural Research Council, Roodeplaat, South Africa. Seed of the respective weed species were planted in pots with a volume of 20 L, which contained a 10:1 mixture of garden soil and vermiculite. Small seedlings of *Malva parviflora* (Malvaceae) and *Galinsoga parviflora* (Gallant soldier) (Asteraceae) were removed from uncultivated crop fields and transplanted into 500 mL pots that contained a 10:1 mixture of garden soil and vermiculite. The pots were kept under natural climatic conditions. Neither fertilizers nor pesticides were applied.

2.2.1 *Tuta absoluta* larval feeding preference

Choice tests (no-choice, two-choice and multi-choice tests) were conducted using the plant species listed in table 2.1. The weed species as well as the combinations used in the respective tests with *T. absoluta* larvae are provided in table 2.1. For use in the choice tests, the first completely expanded leaf was removed from plants of the respective weeds, when the plants had eight completely expanded leaves. Seedlings of tomato plants were used in this study and leaf material was removed from these plants when they had six to eight completely expanded leaves.

All the choice tests were conducted under controlled, laboratory conditions at 26 ± 1 °C, 65 ± 5 % RH and a 14L: 10D photoperiod. Second-instar larvae used in all the experiments were maintained on tomato plants (var. Moneymaker).

Table 2.1: Weed species and tomato in combinations used in the choice tests with *Tuta absoluta* second-instar larvae.

Weed species and combinations used in choice-tests		
No-choice	**Two-choice**	**Multi-choice**
Amaranthus hybridus (Amaranthaceae)		
Solanum scabrum (Solanaceae)	*S. scabrum* x *S. retroflexum* *S. scabrum* x *M. parviflora* *S. scabrum* x *G. parviflora* *S. scabrum* x *S. lycopersicum*	*S. scabrum* x *S. retroflexum* x *S. lycopersicum*
Solanum retroflexum (Solanaceae)	*S. retroflexum* x *M. parviflora* *S. retroflexum* x *G. parviflora* *S. retroflexum* x *S. scabrum* *S. retroflexum* x *S. lycopersicum*	
Malva parviflora (Malvaceae)	*M. parviflora* x *G. parviflora* *M. parviflora* x *S. scabrum* *M. parviflora* x *S. retroflexum* *M. parviflora* x *S. lycopersicum*	
Galinsoga parviflora (Asteraceae)	*G. parviflora* x *M. parviflora* *G. parviflora* x *S. scabrum* *G. parviflora* x *S. retroflexum* *G. parviflora* x *S. lycopersicum*	
Solanum lycopersicum (Solanaceae)	*S. lycopersicum* x *S. retroflexum* *S. lycopersicum* x *S. scabrum* *S. lycopersicum* x *M. parviflora* *S. lycopersicum* x *G. parviflora*	

2.2.1.1 No-choice test

A single disk of leaf material (3 cm diameter) from each of the plant species (Table 2.1), was placed in the center of a Petri dish (9 cm diameter) on a similar sized agar-agar disk (1.5 cm diameter, 3.0 mm thick).

The agar was used to keep the leaf material turgid for the duration of the experiment. One second-instar larva was placed next to the side of the Petri dish. The position of the larva in each Petri dish was recorded after 48 hours and each leaf disk was individually scanned with a smartphone (Samsung Note 8, Resolution: 4032 x 1960) to record the leaf area consumed per plant species, for foliar analysis using the BioLeaf application (http://bioleaftech.co.za/) (Machado *et al.,* 2016). The percentage leaf surface consumed was calculated (Figure 2.2). There were 30 replicates for each plant species.

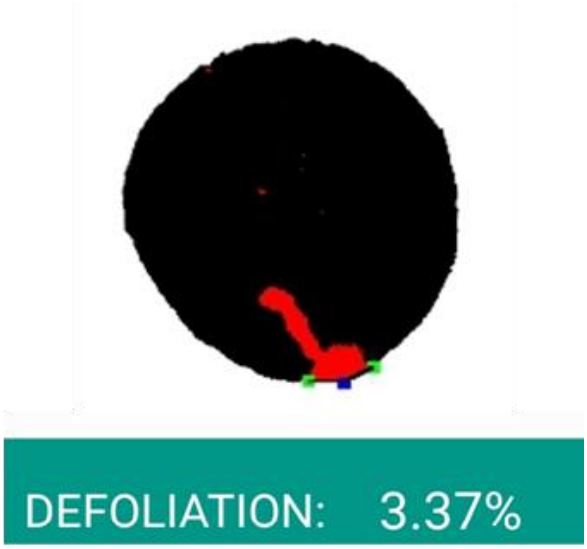

Figure 2.2: Percentage damage to a leaf disk calculated with the BioLeaf application. The damaged area is presented in red and calculated as percentage 'defoliation' of the leaf area.

2.2.1.2 Two-choice test

The same plant species used in the no-choice tests, were tested in different combinations in the two-choice tests (Table 2.1). *Amaranthus hybridus* was omitted from the two-choice tests, since *T. absoluta* larvae did not feed on this plant species in the no-choice tests. A single leaf disc (3 cm diameter) of each of the two plant species were placed on agar disks inside the Petri dishes. The leaf disks were placed next to the sides of the Petri dish, with a 3 cm spacing in between.

Three second-instar *T. absoluta* larvae were placed in the center of the Petri dish. The position the larvae was recorded on the leaf disks and feeding damage was calculated after 48 hours as described in 2.2.1.1. There were 10 replicates for each combination.

2.2.1.3 Multi-choice test

Only the plant species that were preferred in the two-choice tests, were included in the multi-choice tests (Table 2.1). These were tomato, *S. scabrum* and *S. reteroflexum.* Leaf discs from each of these species were placed in a single Petri dish, and replicated 10 times. The leaf discs were placed next to the sides of the Petri dish, with a 3 cm spacing between the respective discs. Three second-instar larvae were placed in the center of the Petri dish. The position of the larvae was recorded inside the Petri dish and feeding damage to the disks of each plant species was calculated after 48 hours as described in 2.2.1.1.

2.2.2 Oviposition preference tests

Oviposition preference of *T. absoluta* moths was investigated in no-choice and multi-choice tests with *A. hybridus, S. scabrum, S. reteroflexum, M. parviflora, G. parviflora* and tomato. Only one plant was placed per container in the no-choice test, and one plant of each of the three different plant species per container, in the multi-choice test. Only the plant species on which eggs were laid in the no-choice tests, were included in multi-choice tests. The size of the containers was 37(L) × 26(W) × 28(H) cm. The plants were placed approximately 20 cm apart and watered daily. There were 10 replicates per plant species in the no-choice test and 10 replicates per multi-choice combination. On the day of eclosion, six *T. absoluta* moths (3 males and 3 females) were released inside each container, that were kept under laboratory conditions in a controlled environment (25 ± 2 °C, 70 ± 10% RH).

All leaves from each plant were removed five days after the moths were released into the cages. The leaves were examined using a stereomicroscope (Nikon SMZ 1500) (100 X magnification) and the number of eggs per plant was recorded.

2.2.3 Development of *T. absoluta* larvae on host plants

Plants with *T. absoluta* eggs obtained from the oviposition preference test (see 2.2.2), were placed separately in plastic containers with gauze infused lids to allow for air flow. Each container served as a replicate. The containers were kept in a climate controlled chamber at 25 ± 1 °C, 65 ± 5% RH.

When the leaves of the seedlings were almost consumed by the feeding larvae or became dry, a fresh plant was introduced into the container. The previously infested seedlings were removed as soon as the new ones were colonized by the larvae. For each host plant species, the development stage (from L1 to pupae), was checked daily and noted. Pupal mass was determined 48h after pupation.

Statistical analysis

The proportion of *T. absoluta* larvae that made a choice in the different choice tests was calculated, and data were analyzed by means of binomial distribution tests. Bonferroni correction was used to adjust for multi means comparisons in the no-choice and multi-choice tests, while choices made in the two-choice tests were tested against a 50% preference ratio. Data for all choice tests were compared between plant species after 48 hours. Data on the percentage leaf disk consumption in no-choice-, two-choice– and multi-choice tests were tested for homogeneity of variance (Levene's test) and normality (Shapiro-Wilk test), but did not met these assumptions and were analyzed by means of the non-parametric, Kruskal-Wallis test followed by Duncan's multiple comparison post hoc test. The mean number of eggs laid by *T. absoluta* females on the respective host plant species did also not meet these assumptions of normality and homogeneity and were analyzed of by means of the non-parametric, Kruskal-Wallis test followed by Duncan's multiple comparison post hoc test. Data for mean larval and pupal development time was not normally distributed, but homogenous and were therefore analysed by means of Welch's ANOVA followed by Tukey HSD test.

2. 3 Results

2.3.1 Feeding preference of *Tuta absoluta*

2.3.1.1 No-choice test

In the no-choice tests, *T. absoluta* larvae fed on all plant species, except on *A. hybridus*. There was, however, no significant difference in preference for *S. scabrum*, *S. retroflexum* and *S. lycopersicum* (tomato).

The latter three plant species were significantly more preferred compared to *M. parviflora* and *G. parviflora* (Figure 2.3), with no preference for *A. hybridus* by the larvae. Larval survival on *M. parviflora* and *G. parviflora* was low.

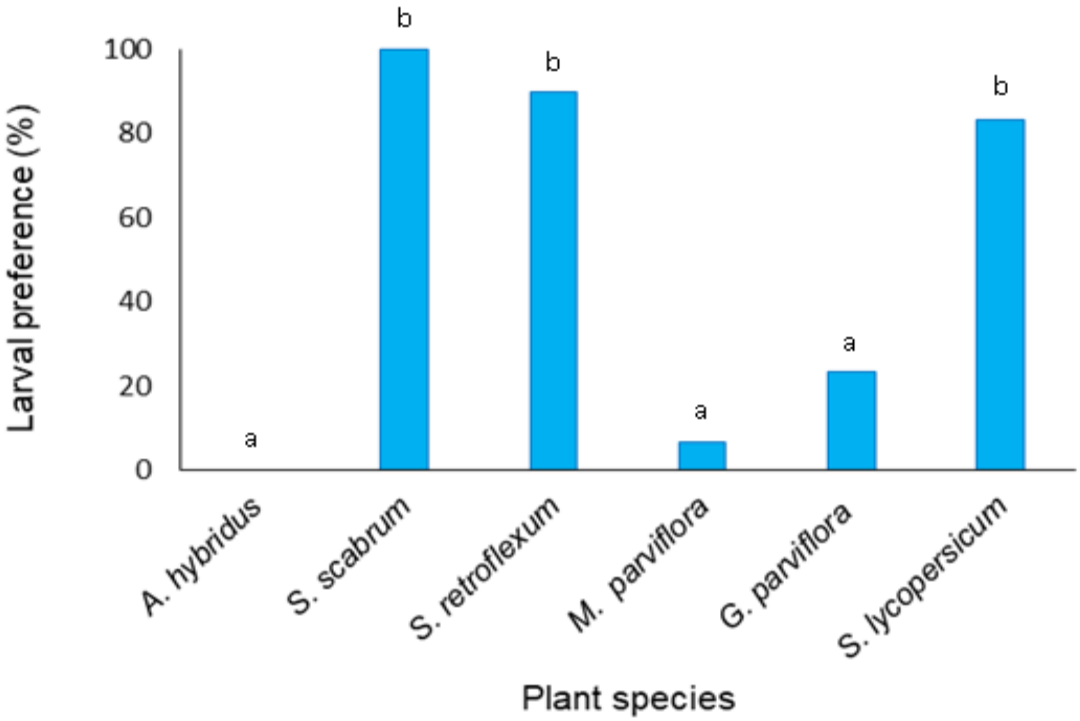

Figure 2.3: *Tuta absoluta* larval preference for selected plant species in a no-choice test after 48 hours. Bars capped with the same letter do not differ significantly (Binomial distribution tests followed by Bonferroni correction at $P < 0.05$).

The percentage of leaf area consumed in the respective no-choice tests is provided in table 2.2. Significantly more of the leaf area of *S. scabrum*, *S. reteroflexum* and *S. lycopersicum* was consumed, compared to *A. hybridus, M. parviflora* and *G. parviflora*. The larvae did not feed on *A. hybridus*.

Table 2.2: Estimated mean percentage leaf area (±SE) of different host plants consumed by second-instar *Tuta absoluta* larvae (n = 30) over a 48h period under no-choice conditions.

Treatment	Mean % leaf area consumed ± SE
Amaranthus hybridus	0 ± 0b
Solanum scabrum	1.935 ± 0.356a
Solanum reteroflexum	1.854 ± 0.308a
Malva parviflora	0.600 ± 0.210b
Galinsoga parviflora	0.270 ± 0.074b
Solanum lycopersicum	6.556 ± 1.941a
	H (5, N= 180) =121,626; P < 0.001

Means within the column followed by the same letter are not significantly different (Duncan's multiple comparison test at P < 0.05)

2.3.1.2 Two-choice test

When two plant species were provided together in the two-choice tests, only Solanaceous species were preferred (Figure 2.4), with no significant differences in preference among these species (Table 2.3).

Table 2.3: Estimated mean percentage leaf area (±SE) consumed over a 48h period by second-instar *Tuta absoluta* larvae (n = 30) in two-choice tests.

Treatment	Mean % leaf area consumed ± SE
Solanum scabrum	5.766 ± 0.862a
Solanum reteroflexum	9.507 ± 1.617a
Malva parviflora	0 ± 0b
Galinsoga parviflora	0 ± 0b
Solanum lycopersicum	15.870 ± 2.198 a
	H (4, N= 200) = 148,896; P < 0.011

Means within the column followed by the same letter are not significantly different (Duncan's multiple comparison test at P < 0.05)

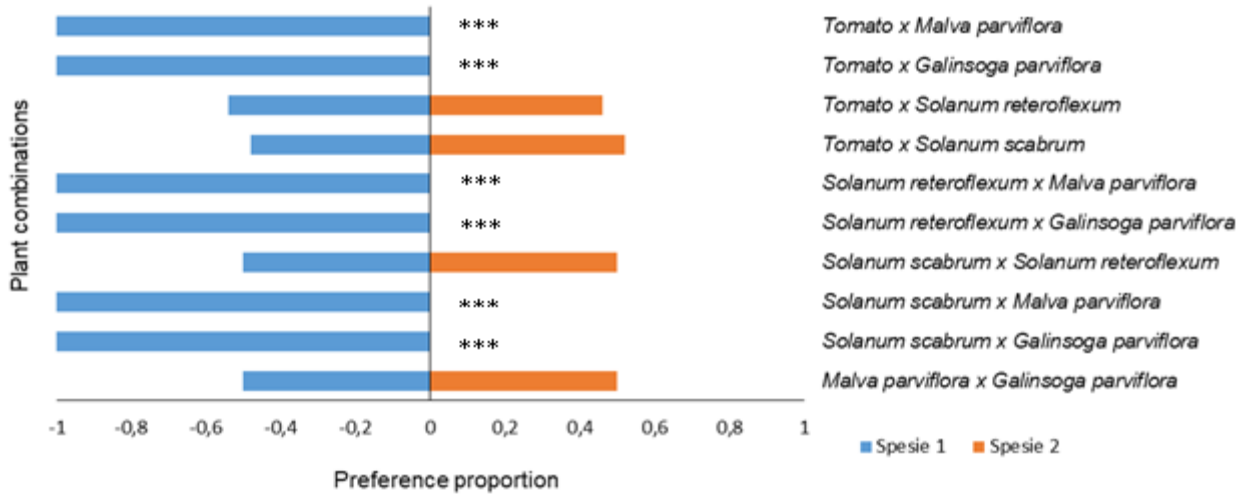

Figure 2.4: *Tuta absoluta* larval preference for leaf tissue of different host plant species in two-choice tests after 48 hours. (Binomial distribution tests followed by Bonferroni correction at P < 0.05). Significance indicated by *** P < 0.001.

2.3.1.3 Multi-choice bioassay

In the multi-choice experiment using second-instar larvae, an equal feeding preference was demonstrated for tomato, *S. scabrum* and *S. reteroflexum*, 48 hours after inoculation (Figure 2.5). Although a much smaller *S. scabrum* leaf area was consumed, larval consumption of the leaves of the three *Solanum* sp. did not differ significantly (Table 2.4).

Table 2.4 Estimated mean percentage leaf area (±SE) consumed by second-instar *Tuta absoluta* larvae (n = 20) in multi-choice tests 48 hours after inoculation.

Treatment	Mean % leaf area consumed ± SE
Solanum scabrum	0.71 ± 0.33ac
Solanum reteroflexum	5.55 ± 1.21ab
Solanum lycopersicum	4.18 ± 1.41a
	H (2, N= 30) = 8,183 P < 0,02)

Means within the column followed by the same letter are not significantly different (Duncan's multiple comparison test at P < 0.05).

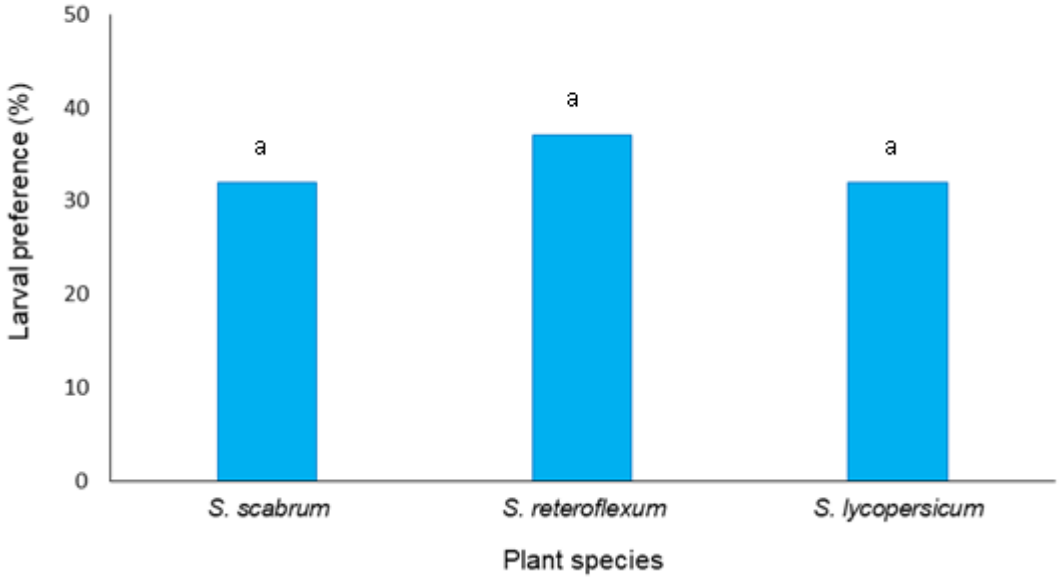

Figure 2.5: *Tuta absoluta* larval preference for leaf tissue of different host plant species in multi-choice tests after 48 hours. (Binomial distribution tests followed by Bonferroni correction at $P < 0.05$). Bars capped with the same letter do not differ significantly.

2.3.2 Oviposition preference of *Tuta absoluta*

2.3.2.1 No-choice oviposition tests

The number of eggs per plant in the no-choice assays did not differ significantly between *S. lycopersicum*, *S. scabrum* and *S. reteroflexum*, but it differed significantly from *G. parviflora, M. parviflora* and *A. hybridus* (Figure 2.6). No eggs were laid on *M. parviflora* and *A. hybridus*.

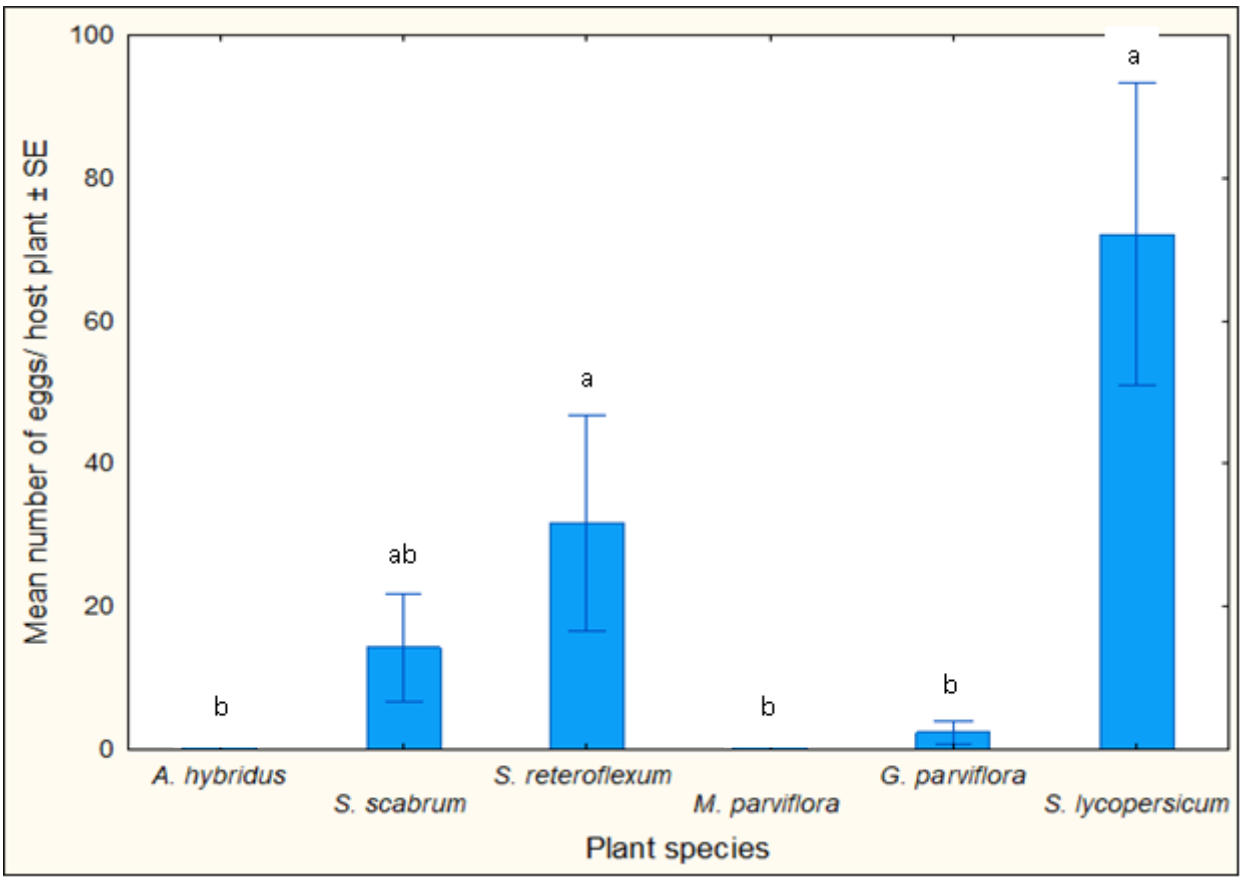

Figure 2.6: Mean number of eggs (±SE) laid by *Tuta absoluta* on different host plants species in no-choice tests after 48 hours. Bars capped with the same letter do not differ significantly at P < 0.05 (Duncan's multiple comparison test).

2.3.2.2 Multi-choice oviposition test

The oviposition preference of females in the multi-choice test varied depending on the host plant species. The number of eggs laid on tomato plants was significantly higher than those on *S. scabrum* and *S. reteroflexum,* which was similar (Figure 2.7). No eggs were laid on *G. parviflora*.

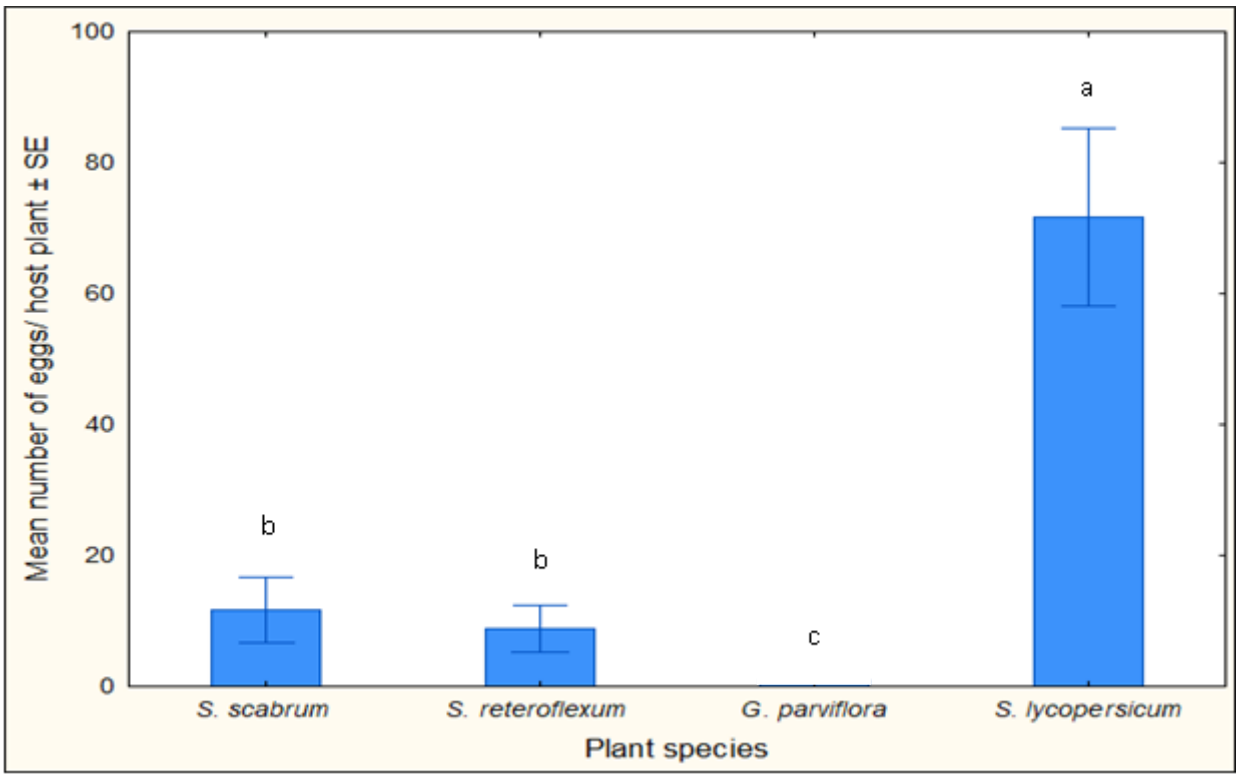

Figure 2.7: Mean number of *Tuta absoluta* eggs (±SE) laid on different host species in multi-choice tests. Bars capped with the same letter do not differ significantly at P ≤ 0.05. (Duncan's multiple comparison).

2.3.3 Development of *T. absoluta* larvae on host plants

First and second-instar *T. absoluta* larvae developed at an almost similar rate on *S. scabrum* and *S. reteroflexum* and *S. lycopersicum* (Table 2.5). Third-instar larvae, did, however, develop significantly faster on *S. scabrum* and *S. reteroflexum* compared to *S. lycopersicum.* Development of fourth-instar larvae was also significantly more rapid on *S. scabrum* than on *S. lycopersicum* (Table 2.5). Mean development time of *T. absoluta* pupae from larvae that fed on the two weed species was, however, significantly longer than pupae of larvae that fed on tomato (Table 2.5). The mean mass of pupae from the larvae that fed on *S. scabrum* and *S. reteroflexum* was similar, but these pupae were significantly heavier than pupae from larvae that fed on *S. lycopersicum* (Table 2.6).

Table 2.5: Mean development time (days±SE) of *Tuta absoluta* larvae that fed on different host plants, as well as pupae from these larvae.

Treatment	Mean development period (days)				
	Larvae				Pupae
	1st instar	2nd instar	3rd instar	4th instar	
Solanum scabrum	3.90 ± 0.28a	3.90 ± 0.23a	5.60 ± 0.58b	4.30 ± 0.42b	9.60 ± 0.50b
Solanum reteroflexum	4.00 ± 0.21a	3.80 ± 0.25a	4.40 ± 0.27b	5.00 ± 0.33b	9.60 ± 0.45b
Solanum lycopersicum	3.70 ± 0.15a	3.40 ± 0.16a	7.80 ± 0.36a	6.50 ± 0.37a	7.30 ± 0.30a
F-statistic	0.69	1.82	27.79	8.08	12.59
df	2; 17.04	2; 17.31	2; 16.73	2; 17.84	2; 17.05
P-value	0.52	0.19	< 0.0001	< 0.01	< 0.001

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

Table 2.6: Mean *Tuta absoluta* pupal mass (mg) (±SE) from larvae that fed on different host plants under controlled conditions.

Treatment	Mean pupal mass (mg) after 48h
Solanum scabrum	2.97 ± 0.152b
Solanum reteroflexum	2.81 ± 0.299b
Solanum lycopersicum	1.86 ± 0.232a
	$F_{(2, 42)}$=8.332; P < 0.001

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

2.4 Discussion

This is the first study to report the suitability of *S. scabrum* and *S. reteroflexum* as alternative hosts for *T. absoluta*. In this study, *T. absoluta* larvae preferred plant species belonging to the Solanaceae, *viz*. *S. scabrum*, *S. reteroflexum* and *S. lycopersicum* significantly more than plants belonging to the Amaranthaceae, Malvaceae and Asteraceae, *viz*. *A. hybridus, M. parviflora*, and *G. parviflora,* respectively. *Tuta absoluta* larvae did not develop on *M. parviflora*, *A. hybridus* and *G. parviflora*. *Tuta absoluta* larvae also consumed the largest leaf areas from the solanaceous species. Larvae did not prefer and consume any leaf material of *M. parviflora* and *G. parviflora* and did also not feed on these species when provided in combination with tomato, *S. scabrum* or *S. reteroflexum*. When leaf material of tomato, *S. scabrum* and *S. reteroflexum* plants were provided in combination in a multi-choice test, preference for and leaf area of these plant species consumed by the larvae, did not differ significantly. These host plants may therefore permit populations of this pest to be sustained in areas where the primary host does not occur abundantly, similar to what Tonnang *et al*. (2015) and Sylla *et al*. (2019) reported for other wild host plant species.

No eggs were laid on *M. parviflora* and *A. hybridus* plants but a few eggs were laid on *G. parviflora*. Neonate larvae that emerged from these eggs did, however die within 48 hours after emergence on *G. parviflora.* These larvae did not tunnel into the leaves and no galleries were therefore present on *G*. *parviflora* leaves. Differences in oviposition and feeding preference may indicate potential variation in the host range of *T. absoluta* (Sylla *et al.*, 2019). The suitability of solanaceous plants as host plants for *T. absoluta* was apparent from their oviposition and feeding preference. The most preferred host plant for oviposition, was the primary host, tomato. Despite the fact that the moths preferred tomato for oviposition, the alternative hosts *S. scabrum* and *S. reteroflexum* were equally preferred, and fed upon compared to tomato, by *T. absoluta* larvae.

The development time of *T. absoluta* larvae on the secondary hosts, *S. scabrum* and *S. reteroflexum,* did not differ from that on tomato. These species are therefore suitable for development of this pest, since development time was not significantly affected. The pupal mass of larvae that developed on tomato was less than those that developed from larvae that fed on *S. scabrum* and *S. reteroflexum.* Successful development and the high level of preference of *T. absoluta* larvae for *S. scabrum* and *S. reteroflexum* suggest that these species may serve as alternative hosts when tomato crops are unavailable or when infested tomato fields cannot sustain the population.

In this study, *T. absoluta* females laid eggs on *G. parviflora.* Larvae could, however, not survive and develop on this plant species. This incompatibility between adult preference and larval development is not uncommon (Hilker and Fatouros, 2015; Arnó *et al.*, 2019), and has also been reported for other Lepidoptera – plant associations (Arnó *et al.,* 2019). For example, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is highly attracted to *Barbarea vulgaris* (R. Br.) for oviposition, even though larval development cannot be sustained. It therefore acts as an 'dead end' trap crop for this particular pest (Shelton and Badenes-Perez, 2006; Arnó *et al.*, 2019).

Knowledge of potential host plants of the tomato leafminer, is important for its control. To avoid growth and spread of *T. absoluta*, crop rotation and weed control approaches should be incorporated together with other IPM strategies (Portakaldali *et al*., 2013). Weed species that could act as host plants for *T. absoluta* should be removed as a precautionary method (Portakaldali *et al.*, 2013). The results of the laboratory tests in this study indicated that the two solanaceous species, *S. scabrum* and *S. reteroflexum,* have the potential to serve as both developmental and reproductive hosts for *T. absoluta*. Previous studies documented that nightshade species such as *S. nigrum* (Garcia and Espul 1982; Desneux *et al*., 2010) can sustain development of *T. absoluta* and may therefore act as an alternative host species (Arnó *et al.,* 2019).

Migration of *T. absoluta* moths between crops and alternative hosts can have a significant influence on the management of this pest (Silva *et al.*, 2017). Additionally the coexistence between crops and weeds in the agro-ecosystem can initiate new feeding preferences in the absence of the preferred host (Barros *et al.*, 2010; Silva *et al.*, 2017). Wild plants together with home gardens can contribute to the ongoing risk of spread and establishment of *T. absoluta* (Arnó *et al.,* 2019).

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Chapter 3: Susceptibility of *Tuta absoluta* to four insecticides

Abstract

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a devastating tomato pest which is mainly controlled with insecticides. The pest has already developed resistance towards insecticides in various countries. A number of insecticides are registered in South Africa for control of *T. absoluta*, but its susceptibility to these insecticides is unknown. The leaf dip bioassay Method 022 adopted by IRAC was used in this study to estimate baseline toxicity of selected insecticides for *T. absoluta* populations from Mareetsane (North-West province), Polokwane (Limpopo province) and Swartwater (Limpopo province) in South Africa. The potential for control failure of insecticides was also determined. The insecticides used were indoxacarb, emamectin benzoate, spinetoram and lufenuron. All three *T. absoluta* populations were highly susceptible to these active ingredients. Low variability in the responses of *T. absoluta* to indoxacarb, emamectin benzoate and spinetoram from all three localities were recorded with differences in LC_{50} values below 2.5 fold. No control failure with these active ingredients are therefore expected. A significantly lower susceptibility to lufenuron was found for the Polokwane population, compared to the populations from Swartwater and Mareetsane. This population was 47 times less susceptible than the Swartwater and 18 times less than the population from Mareetsane. All three populations were, however, susceptible to lufenuron and, although not registered for control of *T. absoluta* in South Africa, effective control will be achieved when it is applied at the registered dosage rate for control of potato tuber moth. Baseline data determined in this study, can be used in future resistance monitoring of *T. absoluta* to indoxacarb, emamectin benzoate, spinetoram and lufenuron in South Africa.

3.1 Introduction

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a major pest of tomato (*Solanum lycopersicum* L.) crops, in open fields as well as in greenhouses (Desneux *et al*., 2010).

Control of *T. absoluta* is problematic under both these cultivation methods in the Mediterranean countries (Roditakis *et al.,* 2013a). This has been ascribed to high infestation pressure (Roditakis *et al*., 2013b), the multi-voltinism of the insect, with 10-12 continuous generations per annum, and females which can lay approximately 260 eggs during their lifespan (Roditakis *et al.,* 2013a).

Insecticides are key components of pest management systems (Roditakis *et al*., 2018), and chemical control is also the most common method used for suppression of *T. absoluta* (Roditakis *et al*., 2013a).

The number of cases of resistance to pesticides, increased as a result of extensive and repeated use of these chemicals (Sparks and Nauen, 2015). There were more than 15 000 cases of insecticide resistance and almost 500 cases for weed and plant diseases, respectively reported by Sparks and Lorsbach (2017). Roditakis *et al.* (2013a) emphasized that the rate at which *T. absoluta* evolved resistance to insecticides, was a major concern for control of this pest. Many cases of resistance to insecticides were reported for *T. absoluta* by 2015, due to the misuse of insecticides particularly on tomato crops (Campos *et al.*, 2015). Resistance of *T. absoluta* to abamectin, cartap, methamidophos and permethrin was reported in Brazil (Siqueira *et al*., 2000; Silva *et al.*, 2011), and to organophosphates and pyrethroids in Chile (Salazar and Araya, 1997; Silva *et al.*, 2011). Although cases of resistance were also reported to indoxacarb in Brazil (Silva *et al*., 2011; Michaelides *et al.,* 2019) and Italy (Roditakis *et al*., 2013a), no resistance to chlorantraniliprole, flubendiamide, emamectin benzoate, spinosad or indoxacarb was found in populations from Greece by 2011 (Roditakis *et al*., 2013b). However, soon after, resistance to diamides in Italy was reported by Roditakis *et al*. (2015) and in Brazil by Silva *et al*. (2016). By 2016, resistance was also reported to emamectin benzoate in Italy and Greece, and to spinosad in the UK (Roditakis *et al*., 2018).

When *T. absoluta* is mainly controlled with insecticides, the threat of natural selection for resistance to these insecticides increases (Guedes *et al.*, 2019). Cifuentes *et al*. (2011) reported that a single genetically uniform and invasive population with high insecticide resistance, spread through South America and the Mediterranean basin. Idriss (2019) also reported a high level of genetic homogeneity in *T. absoluta* populations from Uganda, Senegal and Tanzania. It is not known from where the populations that initially invaded South Africa, originated. The status of susceptibility of these populations to insecticides is therefore also unknown.

With the invasion of *T. absoluta* into South Africa, the Department of Agriculture, Forestry and Fisheries (DAFF) granted emergency registration of insecticides for control of this pest. These insecticides belong to 7 Insecticide Resistance Action Committee (IRAC), Mode of Action (MoA) groups (DAFF, 2017). Four of these insecticides are indoxacarb, emamectin benzoate, spinetoram and lufenuron (DAFF, 2017)

Indoxacarb is a broad-spectrum insecticide for control of pests in 10 insect orders and over 30 families, and is especially effective against lepidopteran pests such as cutworms, armyworms and loopers (Wing *et al*., 1998; Bird, 2015). Indoxacarb is an oxadiazine that acts as a voltage dependent sodium channel blocker, in MoA group 22A (IRAC, 2020). This compound acts on the movement of sodium ions in the nerve cells, blocking the action potential in the voltage gates of sodium channels (IRAC, 2020). The insecticide is primarily taken in through ingestion, but absorption through the cuticle is also possible (Lasota, 1991; Bird, 2015).

Emamectin benzoate, is a semisynthetic, second-generation insecticide from the family of avermectins and it is classified in MoA group 6 (IRAC, 2020). Emamectin benzoate causes irreversible activation of the chloride channels of the nervous system of insects (Sial and Brunner, 2010). It causes neuronal as well as muscular system malfunctions (Ishaaya *et al*., 2002), preventing muscle contraction and causing cessation of feeding and death (Sial and Brunner, 2010).

Spinetoram is a broad spectrum insecticide with activity against numerous pest species such as whiteflies, leafrollers, thrips and leafminer flies (Sparks *et al*., 1998; Bacci *et al.*, 2016). Spinosyns, which include spinosad and spinetoram belong to MoA group 5 (IRAC, 2020). This group of insecticides affects the nicotinic acetylcholine receptors and γ-aminobutyric acid (GABA) receptors on the postsynaptic membranes in insect nervous systems causing abnormal neural transmission (Salgado and Sparks, 2005; Bacci *et al.*, 2016).

Lufenuron is classified in group 15 of the MoA classification (IRAC, 2020), which is inhibitors of chitin biosynthesis. A chitin synthesis inhibitor (CSI) acts on the integration of N-acetyl glucosamine monomer into chitin in the integument (Nakagawa *et al*., 1996; Islam *et al*., 2015), resulting in unsuccessful moulting and death of the insect (Cohen, 2001). Chitin synthesis inhibitors largely act as larvicides and ovicides for most insect species, but their main activity is against larvae (Cohen, 2001). A key component of insecticide resistance management (IRM) is to determine baseline toxicity of insecticides to a pest (Roditakis *et al*., 2012b).

It is also important to address insecticide resistance in pest populations and to be able to achieve this, the presence of insecticide-resistant strains should be reliably detected (Sparks and Nauen, 2015). The IRAC recommends the use of a leaf dipping method (Method 022) in toxicological bioassays to test the susceptibility of *T. absoluta* to insecticides (Roditakis *et al.*, 2013a;b)

The aim of this study was to estimate the base line susceptibility of *T. absoluta* populations to indoxacarb, emamectin benzoate, spinetoram and lufenuron, from three tomato production areas in South Africa.

3.2 Material and Methods

Tuta absoluta populations

Tuta absoluta populations were collected from infested tomato crops in three tomato production areas of South Africa during 2019, *viz*. Mareetsane (-26.697200, 25.440079), North-West province, Polokwane (-23.8962, 29.4486), and Swartwater (-22.8592, 28.2072), Limpopo province, South Africa (Fig. 1).

At each sampling site approximately 250 larvae were collected from tomato leaves infested with *T. absoluta*. The infested leaves were placed in insect-proof rearing cages (75 cm (L) x 75 cm (W) and 80 cm (H)), containing three insect-free potted tomato plants. The cages were kept in a laboratory at 26 ± 1 ºC, 65 ± 2% relative humidity (RH) and a 14L:10D photoperiod. When the sampled leaves desiccated, larvae moved to the potted plants where they resumed their development.

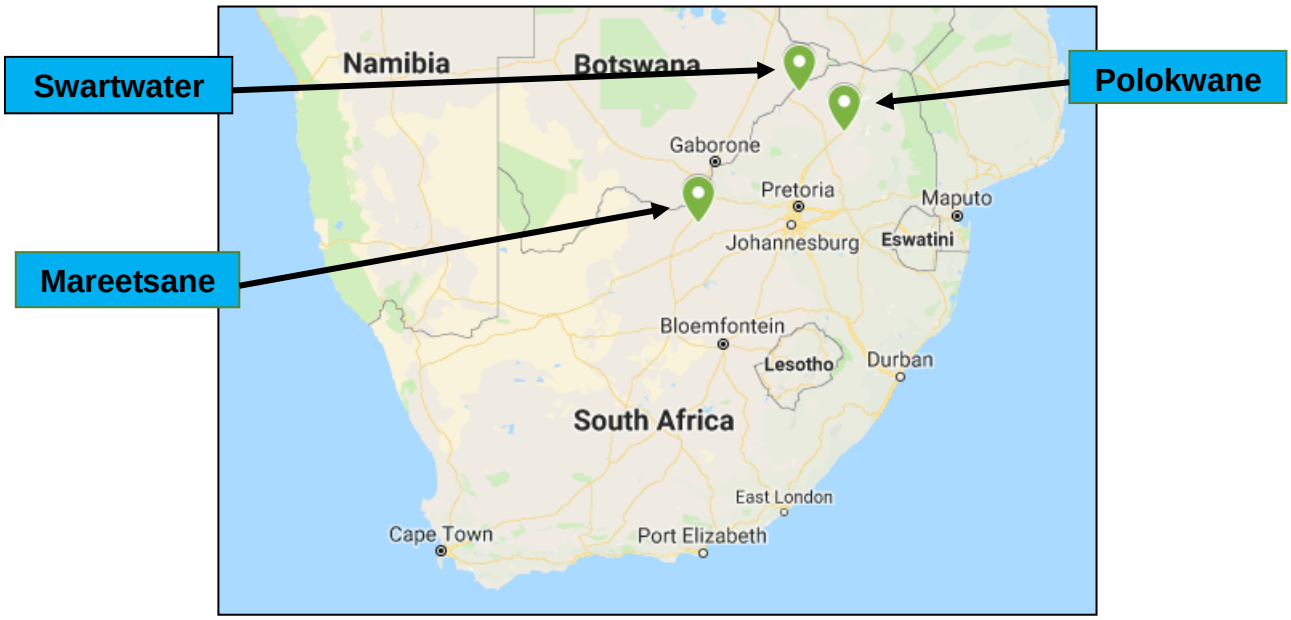

Figure 3.1: Sites where *Tuta absoluta* populations were sampled on tomato crops in South Africa.

3.2.1 Susceptibility testing

Baseline susceptibility of the three *T. absoluta* populations to emamectin benzoate, spinetoram, indoxacarb and lufenuron, was estimated by means of recording the responses of larvae in susceptibility tests. The procedures were the following:

3.2.1.1 Plant material

Leaves of tomato plants (cv. Moneymaker) were used in the bioassays. Plants were maintained insect free in pots kept in a 3 x 3 m insect-proof cage under ambient conditions with no insecticides applied onto these plants.

3.2.1.2 Insecticides

Commercial formulations, provided by the respective companies, were used for susceptibility testing of *T. absoluta* to emamectin benzoate (Proclaim®, Syngenta), spinetoram (Delegate™, Dow AgroSciences), indoxacarb (Steward 30WG, DuPont) and lufenuron (Sorba®, Syngenta).

3.2.1.3 Bioassay method

Tuta absoluta moths, reared from the field collected larvae (F0), were sampled from the rearing cages and transferred to oviposition cages (similar to the rearing cages described above), and allowed to lay eggs on insect-free plants. Larvae that hatched from these eggs (F1), were reared to the second instar (L2) (4-5 mm in size) for use in susceptibility bioassays.

The standard bioassay method recommended by IRAC (IRAC method no. 22) was used. A stock solution (1 L) was prepared from which seven serial dilutions (250 mL) were prepared. Triton X-100, a non-ionic surfactant, was added to the respective dilutions (0.25 ml L^{-1}) to obtain optimal leaf coverage. The dilution range used was determined through rangefinder assays conducted 3-4 days prior to the toxicological experiments. The range of dosages was sufficient to obtain six points on a regression line between the LC_{20} and LC_{80}. A higher concentration was also included where 100% mortality was expected. The concentration range used for the Mareetsane, Polokwane and Swartwater populations were as follows: emamectin benzoate (0.01 - 0.85 mg L^{-1}), spinetoram (0.01 - 0.85 mg L^{-1}), indoxacarb (1.5 - 13.3 mg L^{-1}) and lufenuron (0.01 - 4.28 mg L^{-1}).

Assays were performed in 32-well bioassay trays (Frontier: Scientific services, Newark, Delaware, United States). Tender, young, uninfested leaflets of uniform size were immersed individually in the insecticide aqueous solutions.

The leaflets were immersed for five seconds with gentle agitation to ensure that the entire surface was equally covered. The treatments (dosage rates) per insecticide solution were as follows: seven serial concentrations and an untreated control treatment consisting of water and 0.025% Triton X-100.

The leaflets were then left to air dry on a wired net with the adaxial leaf surface facing upwards. A 2.8% agar-agar solution was prepared and 5 mL was poured onto the base of each well of the bioassay trays to ensure that the leaf material remained turgid throughout the bioassay period. After the agar was set, treated air dried leaflets were placed individually per well of the labeled bioassay trays, onto the agar. One L2 *T. absoluta* larva was placed onto the treated tomato leaflet in each well, by mean of a fine artist brush. Bioassay trays were firmly sealed with transparent ventilated adhesive lids (Frontier: Scientific services), and kept at 26 ± 1°C, 60-70% RH, and a 14L:10D photoperiod.

Each concentration was tested in three replicates and 24 larvae were used per replicate, corresponding to 72 larvae per concentration. A fresh stock solution was prepared for each replicate of each bioassay and used immediately after preparation. Mortality assessments were done 72 hours after inoculation of larvae for emamectin benzoate, indoxacarb and spinetoram and after 96 hours for lufenuron by gently touching the larvae with a fine brush. Larvae which were unable to respond were considered dead.

3.2.2 Data analysis

Mortality was expressed as percentage of insects at each concentration. Control mortalities were corrected for using Abbott's formula (Abbott, 1925). Mortality data from the respective dose–response bioassays were subjected to probit analysis using POLO SUITE® (LeOra software). The latter software tests the linearity of the dose–mortality response and determines the slope, lethal concentrations (LCs) and the 95% confidence limits (CL) of the LCs for each mortality line. Outliers causing a lack of fit were identified and removed after studying a plot of the residuals. Residuals outside the -2 and +2 boundaries indicated these outliers. The relative potency ratio among responses was also estimated. Responses were considered significantly different when the 95% confidence interval of the relative potency ratio did not include the value 1. The likelihood of control failure with an insecticide was calculated according to Guedes (2017) as follows: control failure likelihood (CFL) = 100 - [achieved mortality (%) x 100]/expected mortality (%) (e.g. 80%). The likelihood of insecticide control failure was estimated by comparing the estimated LC_{80} and LC_{95} to the maximum recommended label rate (RLR_{max}) according to Silva *et al*. (2011) and Roditakis *et al*. (2013b).

The maximum recommended label rates for control of *T. absoluta* in South Africa for the insecticides used in this study were: emamectin benzoate 50 mg L^{-1}, spinetoram 100 mg L^{-1} and indoxacarb 90 mg L^{-1}. Lufenuron is not registered against *T. absoluta*, but it is applied against potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) on tomato crops at a maximum recommended label rate of 40 mg L^{-1}. (Labels of the respective insecticides available at https://www.agri-intel.com/label-information /search-registration-information/).

3.3 Results

Results from the probit analyses of the response of *T. absoluta* larvae in the susceptibility tests are provided in Table 3.1. The responses of the tested populations to all insecticides were homogeneous and fitted the log (dose)/probit (mortality) model.

Indoxacarb

With regard to the responses of the three populations to the respective insecticides, the steepest slopes were found in exposure response to indoxacarb. The slopes ranged from 2.36 to 3.41. The LC50 for indoxacarb ranged from 1.96 to 3.47 mg L^{-1} (Table 3.1). The variability of responses of the respective populations were therefore very low. Only a 1.77 fold difference between the Mareetsane and Swartwater populations was exhibited, representing the most and least susceptible populations to indoxacarb. The LC_{50} of the Mareetsane population was, however, significantly lower compared to the Polokwane and Swartwater populations ($P<0.05$).

The LC_{80} and LC_{95} were lower than the recommended label rate for indoxacarb (90 mg L^{-1}), suggesting that this insecticide would provide the expected control of *T. absoluta* populations at all three localities. Mortality at the recommended label rate was estimated at 100%.

Emamectin benzoate

The slopes of the response lines to emamectin benzoate ranged from 1.16 to 1.34 (Table 3.1). The LC_{50} ranged from 0.02 to 0.05 mg L^{-1}, indicating a 0.03 mg L^{-1} (or 2.5 fold) difference in susceptibility to emamectin benzoate between the population from Mareetsane and the two populations from Polokwane and Swartwater, respectively. The response of the Mareetsane population differed significantly from the Polokwane and Swartwater populations ($P < 0.05$), with the Swartwater population exhibiting the steepest slope of 1.34. The low variability in LC_{50} and overlapping of the confidence limits between the Mareetsane, Polokwane and Swartwater populations suggest low variability in terms of susceptibility to emamectin benzoate between these populations.

The recommended label rate (50 mg L^{-1}) was higher than the LC_{80} and LC_{95} for the Mareetsane, Polokwane and Swartwater populations and will, therefore result in 100% mortality based on the estimations of the probit model.

Spinetoram

The LC_{50} values (0.02 to 0.04 mg L^{-1}) of all three *T. absoluta* populations for spinetoram, were low, with a twofold difference in susceptibility between the Polokwane and Mareetsane populations, with the Polokwane population being the most susceptible. The slopes of these regression lines were also small and ranged between 0.80 and 1.16, which is lower than the slopes obtained with indoxacarb (Table 3.1). The small difference in LC_{50} and extensive overlapping of the confidence limits for the Mareetsane, Polokwane and Swartwater populations, suggest low variability in terms of susceptibility to spinetoram between these populations. There were no significant differences between the LC_{50} values of the three different populations ($P > 0.05$). The LC_{80} and LC_{95} were much lower than the recommended label rate and no control failure is therefore expected.

Lufenuron

Slopes of the regression lines for lufenuron ranged from 0.67 to 2.12 (Table 3.1). The LC_{50} for lufenuron was highly variable and ranged between 0.01 and 0.47 mg L^{-1}. High variability in terms of susceptibility therefore occurred between the populations, with the Polokwane population being 47 times less susceptible than the Swartwater and 18 less than the Mareetsane population. The three populations differed significantly in their susceptibility to lufenuron. The recommended label rate for lufenuron (200 mg L^{-1}) for lepidopteran pests on tomato is, however, much higher. This suggests that effective control of *T. absoluta* will be obtained with lufenuron (when this insecticide is applied for control of potato tuber moth), at all three localities.

Table 3.1: Log dose probit mortality data for *Tuta absoluta* populations from three localities in South Africa for emamectin benzoate, spinetoram, indoxacarb and lufenuron.

Population	n	LC_{50}	CL95%	LC_{80}	CL95%	LC95	Slope	SE	χ^2	df
Indoxacarb										
Mareetsane	360	1.96	1.38-2.51	3.69	2.91-4.73	6.77	3.05	0.3	2.56	3
Polokwane	360	2.92	1.80-3.86	6.64	5.06-10.36	14.54	2.36	0.23	3.95	3
Swartwater	432	3.47	2.83-4.08	6.13	5.24-7.43	10.54	3.41	0.29	4.46	4
Emamectin benzoate										
Mareetsane	288	0.05	0.02-0.09	0.24	0.13-0.54	1.14	1.18	0.13	1.37	2
Polokwane	360	0.02	0.01-0.04	0.1	0.05-0.24	0.52	1.16	0.11	4.20	3
Swartwater	432	0.02	0.01-0.02	0.07	0.06- 0.10	0.29	1.34	0.19	2.04	4
Spinetoram										
Mareetsane	288	0.04	0.02-0.06	0.19	0.11-0.43	0.94	1.16	0.13	1.15	2
Polokwane	360	0.02	0.01-0.04	0.26	0.18-0.38	2.60	0.80	0.10	0.69	3
Swartwater	432	0.03	0.01-0.06	0.35	0.20-0.78	3.47	0.81	0.10	4.37	4
Lufenuron										
Mareetsane	288	0.18	0.10-0.24	0.44	0.32-0.96	1.06	2.12	0.31	1.38	2
Polokwane	288	0.47	0.05-1.27	4.39	1.56-197.73	37.06	0.87	0.13	2.34	2
Swartwater	288	0.01	0.00-0.03	0.13	0.02-0.79	2.15	0.67	0.11	1.81	2

Table 3.2: Confidence intervals (95%) of the relative potency ratios at the LC_{50} response level for the respective insecticides.

Locality	Confidence intervals (CI) of LC50 ratios of active ingredients			
	Indoxacarb	**Emamectin benzoate**	**Spinetoram**	**Lufenuron**
Mareetsane	1a	1a	1a	1a
Polokwane	0.514 - 0.872b	1.213- 4.462b	0.701- 3.367a	8.02- 76.209b
Swartwater	0.449 – 0.706c	1.573- 4.543b	0.549- 2.236a	0.229- 0.632c

***** Responses were considered significantly different when the 95% confidence interval of the relative potency ratio did not include the value 1. Values within columns followed by the same letter did not differ significantly.

3.4 Discussion

Baseline susceptibility data of *T. absoluta* populations from Mareetsane, Polokwane and Swartwater to indoxacarb, emamectin benzoate, spinetoram and lufenuron were estimated. This is the first report of baseline susceptibility of *T. absoluta* to these insecticides in South Africa, and will therefore serve as baseline data for resistance monitoring in future. *Tuta absoluta* populations from these localities in South Africa, were highly susceptible to these active ingredients. Reference baseline dose-mortality response data is important for future monitoring of the pests (Cook *et al*., 2004).

It is, however, important to correlate the bioassay data with the expected field effectiveness of a pesticide (Ball, 1981). Insecticide resistance is a potential cause, or the potential consequence, control failure (Guedes, 2017). Many other factors may, however, also lead to control failure, such as inappropriate insecticide application and weather conditions (Guedes, 2017). Both concepts should be addressed, either by directly linking laboratory and field results, or by simulating field applications under standardized conditions (ffrench-Constant and Roush, 1990; Guedes, 2017). Recognition of control failure due to insecticide resistance or the assessment of control failure likelihood remains a challenge (Guedes, 2017).

Comparisons between the LC_{50} values of the *T. absoluta* populations indicated that, although susceptibility was high, differences existed in susceptibility of the respective populations. The responses of *T. absoluta* to indoxacarb, emamectin benzoate and spinetoram, from all three localities, exhibited low variability, with differences in LC_{50} values < 2.5 fold. No control failure is therefore expected. Further investigation into the prior insecticide application history of the Polokwane population is, however, needed to explain the significantly lower susceptibility to lufenuron, compared to the populations from Swartwater and Mareetsane. Lufenuron is not registered for control of *T. absoluta* in South Africa and applications for control of potato tuber moth should be taken into account.

Information regarding the phenotypic variation within a population is provided by the slope of the concentration-mortality lines, together with environmental and genetic variation. A steeper slope indicates reduced phenotypic variation in reaction to insecticides within a particular population (high degree of homogeneity) (Plapp, 1979). A slope value exceeding the value of one signifies a progressively homogeneous population whereas a slope value of less than one signifies a progressively heterogeneous population (Plapp, 1979).

The slopes of the concentration-mortality lines of spinetoram were low indicating the populations to be heterogeneous in their responses to spinetoram. The insecticidal activity of spinetoram is effectively executed during all life stages of lepidopteran pests, *viz*. eggs, first to late instar larvae and adults (Shimokawatoko *et al*., 2012). This insecticide was reported to be effective in controlling *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) in a crop protection scenario, because various growth stages of the pest are usually present simultaneously in infested fields (Shimokawatoko *et al*., 2012). This can, therefore, also be advantageous to farmers of solanaceous crops such as potatoes, since overlapping generations of *T. absoluta* are also usually found on these crops.

Shimokawatoko *et al*. (2012) demonstrated the insecticidal activity of spinetoram with a study on the common cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). Two pathways, *viz*. direct contact (spray) as well as feeding on treated leaves were reported to be involved. Spinetoram will therefore provide control of pests through both these pathways (Shimokawatoko *et al*., 2012).

The slopes of the concentration-mortality lines of indoxacarb was the steepest amongst the insecticides evaluated in this study. This indicates that the populations were more homogeneous in their responses to indoxacarb, compared to the other insecticides. Nonetheless the inconsistencies in the response of *T. absoluta* in populations from Brazil to indoxacarb reported by Silva *et al*. (2011), and populations from Italy by Roditakis *et al*. (2013b), resistance was reported in Brazil (Silva *et al*., 2011) and later in Italy and Greece (Roditakis *et al*., 2018).

Tuta absoluta demonstrates high reproductive fitness in warm, arid conditions, resulting in the use of insecticides on a regular basis to control populations (Silva *et al.*, 2011). Geographical distribution of insecticide resistance may be a consequence of dispersal and therefore, spatially dependent among populations. (Fragoso *et al*., 2003; Silva *et al.*, 2011). This implies that insecticide resistance profiles of populations in close-by sampling sites will be more comparable (Silva *et al.*, 2011). This is an aspect of *T. absoluta* resistance monitoring and IRM which should be investigated in South Africa in future.

Successful control of *T. absoluta* may require repeated applications of insecticides during a cropping season, enhancing the risk of resistance evolution (Roditakis *et al*., 2013a). Low levels of resistance to the insecticides evaluated in this study, was estimated. Roditakis *et al*. (2013a) recommended that an appropriate resistance monitoring scheme should to be in place under such conditions.

The spread of insecticide resistance to new areas of infestation by *T. absoluta* should be considered when monitoring populations of this species, and existing genotypes should be identified to minimize insecticide control failure (Guedes and Picanço, 2012). Since the risk is higher during the warm and dry season, only insecticides for which no control failure was reported should be used under such conditions (Guedes and Picanço, 2012). More diverse insecticides should be rotated to minimalize the threat of insecticide resistance evolution in *T. absoluta* populations (Guedes and Picanço, 2012). These insecticides should however not include those in the cross- and multiple-resistance range. These are aspects that need to be communicated to farmers in South Africa to keep the insecticides that are currently applied, available for effective control of this pest, for as long as possible.

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Chapter 4: Conclusion and Recommendations

4.1 Conclusion

Biological invasions by alien pest species threaten agricultural production (Pyšek and Richardson 2010; Goergen *et al*., 2016; Karlsson *et al.,* 2018). The impact of invasive species is apparent from increased crop production costs, including supplementary pest management costs, the reduction in marketability of crops, as well as from restrictions on the export of products to non-infested countries that result in loss of trading associates (Campos *et al*., 2017). The main limitation for tomato cultivation is a decrease in yield and quality of the crops caused by insect pest damage (Balzan and Moonen, 2012; Roditakis *et al.,* 2015). Cultivation of these crops also plays a vital role in providing an income for resource-limited growers, particularly those in urban regions (FAO, 2012; Brévault *et al.,* 2014). Ever since the detection of *T. absoluta* in the Mediterranean region, the tomato leafminer has become a widespread and important pest of Solanaceous species (Balzan and Moonen, 2012), in open-field and greenhouse production (Han *et al*., 2019). The decrease in tomato production in Europe can mainly be ascribed to *T. absoluta* damage to the leaves, reducing the photosynthetic ability of the plant (Campos *et al*., 2017).

The main method applied to control populations of *T. absoluta* in tomato fields is chemical control (Lietti *et al*., 2005; Brévault *et al.,* 2014). Effective control of *T. absoluta* is problematic, because of its endophytic behaviour (Lietti *et al*., 2005; Brévault *et al.,* 2014), and rapid insecticide resistance development due to its short generation time and high biotic potential (Balzan and Moonen, 2012). Efficacy of insecticides for control of the tomato leafminer has also decreased over the years due to the misuse of chemicals for its control (Silva *et al.,* 2011). Consequently, it is very important to monitor the insecticide susceptibility levels of the pest for early detection of resistance development (Roditakis *et al.,* 2015).

No resistance to indoxacarb, emamectin benzoate, spinetoram and lufenuron by *T. absoluta* was found in this study. Cases of resistance were, however, reported to indoxacarb in Brazil (Silva *et al*., 2011; Michaelides *et al.,* 2019) and Italy (Roditakis *et al*., 2013a), to diamides in Italy (Roditakis *et al*., 2015) and in Brazil (Silva *et al*., 2016), to emamectin benzoate in Italy and Greece, and to spinosad in the UK (Roditakis *et al*., 2018).

Nevertheless, baseline data acquired from this study can be used for future studies to monitor the development of resistance of *T. absoluta* populations in South Africa.Resistance development unlocks possibilities for new research into other control methods including biological and cultural tactics. For example, biological pest management strategies for *T. absoluta* by means of natural enemies introduced into a problem area (Balzan and Moonen, 2012). A harmonizing approach should include farm management tactics to increase the habitat as well as resources for natural enemies in surrounding areas in order to develop conservation biological control (Rusch *et al*., 2010; Balzan and Moonen, 2012). An IPM strategy is the only sustainable solution to ensure the susceptibility of insecticides for *T. absoluta* for as long as possible. Desneux *et al.* (2010) indicated, that in order for an IPM strategy to be effective, it should include a thorough sampling procedure in combination with pheromone trapping to compare yield loss with the abundance of moths (Balzan and Moonen, 2012). For this reason, the continuation of research, particularly in countries such as South Africa which is still in the developing stage, remains essential.

Plant volatiles are mainly used by herbivorous insects to identify and resourcefully locate their host plant (Bengtsson *et al.*, 2001). These volatiles facilitate host finding by herbivores from a distance which in turn leads to host reliability for feeding and habitat-specific mating (Bengtsson *et al*., 2006; Proffit *et al*., 2011). Both genders distinguish these volatiles through dedicated olfactory receptor neurons, and use them to separate sustenance resources, engagement locations or food sources for larvae, from other chemicals in the environment (Dethier, 1982; Bengtsson *et al*., 2006). The host range of an insect depends on its capability to find possible host plants as well as behavioural, neurophysiological and physiological qualities, by the collection of plant species within the environmental range of the population (Suckling *et al.,* 2014).

Chemical signals released by plants can clarify behavioural discrimination of herbivores between plant species (Bawin *et al.,* 2015). From this study it can be speculated that the two weed species (*Solanum reteroflexum* and *Solanum scabrum*) may have similar volatile chemical profiles than tomato which is preferred by *T. absoluta* for oviposition. Therefore, these findings highlight the probability that these two species can act as alternative hosts for the leafminer when tomato is not available, especially when they are in close proximity to tomato fields. As a result, reinfestation of tomato crops will take place in the following season when *T. absoluta* moths migrate from the weeds back to the tomato crops.

Alternative host plants can play an important role in the preservation and spread of agricultural pests, predominately when these insects attack a variety of hosts, are ecologically plastic and exhibits a high reproductive capability (Abbes *et al.,* 2016). During the oviposition phase of females, they are exposed to a diversity of signals of which plant volatiles, contact chemicals and visual signals assist them to determining the characteristics of plants (Caparros Megido *et al.,* 2014). By taking advantage of food resources adjacent to crops and weeds, rotation of crops may also play an important role in the dynamics and outbreaks of a pest population (Barros *et al.,* 2010).

4.2 Recommendations

When considering the alternative hosts plants of *T. absoluta* tested in this study, it becomes clear that the probability of development on weeds as an alternative host for tomato, seems possible. Therefore, it would be recommended to remove any unnecessary weeds from the immediate vicinity of tomato crops to prevent reinfestation in the next season. Some weeds may also act as dead end trap crops for *T. absoluta* as seen with *Galinsoga parviflora* where eggs were laid on leaves, yet larvae could not develop. Further studies are however, necessary to confirm this. Since the oviposition preference tests indicated *G. parviflora* as less preferred compared to tomato, it can however, not be considered as a pull plant species in a push-pull system.

Aspects regarding insecticide resistance need to be communicated to farmers in South Africa to keep the insecticides that are currently applied, available for effective control of this pest, for as long as possible. These recommendations were summarized by Guedes and Picanço (2012). The spread of insecticide resistance to new areas of infestation by *T. absoluta* should be considered when monitoring populations of this species, and existing resistant populations should be identified to minimize insecticide control failure Since the risk is higher during the warm and dry season, only insecticides for which no control failure was reported should be used under such conditions (Guedes and Picanço, 2012). Insecticides with different MoA's should be rotated to minimize the threat of insecticide resistance evolution in *T. absoluta* populations (Guedes and Picanço, 2012). These insecticides should however not include those in the cross- and multiple-resistance range. This study provided baseline susceptibility data of *T. absoluta* to indoxacarb, emamectin benzoate, spinetoram and lufenuron. Future research should include subsequent monitoring of the susceptibility levels of *T. absoluta* to these insecticides. It should be conducted continuously to effectively implement insecticide resistance management (IRM) of *T. absoluta* in South Africa.

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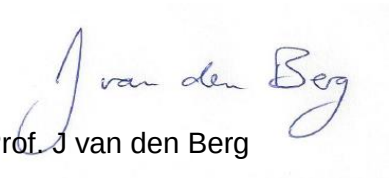
APPENDIX A

Declaration of language editing

Language editing statement

To whom this may concern,

I, Prof. J van den Berg, hereby declare that the thesis titled: “Host plant suitability and baseline susceptibility of *Tuta absoluta* (Lepidoptera: Gelechiidae) to insecticides in South Africa” by JP Hefer 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. J van den Berg

18 November 2020

Date

APPENDIX B

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ETHICS APPROVAL LETTER OF STUDY

Based on the review by the **Faculty of Natural and Agricultural Sciences Ethics Committee (FNAS-REC)**, the Committee hereby clears your study as no ethical risk. This implies that the FNASREC grants permission that, provided the general conditions specified below are met, the study may be initiated, using the ethics number below.

Study title: Host plant suitability and baseline susceptibility of *Tuta absoluta* (Lepidoptera: Gelechiidae) to insecticides in South Africa
Study Leader/Supervisor: Prof MJ du Plessis
Student: JP Hefer

Ethics number:

N	W	U	-	0	0	3	6	1	-	1	9	-	A	9
Institution				Study Number						Year			Status	

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application type: Single **Risk Category: No Risk**
Commencement date: 01/02/2020
Expiry date: 01/04/2021

General conditions:
The following general terms and conditions apply:

- *The commencement date indicates the date when the study may be started.*
- *In the interest of ethical responsibility, the NWU-SCRE and FNASREC reserves the right to:*
  - *request access to any information or data at any time during the course or after completion of the study;*
  - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the study are revealed or suspected;*
    - *it becomes apparent that any relevant information was withheld from the FNASREC or that information has been false or misrepresented;*
    - *submission of the annual (or otherwise stipulated) monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and / or*
    - *new institutional rules, national legislation or international conventions deem it necessary.*
- *FNASREC can be contacted for further information or any report templates via Roelof.Burger@nwu.ac.za 018 299 4269*

The FNASREC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the FNASREC or the NWU-SCRE for any further enquiries or requests for assistance.

Yours sincerely,

Prof Roelof Burger
Chairperson Faculty of Natural and Agricultural Sciences Ethics Committee (FNASREC)
File reference: 9.1.5.4.2