

# **Evaluation of tomato cultivars for resistance to *Tuta absoluta* (Lepidoptera: Gelechiidae)**

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## DECLARATION BY THE CANDIDATE

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

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Date 27 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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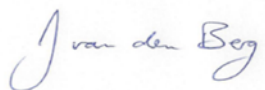


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## ABSTRACT

Cultivated tomato, *Solanum lycopersicum* L., is the second-most important herbaceous vegetable after potato, both in the world and South Africa. It is cultivated in all nine provinces of South Africa with an estimated annual gross value of over R2 billion. However, the introduction of the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), during late 2016, brought uncertainties for the tomato industry. *Tuta absoluta* causes significant crop losses due to its leaf-mining activity. The current pest management tactics rely on chemical control, which cannot be sustained indefinitely. Host plant resistance is a valuable component of any integrated pest management strategy. It affects important life history parameters of herbivorous species through antixenosis and antibiosis, or a combination of these two resistance mechanisms. The aim of this study was to evaluate tomato accessions for resistance to *T. absoluta*. In doing so, behavioural responses of *T. absoluta* were determined and morphological characteristics (leaf trichome type and density) responsible for resistance identified. The accessions included four commercially available cultivated accessions viz. Moneymaker, Commander, Emerald and Esty, one interspecific cross (*L. lycopersicum* × *L. habrochaites* f. *glabratum*, accession PI 134417, and two wild species (*L. habrochaites* f. *typicum*, accession LA 1777 and *L. habrochaites* f. *glabratum*, accession PI 134417). To determine the feeding preference of *T. absoluta* larvae, third-instar larvae were used for larval feeding bioassays conducted under laboratory conditions at  $26 \pm 1$  °C,  $65 \pm 5\%$  RH and 14L: 10D photoperiod. Larvae showed equal preference for the cultivated accessions, LA 1777 and the Cross. Wild accession PI 134417 was significantly less preferred when compared to cultivated accessions. The results for larval development times showed that neonate larvae had difficulty in establishing (antixenosis) and developing (antibiosis) on LA 1777, PI 134417 and the Cross. Only a few larvae were able to complete their life cycles on wild accession LA 1777 and none was able to do so on PI 134417 and the Cross. Cultivated accessions were suitable host plants for *T. absoluta* as larvae had shorter development times when compared to LA 1777. The mass of pupae reared on this accession and the cultivated accessions showed no significant difference despite the low survival rate on LA 1777. Female moths showed low preference for wild accessions. When the extent to which *T. absoluta* larvae can feed on these accessions was quantified under semi-field conditions, larvae showed similar responses as when under laboratory conditions, except for the Cross, which was not included. The morphological characteristics assumed to be responsible for resistance to *T. absoluta* in wild accessions and the Cross is most likely the presence of glandular trichome type 4, which was not present in cultivated accessions. Despite the high density of trichome type 4 observed on LA 1777, *T. absoluta* resistance in this study was most likely related to the kind of exudates produced, rather than density. Further studies are required to understand the mechanisms of resistance that are present in the wild accessions and the Cross.

**Keywords:** Antibiosis, antixenosis, behavioural response, cultivated accessions, trichomes, wild accessions.

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## CHAPTER 1

### Introduction and literature review

#### 1.1 Introduction

Cultivated tomato (*Solanum lycopersicum* L.), which belongs to the poisonous nightshade family Solanaceae is the second most important herbaceous vegetable after potato both in the world and South Africa (Jenkins, 1948; Albrecht and Chetelat, 2009; DAFF, 2017; FAO, 2020). It is consumed in diverse ways including as a fresh vegetable, as an ingredient in many dishes and sauces, and in drinks (Perveen *et al.*, 2015). It is an excellent source of a wide variety of nutrients including vitamins, minerals, sugars, essential amino acids, iron, dietary fibres and phosphorus that are important for the human diet (Arah *et al.*, 2015; Elbadrawy and Sello, 2016). Tomato contains higher amounts of lycopene that is linked to the reduced incidence of some chronic diseases such as cancer, cardiovascular disorders and diabetes (Bhowmik *et al.*, 2012; Arah *et al.*, 2015; Quinet *et al.*, 2019). Globally, as well as in South Africa, it is cultivated by both commercial and subsistence farmers. It serves as a source of income for most rural and semi-urban producers (Tshiala and Olwoch, 2010; Retta and Berhe, 2015; Swetha and Banothu, 2018).

Tomato is a warm-season crop that is sensitive to freezing temperatures and frost (Akinfasoye *et al.*, 2011; Gebremariam, 2015). Areas prone to unsuitable climatic conditions produce most of their tomatoes under controlled greenhouse conditions due to the increased consumption (Bergougroux, 2014). The global tomato production area reported in 2020, was approximately 4.8 million hectares with an average production of 182 million tons (FAO, 2020). From this, Asia produces 61.1%, America, 13.7%, Europe, 13.5%, Oceania, 0.2% and Africa 11.8%. In the 2016/17 production season, China produced most of the world's tomatoes with an estimated 59 million tons followed by India, Turkey, the United States of America (USA), Egypt, Iran, Italy, Spain, Mexico and Brazil (FAO, 2020). In Africa, particularly the Southern African Development Corporation (SADC) region, South Africa remains a major regional tomato producer despite ranking 35th in the world (Malherbe and Marais, 2015). Although it is cultivated in all provinces, more than 75% of the total area planted is in the Limpopo province (Daiber, 1996; Visser, 2015; DAFF, 2017). Approximately 20-30% of tomato for the South African fresh market is produced in the Mooketsi region near Tzaneen, Limpopo province (Daiber, 1996). Other

main tomato producing regions include areas in Mpumalanga, KwaZulu-Natal and Eastern Cape provinces (Fig. 1.1) (Daiber, 1996; DAFF, 2017).

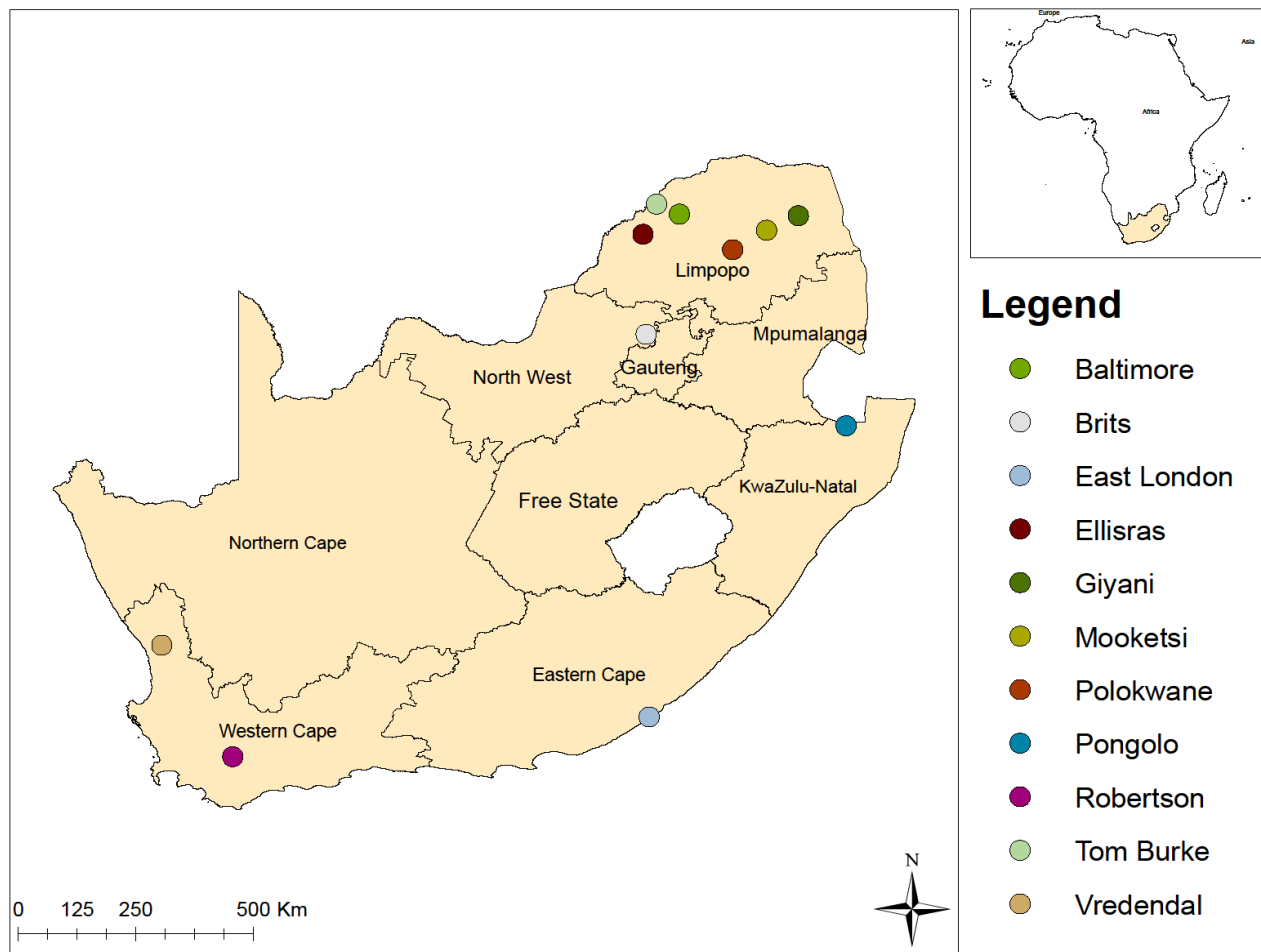


**Figure 1.1:** Main tomato production areas of South Africa

## 1.2 Tomato crop loss caused by pests throughout the world

Despite its benefits, tomato is highly susceptible to a number of pests (Afreen *et al.*, 2017). This is because the perception of improving crop yield and quality and not defense mechanisms dominated trait selection and modification during domestication (Mitchell *et al.*, 2016; Follett, 2017). Specific biosynthetic capabilities were lost during crop breeding and the domesticated plants produce volatiles in larger quantities, but of simpler composition compared to their wild relatives (Mitchell *et al.*, 2016). Crop production indicated to be conducive in transforming selected herbivores into pests, particularly herbivores that were hosted by wild relatives of the crop (Bernal and Medina, 2018). Global estimates for losses caused by pests differ significantly on an annual basis. The changes in cultivation techniques, use of varieties with high yield potential but with high susceptibility to pests and expansion of crops into less suitable regions with higher

incidences of other pests, are amongst the factors contributing to crop losses. Human activities into regions without natural restrictions also influence the import and spread of pests (Oerke, 2006).

The availability of quantitative data of crop losses caused by pests is very limited and the assessment of crop losses is difficult (Dhaliwal *et al.*, 2015). This is mainly because pest outbreaks depend on several variables related to environmental conditions, socioeconomics of farmers and the level of technology available (Oliveira *et al.*, 2014). However, losses caused by insect pests in both developed and developing countries are severe (Dhaliwal *et al.*, 2015). Although the majority of insect species are beneficial to humans and the environment, about 0.5% of insects are pests. Insect pests alone are responsible for reduced crop productivity and annual crop loss estimated between 18-26% worldwide (Culliney, 2014; Mitchell *et al.*, 2016). In tomato production, significant yield and quality losses due to insect pests are reported (Singh *et al.*, 2017; Diatte *et al.*, 2018; Rajam and Yogindran, 2018; Vosman *et al.*, 2018). Crop losses may be identified as either quantitative or qualitative. Quantitative losses occur as a result of reduced productivity, while qualitative losses result from reduced content of valuable ingredients, market quality, and storage characteristics, or as a result of contamination of the harvested product with the pest (Oerke, 2006).

For tomato crops, more than 200 insect pests, that cause significant yield and quality losses, are reported throughout the world (Ebadah *et al.*, 2016; Singh *et al.*, 2017; Diatte *et al.*, 2018; Rajam and Yogindran, 2018). In South Africa, more than 45 insect pest species are already reported to attack tomato (Table 1.1) (Visser, 2015). Amongst those causing severe damage and yield losses is tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Sylla *et al.*, 2017). It is considered to be a serious threat to tomato production in the world (Brévault *et al.*, 2014).

**Table 1.1: Insect pests of tomato in South Africa (Visser, 2015).**

| Order               | Common names            | Family and scientific names                         | Feeding area |
|---------------------|-------------------------|-----------------------------------------------------|--------------|
| <b>Hemiptera</b>    | <b>Whiteflies</b>       | <b>Family: Aleyrodidae</b>                          |              |
|                     | Sweet potato whitefly   | <i>Bermisia tabaci</i> (Gennadius)                  | L, S, V      |
|                     | Aphids                  | Family: Aphididae                                   |              |
|                     | Potato aphids           | <i>Macrosiphum euphorbiae</i> (Thomas)              | F, L         |
| <b>Thysanoptera</b> | <b>Thrips</b>           | <b>Family: Thripidae</b>                            |              |
|                     | Onion thrips            | <i>Thrips tabaci</i> (Lindeman)                     | F, L         |
|                     | Western flower thrips   | <i>Frankliniella occidentalis</i> (Pergande)        | F, L         |
| <b>Diptera</b>      | <b>Leaf miner flies</b> | <b>Family: Agromyzidae</b>                          |              |
|                     | American leaf-miner     | <i>Liriomyza trifoli</i> (Burgess)                  | L            |
| <b>Lepidoptera</b>  | <b>Twirler moths</b>    | <b>Family: Gelechiidae</b>                          |              |
|                     | Potato tuber moth       | <i>Phthorimaea operculella</i> (Zeller)             | L            |
|                     | <b>Owlet moths</b>      | <b>Family: Noctuidae</b>                            |              |
|                     | Tomato semi-looper      | <i>Chrysodeixis acuta</i> (Walker)                  | Fr, L        |
|                     | Plusia semi-looper      | <i>Thysanoplusia orichalcea</i> (Fabricius)         | Fr, L        |
|                     | Tomato moth             | <i>Spodoptera exigua</i> (Hübner)                   | Fr, L        |
|                     | African bollworm        | <i>Helicoverpa armigera</i> (Hübner)                | F, Fr, L, S  |
|                     | Grey cutworm            | <i>Agrotis subalba</i> Walker                       | L, S         |
|                     | Common cutworm          | <i>Agrotis segetum</i> ([Denis and Schiffermüller]) | L, S         |

Feed area: F= flower; Fr= fruit; L= leaves; S= stem; V= virus vector

### **1.3 *Tuta absoluta*: habitat origin, spread and distribution in Africa**

*Tuta absoluta* was first described by Edward Meyrick in 1917 after capturing one male specimen in Huancayo (Central Peru) (Sannino and Espinosa, 2010). Since the collection, there has been a series of genera changes (Biondi *et al.*, 2018). The pest was first named *Pthorimaea absoluta* by Meyrick in 1917. Later, it was described under the genus

*Gnorimoschema* (Clark) in 1962 and *Scrobipalpuloides* (Povolný) in 1964. In 1993, however, Povolný re-instituted the old genus created by Strand, *Tuta* (Sannino and Espinosa, 2010). It was observed in Spain (eastern region) for the first time outside its native region in 2006 (Campos *et al.*, 2017). Since the Spain invasion, it has rapidly spread at an average speed of 800 km per year invading most countries throughout Europe, Middle East, Asia and Africa (Tonnang *et al.*, 2015; Campos *et al.*, 2017). According to Guillemaud *et al.* (2015), Chile is the source of the *T. absoluta* population that invaded the Mediterranean region. A model proposed by Guimapi *et al.* (2016), accurately predicted *T. absoluta* to reach South Africa in 2016. Ten years after the initial invasion in Spain, *T. absoluta* was confirmed to have invaded South Africa in August 2016, and was first reported in the Mpumalanga province (Fig. 1.2) (Visser *et al.*, 2017).

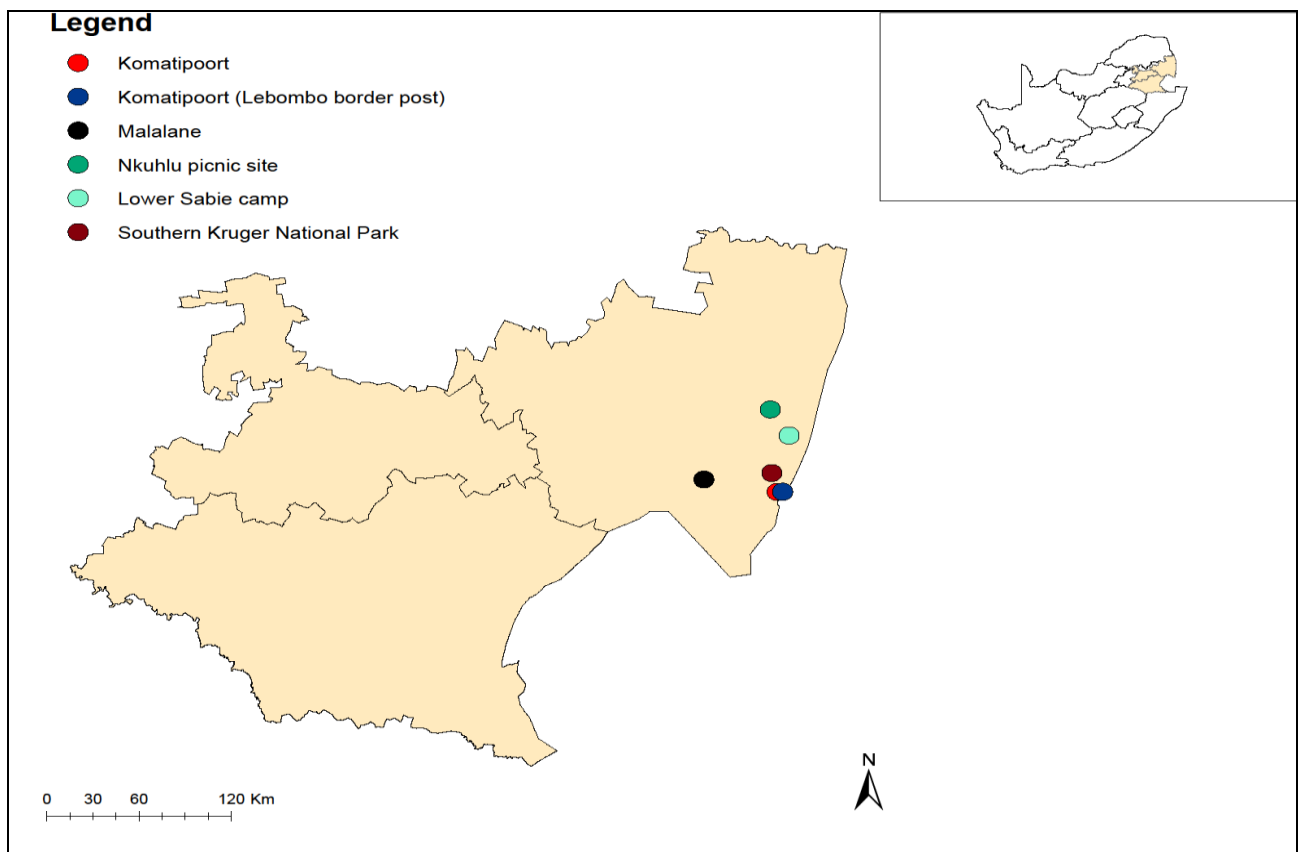


**Figure 1.2:** Mpumalanga province, the area where *Tuta absoluta* was initially reported in South Africa.

Tonnang *et al.* (2015), modelled the potential establishment and spread of *T. absoluta* in Africa, using the CLIMEX model. For *T. absoluta* establishment, these authors found the ecological and climatic conditions of Africa to be similar to those of South America, the native region of the pest (Fig. 1.3). The average temperatures of most areas are regarded to be considerably warm and near to the threshold temperatures for optimal development

and survival of this pest. According to Guimapi *et al.* (2016), relative humidity also has the greatest influences on the suitability of the area for *T. absoluta* establishment. Although the pest has a high potential of wind-assisted natural spread, its long distance dissemination is considered to be through human activities, rather than natural means (Ayalew, 2015). This is because empirical evidence on the flight ability of the pest for long-range movement, such as dispersal across geographic barriers is still missing (Biondi *et al.*, 2018). The human-mediated activities such as the cross border trade and intensive cultivation of *T. absoluta* host plants and fruits are responsible (Tonnang *et al.*, 2015; Campos *et al.*, 2017; Biondi *et al.*, 2018). Also, the areas with large monocultures of tomato and alternative host plants are prone to *T. absoluta* infestation (Biondi *et al.*, 2018).

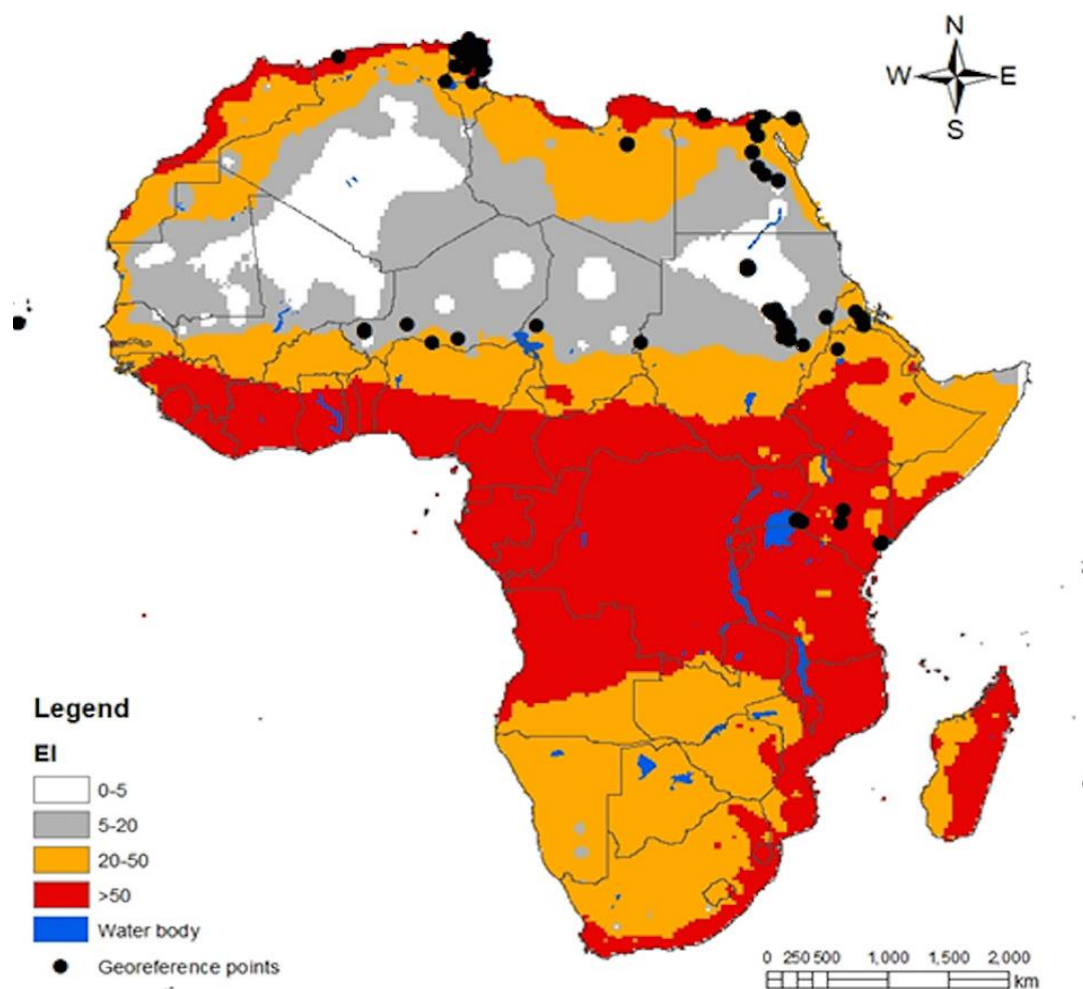


**Figure 1.3:** CLIMEX climatic suitability indices for *Tuta absoluta* in Africa. Predictions are based on the eco-climatic index (EI), a measure of climatic suitability scaled from 1–100, for locations within CLIMEX’s station database. EI = (0-5) location is not suitable; EI = (5-20) moderate level of suitability; EI = (20–50) high risk of establishment and EI > 50 very high likelihood of long-term survival. The black dots indicate the geo-reference location points obtained from surveys (Tonnang *et al.*, 2015).

#### 1.4 Host plant preference of *Tuta absoluta*

Tomato is the primary host plant of *T. absoluta* and it is consequently known as tomato leafminer, tomato pinworm or tomato borer (Sannino and Espinosa, 2010). In the absence of tomato, *T. absoluta* has been reported to cause significant damage to potato (*Solanum tuberosum*) and eggplant (*Solanum melongena*) (Solanaceae) (Unlu, 2012; Mohamed *et al.*, 2015; Sridhar *et al.*, 2015). The moth affects the aboveground parts of the crop (Đurić *et al.*, 2016), causing severe foliage damage (Mohamed *et al.*, 2012; 2015). Potato was also confirmed as a suitable host for oviposition and survival of *T. absoluta* (Galarza, 1984; Pereyra and Sánchez, 2006; Bawin *et al.*, 2016). In warm regions, it is considered to be a major pest of potato (Mohamed *et al.*, 2012; 2015). Abbes *et al.* (2016) reported that the pre-imaginal development period (egg-pupa) of *T. absoluta* on potato does not differ from that on tomato. However, in contrast, Caparros Megido *et al.* (2013) observed this period to be different between these two host plant species but the survival rate did not differ. Meanwhile, heavy *T. absoluta* infestation on eggplant was reported to result in a 30 to 40% yield loss in Italy (Sannino and Espinosa, 2010). Although tobacco (*Nicotiana tabacum*) (Solanaceae) was attacked by *T. absoluta* in Argentina, no damage was recorded (Sannino and Espinosa, 2010). The nutritional value of host plants affects the survivorship, growth, and fecundity of this pest whilst a shift in diet generally represents a rich and concentrated food source (Steppuhn *et al.*, 2004; Pereyra and Sánchez, 2006; Kumar *et al.*, 2014).

Other alternative host-plants include horticultural crops viz. pepper (*Capsicum annuum*) (Solanaceae) (Portakaldali *et al.*, 2013; Abbes *et al.*, 2016) and broad bean (*Vicia faba*) (Fabaceae) (Hossain *et al.*, 2016), several *Solanaceae* species viz. Cape gooseberry (*Physalis peruviana*) (Garzia, 2009), nightshade (*Solanum nigrum*), dense-thorn bitter apple (*Solanum sisymbriifolium*), soap-scented, toadstool (*Solanum saponaceum*) (Desneux *et al.*, 2010), jimson weed (*Datura stramonium*) (Tropea Garzia *et al.*, 2012), and other plant species viz. goosefoot (*Chenopodium album*) (Amaranthaceae) (Mohamed *et al.*, 2015) and bindweed (*Convolvulus arvensis*) (Convolvulaceae) (Bajonero and Para, 2017).

#### 1.5 Growth and development stages of *Tuta absoluta*

*Tuta absoluta* has a high reproduction potential that is capable of producing 10 to 12 generations per year under suitable agro-ecological conditions (Tropea-Garzia *et al.*, 2012; Allache *et al.*, 2015; El-Aassar *et al.*, 2015; Illakwahhi and Srivastava, 2017). It is a

widespread species with the capability to adapt to a wide range of temperatures different from its original habitat (Sannino and Espinosa, 2010; Portakaldali *et al.*, 2013; Bawin *et al.*, 2014). The moths are crepuscular to nocturnal and hide between vegetation during the day (Illakwahhi and Srivastava, 2017). The lifespan of moths ranges between 5-6 days for males, and 10-15 days for females (Illakwahhi and Srivastava, 2017). Its holometabolic life cycle consists of egg, larvae (four-instars), pupa and adult (moth) (Fig. 1.4) (Miranda *et al.*, 1998; Rostami *et al.*, 2017). A female moth can lay approximately 260 eggs on the aerial parts of the host plant either singularly or in small groups. The incubation period of eggs is 5-10 days (Sannino and Espinosa, 2010).

First and second larval instars feed on the leaf parenchyma causing very narrow linear or blotched mines. The third and fourth larval-instars exit mines and continues to feed into the apical buds, fruits and sometimes stems (Bawin *et al.*, 2014; Chidege *et al.*, 2016; Mutamiswa *et al.*, 2017; Rostami *et al.*, 2017). The larval stage takes between 11-20 days to complete with a maximum development period of up to two months in the coolest period. Pupation ranges from 6-8 days under favourable conditions, but it can last up to two months in the over-wintering generation (Speranza and Sannino, 2012). Pupation mostly occurs in the soil where the larva spins and forms a small silky cocoon to which earthy particles or debris stick. The shrivelled leaf, area near the calyx as well as mines are also suitable sheltered areas for pupation (Sannino and Espinosa, 2010; Chidege *et al.*, 2016).

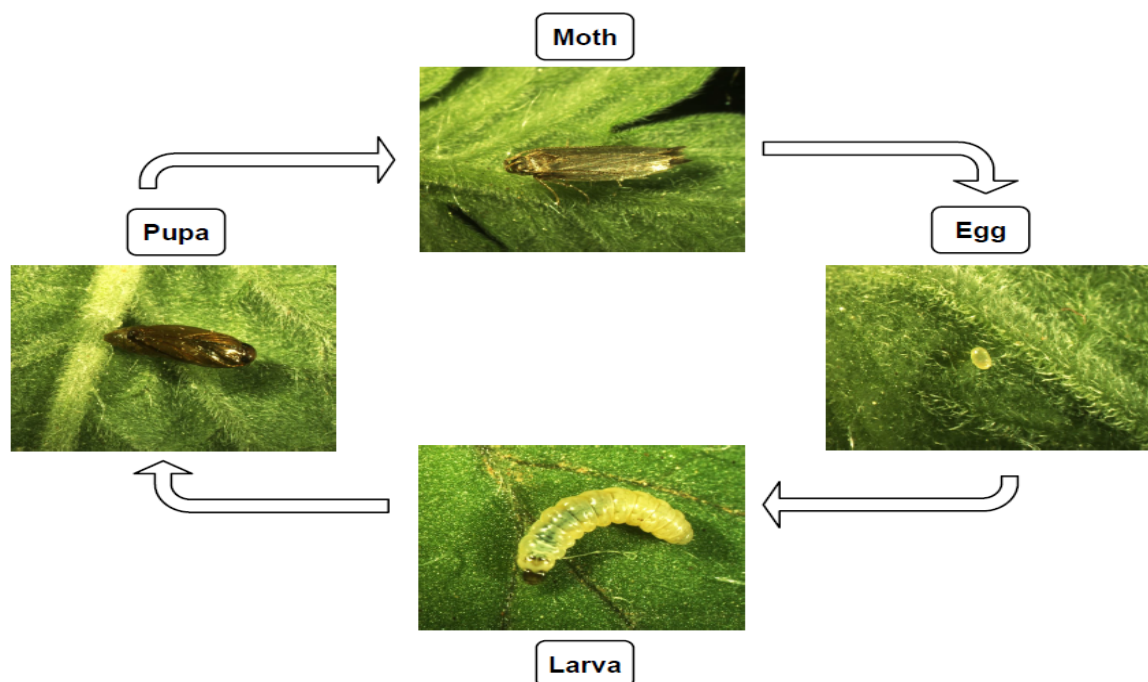


**Figure 1.4:** Development stages of *Tuta absoluta*.

## **1.6 Management tactics for *Tuta absoluta***

### **1.6.1 Likelihood of management failures**

#### **1.6.1.1 Sex pheromone-based control**

Sex pheromones are widely used for disruption of pest mating in Lepidopteran pest populations (Ando *et al.*, 2004). Their preventative control methods include: 1. attraction and annihilation of male moths and, 2. saturation of the atmosphere with synthetic sex pheromones (Megido *et al.*, 2012; Cocco *et al.*, 2013). However, the success of this management tactic in *T. absoluta* is threatened by the ability of female moths to breed asexually via parthenogenesis (Megido *et al.*, 2012; Illakwahhi and Srivastava, 2017). Megido *et al.* (2012) and Abbes and Chermiti (2014) reported *T. absoluta* to be able to produce male and female moths from unfertilized eggs (deuterotokous parthenogenetic reproduction). Skaljic *et al.* (2012) reported this to be due to the presence of *Wolbachia*, a symbiotic microorganism that causes a reproductive disorder in insects. Despite the demonstrations that parthenogenesis has limited impact on population density (Abbes and Chermiti, 2014), Megido *et al.* (2012) reiterated that this poses a threat to the genetic variation of *T. absoluta* populations, as *Wolbachia* is suggested to be transferrable from mother to offspring (Rousset *et al.*, 1992).

Although sex pheromone application may be effective for disrupting male orientation, it is unable to reduce the larva infestations (Cocco *et al.*, 2013; Cherif *et al.*, 2018). Abbes and Chermiti (2011) reported its use to be useful if used in combination with other control measures as it may assist with monitoring programmes.

#### **1.6.1.2 Chemical control**

Since the 1980s, farmers relied heavily on chemical insecticides for *T. absoluta* control. It is a common control tactic employed after the introduction of an invasive species (Guedes and Siqueira, 2013). The difficulty in controlling *T. absoluta* and the overreliance on chemical insecticides to control the high levels of this pest in South American countries, proved that management strategies based on chemical control only, are not sustainable (Abbes *et al.*, 2012; Gebremariam, 2015). The reliance on chemical insecticides leads to resistant populations (Siqueira *et al.*, 2000; Lietti *et al.*, 2005; Guedes and Siqueira, 2013; Cocco *et al.*, 2013; Zekeya *et al.*, 2017; Kamali *et al.*, 2018). The quick chemical insecticide resistance evolution is directly correlated to insecticide applications (Guedes and Siqueira, 2013). Chemical insecticides applied to open-field tomato crops are mostly

inefficient due to weather conditions and spatial dependence (Balzan and Moonen, 2012; Gontijo *et al.*, 2013). As a result, the number of applications increases (Balzan and Moonen, 2012). In Brazil, applications have increased from 10-12 applications to over 30 applications per growing season (Guedes and Picanço, 2012). The use of a limited number of insecticides is not a sustainable management option for *T. absoluta* in South America (Desneux *et al.*, 2010). This is evident from reports of resistance to almost all the insecticide groups used against *T. absoluta* in South America (Siqueira *et al.*, 2000; Lietti *et al.*, 2005; Desneux *et al.*, 2010; Guedes and Picanço, 2012; Bawin *et al.*, 2016; Zekeya *et al.*, 2017). Incidence of resistance to the pyrethroids, deltamethrin, abamectin, cartap, permethrin, as well as to the organophosphate, methamidophos have been reported (Balzan and Moonen, 2012; Biondi *et al.*, 2018).

## **1.6.2 Promising management tactics**

### **1.6.2.1 Biological control**

The use of indigenous natural enemies is one of the most promising control tactics available against exotic pests (Zappalá *et al.*, 2012; 2013). There are three different kinds of biological control agents available, namely predators, parasitoids and entomopathogenic micro-organisms (Salim *et al.*, 2016). The host plants of *T. absoluta* emit volatile compounds that attract either pest predators or parasitoids (Zekeya *et al.*, 2017). Over 50 natural enemy species of *T. absoluta* were documented in South America (Aynalem, 2018). However, there is only limited data available from regions outside the native region of the pest (Table 1.2) (Desneux *et al.*, 2010). The success of biological control depends on the efficacy of the released beneficial organism and the suitability of the hosting agro-ecosystem (Ingegno *et al.*, 2017). *Trichogramma* species are the principal egg parasitoids of lepidopteran agricultural pests (Chailleux *et al.*, 2013). There are reports of successful biological control agents of *T. absoluta* in Europe (Urbaneja *et al.*, 2012; Chailleux *et al.*, 2013). The periodic inundative release of *Trichogramma* species and mirid predators, i.e. *Nesidiocoris tenuis* Reuter and *Macrolophus pygmaeus* (Rambur), is already commercialised and employed successfully in integrated pest management strategies (Borgi *et al.*, 2016).

**Table 1.2:** Natural enemies of *Tuta absoluta* reported in the Mediterranean Basin (Desneux *et al.*, 2010; Urbaneja *et al.*, 2012)

| Natural enemy type | Order       | Family            | Species                                          | Stage preferred                            | References                       |
|--------------------|-------------|-------------------|--------------------------------------------------|--------------------------------------------|----------------------------------|
| Parasitoids        | Hymenoptera | Braconidae        | <i>Necremnus artynes</i> (Walker)                | 2 <sup>nd</sup> and 3 <sup>rd</sup> instar | Ferracini <i>et al.</i> (2012)   |
|                    |             | Eulophidae        | <i>Stenomesus cf. japonicas</i> (Ashmead)        | 2 <sup>nd</sup> and 3 <sup>rd</sup> instar | Chailleux <i>et al.</i> (2017)   |
|                    |             |                   | <i>Neochrysocharis Formosa</i> (Westwood)        | 1 <sup>st</sup> instar                     | Luna <i>et al.</i> (2011)        |
|                    |             | Trichogrammatidae | <i>Trichogramma achaea</i> Nagara and Nagarkatti | Eggs                                       | Oliveira <i>et al.</i> (2017)    |
|                    |             |                   | <i>Trichogramma brassicae</i> (Bezdenko)         | Eggs                                       | Mirhosseini <i>et al.</i> (2019) |
|                    |             |                   | <i>Trichogramma cacoeciae</i> (Marchal)          | Eggs                                       | Cherif <i>et al.</i> (2019)      |
| Predators          | Hemiptera   | Miridae           | <i>Nesidiocoris tenuis</i> Reuter                | Eggs and 1 <sup>st</sup> instar            | Mollá <i>et al.</i> (2014)       |
|                    |             |                   | <i>Dicyphys marrocannus</i> Wagner               | Eggs and 1 <sup>st</sup> instar            | Ingegno <i>et al.</i> (2019)     |
|                    |             |                   | <i>Dicyphus tamaninii</i> Wagner                 | Eggs and 2 <sup>nd</sup> instar            | Mansour <i>et al.</i> (2018)     |
|                    |             | Anthocoridae      | <i>Orius majusculus</i> (Reuter)                 | Eggs                                       | Urbaneja <i>et al.</i> (2012)    |
|                    |             | Nabidae           | <i>Nabis pseudoferus ibericus</i> (Remane)       | Larvae                                     | Matendo (2017)                   |
|                    | Hymenoptera | Sphecidae         | Undetermined species                             | Larvae                                     | Ferracini <i>et al.</i> (2019)   |
|                    | Acari       | Phytoseiidae      | <i>Amblyseius swirskii</i> (Athias Henriot)      | Eggs                                       | Desneux <i>et al.</i> (2010)     |

### **1.6.2.2 Host plant resistance**

Although the ecological and phylogenetic relationships that promote defensive mechanisms of plants against insects are not well understood (War *et al.*, 2012; López-Carretero *et al.*, 2016), the phylogenetic history of a plant and its adaptation to environmental conditions is reported to contribute to the important features that lead to the development of plant defence mechanisms (Le Roux *et al.*, 2010). Plants contain constitutive and induced defences that respond to herbivory through various morphological and molecular defence mechanisms (War *et al.*, 2012). Certain primary mechanisms responsible for natural resistance to herbivorous species occur in wild *Lycopersicon* accessions (Antônio *et al.*, 2011; Lucini *et al.*, 2015; Sohrabi *et al.*, 2017). Many wild relatives and landrace varieties have sources of resistance against various tomato insect pests (Maluf *et al.*, 2007). The survival, reproduction, development time, size, longevity and fecundity success of insects are affected by these resistant sources (War *et al.*, 2012; Hagenbucher *et al.*, 2013; López-Carretero *et al.*, 2016; Mitchell *et al.*, 2016). The constituent resistant traits are physical and chemical with a direct impact on the insect (War *et al.*, 2012; Hagenbucher *et al.*, 2013; Gong and Zhang, 2014; Wang *et al.*, 2015; Mitchell *et al.*, 2016). Although the lethal reactions are not common in the direct impact, the avoidance behaviour in the life-history parameters of the insect is modified to help protect the plant (Hagenbucher *et al.*, 2013). Aljiboy and Chen (2018) emphasised that a direct impact includes morphological defence resistance-traits that reinforce the endogenous ability of plants to counter insect pest attacks. This includes trichomes, morphologically diverse arrays that develop from cells of the aerial epidermis (Dalin *et al.*, 2008; Bickford, 2016). During insect-plant interaction, trichomes act as a first line of defence for physical impedance, abrasion and laceration of the insect body (Dai *et al.*, 2010; Stavrianakou *et al.*, 2010; Wei *et al.*, 2013; Karley *et al.*, 2015; Kariyat *et al.*, 2017). There is a negative correlation response reported between leaf trichome density and insect behaviour on various *Lycopersicon* species (Levin, 1973).

## **1.7 Research aim and objectives**

### **1.7.1 Aim**

The aim of this study was to evaluate wild and commercially available tomato accessions, as well as an interspecific cross, for resistance to *T. absoluta*.

### 1.7.2 Specific objectives of this study were to:

- Determine the effect of trichomes on feeding preference, development, and survival of *Tuta absoluta* larvae on different tomato accessions.
- Determine the suitability of selected tomato accessions for *T. absoluta* oviposition preference and larval host.

## 1.8 References

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## CHAPTER 2

### Effect of trichomes on feeding preference, development, and survival of *Tuta absoluta* larvae on different tomato accessions

#### 2.1 Abstract

Tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a pest of solanaceous crops which causes severe foliage and fruit damage to tomato (*Solanum lycopersicum* L.). One of the management strategies that can be deployed against this pest is host plant resistance. The objectives of this study were to evaluate the suitability of different tomato accessions for development of *T. absoluta* larvae, and to study the trichome types and densities on the leaves of these accessions. The accessions included four commercially available cultivated accessions viz. Moneymaker, Commander, Emerald and Esty, one interspecific cross (*S. lycopersicum* × *Solanum habrochaites* f. *glabratum*, accession PI 134417), and two wild species (*S. habrochaites* f. *typicum*, accession LA 1777 and *S. habrochaites* f. *glabratum*, accession PI 134417). To determine the feeding preferences of *T. absoluta* larvae for each of the seven tomato accessions, bioassays were conducted in which larvae were exposed to leaf material in no-, two- and multi-choice tests. In the no-choice tests, no significant preference by *T. absoluta* for any of the accessions was found. Feeding occurred on all accessions after 24 hours and PI 134417 was significantly less fed on when compared with Moneymaker. In the two choice tests, with combinations including Moneymaker and any other accession, significantly fewer larvae preferred PI 134417 after 6 and 24 hours and Moneymaker was more significantly fed on in combination with Emerald, Esty, PI 134417 and LA 1777. No accession was significantly more preferred when provided in the multi-choice tests, but the commercial accession, Moneymaker, was significantly more preferred when provided in combination with the two wild accessions, PI 134417 and LA 1777. Moneymaker was less preferred in combination with Emerald and the Cross as well as Esty and PI 134417. *Tuta absoluta* larvae were not able to complete their development on the wild accession, PI 134417, as well as the Cross. Larval development on all commercial accessions was significantly shorter, compared to the wild accession LA 1777. The mass of pupae from larvae reared on commercial accessions was similar. Trichome types 1, 3, 4, 5

and 6 were found. Type 3, 4 and 5 trichomes were not present on the Cross, commercial and wild accessions, respectively. Trichome type 6 occurred throughout the accessions but with varied morphological characteristics. The high survival rate of *T. absoluta* on some of the accessions in this study could not be ascribed to the presence or density of a particular trichome type only. However, glandular trichomes, particularly trichome type 4, did affect survival of *T. absoluta* larvae.

**Key words:** Development times, feeding preference, host suitability, pest management, *Solanum* accessions, trichomes

## 2.2 Introduction

Damage to tomato crops by the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a major concern for tomato producers globally (Guedes *et al.*, 2019). Integrated pest management strategies employed against this pest include cultural- (Abbes *et al.*, 2012; Guedes and Picanço, 2012), biological- (Desneux *et al.*, 2010; Khidr *et al.*, 2013; Brévault *et al.*, 2014), bio-technological (Desneux *et al.*, 2010) and chemical control (Kennedy, 2008; Aynalem, 2018). Cultural control practices, biological control, manipulation of pest behaviour and host plant resistance are used to prevent or reduce exposure of the crop to damaging pest populations (Kennedy, 2008). Once the populations have reached threshold levels, chemical control is usually applied to suppress these populations (Kennedy, 2008). Van Emden (2017) suggested that the use of only one method that provides adequate control will subsequently result in a strain with increased tolerance to that particular method. Different control methods should therefore be used in combination, to enhance the potential of the respective methods, compared to when they are used individually.

Plant resistance is an important insect management tactic based on the insect-plant interaction (Acharya *et al.*, 2018). Amongst the benefits of the use of host plant resistance, are increased productions and reduced losses of crops, as well as no adverse effects on the non-target organisms in the environment (Sharma, 2016). Host plant resistance affects insect herbivores through antixenosis, (the effects of plant physical and chemical properties on insect behaviour), antibiosis (the effects that plant traits have on the life-history parameters and/or biology of an insect

herbivore), and tolerance (the ability of the plant to compensate for higher levels of injury by the insect herbivores) (Smith, 2005; Kennedy, 2008).

Fewer large mines caused by *T. absoluta* larvae inside leaves of different tomato accessions may be an indication of plant resistance through antibiosis and/or antixenosis (Oliveira *et al.*, 2009). Resistance traits, which are present in wild accessions of *Lycopersicon* to tomato pests, have to be introgressed into cultivated tomato before it will have commercial value (Maluf *et al.*, 2007). However, to date, there are no commercial accessions with high levels of resistance to *T. absoluta* (Maluf *et al.*, 2007; Oliveira *et al.*, 2009).

Possible resistance to herbivory had been identified in accessions of wild tomato species (Rakha *et al.*, 2017a; Bitew, 2018). Resistance to various arthropod pests such as *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) and *Tetranychus urticae* Koch (Acari: Tetranychidae) were reported on accessions of *Solanum pennellii* Correll (formerly *L. pennellii* Correll) (Resende *et al.*, 2008; Maluf *et al.*, 2010; Lucini *et al.*, 2015), *S. cheesmaniae* (L. Riley) Fosberg (formerly *L. cheesmaniae* L. Riley), *S. galapagense* S.C. Darwin et Peralta (formerly *L. cheesmaniae* f. *minor* (Hook. f.) C. H. Müll.), (Firdaus *et al.*, 2012), *S. habrochaites* f. *glabratum* C. H. Mull (formerly *L. hirsutum* f. *glabratum* C. H. Mull.) (Chatzivasileiadis and Sabelis, 1997) as well as *S. pimpinellifolium* L. (formerly *L. pimpinellifolium* (L.) Mill.) (Rakha *et al.*, 2017a, b, c; Ali *et al.*, 2019), amongst others. The presence of trichomes of different types and densities have been reported to be responsible for plant resistance to herbivory by several insect species (Simmons *et al.*, 2003; Glas *et al.*, 2012; Rakha *et al.*, 2017a). Trichomes interfere with the most important behavioural responses of insects, such as oviposition, orientation and feeding which occur during the initial stages of crop colonisation (Saxena, 1985; Lucini *et al.*, 2015). Glandular trichomes of *Lycopersicon* have 'heads' that release, on contact with pests, sticky and/or toxic exudates which entrap and irritate, and could potentially kill the pest. Non-glandular trichomes have no 'heads' and affect pests by mechanical means (e.g. constituting a barrier to movement or access to nutritious tissue) (Simmons and Gurr, 2004).

The objectives of this study were to evaluate the suitability of different tomato accessions for feeding preference, development and survival of *T. absoluta* larvae and study the trichome types and densities on the leaves of these accessions.

## **2.3 Material and methods**

### ***Tuta absoluta* rearing colony**

*Tuta absoluta* larvae and pupae were collected from infested tomato greenhouses and fields at Ventersdorp (-26.406861, 26.793333) in the North-West province, Pretoria (-25.535894, 28.150865) in Gauteng province, and Mooketsi (-23.665288, 29.7638120) in Limpopo province. The colony was reared in three screen cages (0.8(H) × 0.6(L) × 0.5(W) m) on tomato (commercial accession Moneymaker), under ambient temperature and humidity conditions at the North-West University, Potchefstroom. Separate cages were used to allow for oviposition by moths, larval development, and for pupation and moth emergence. The tomato plants were moved between the cages according to the development stages of the pest. The plants in the oviposition cage were replaced every second day, and developing larvae were continuously provided with additional uninfested plants to ensure a constant availability of food.

Third-instar larvae (L3) for use in experiments were identified using their morphological appearance viewed with a handheld magnifier equipped with an ocular scale. Third-instar larvae leave the mines inside leaves and move onto the surface of the plant to find new feeding sites (Cuthbertson *et al.*, 2013). Injury to larvae sampled for experiments, was reduced by collection of these larvae from the surface of the plant and not from inside the mines.

### ***Tomato accessions***

Seed of seven tomato accessions were obtained from Sakata Seed Southern Africa (Pty) Ltd (Lanseria, Gauteng). These were four commercially available cultivars viz. Moneymaker, Commander, Emerald and Esty, one interspecific cross (*L. lycopersicum* × *L. habrochaites* f. *glabratum* accession PI 134417) (hereafter referred to as “Cross” for ease of reading), and two wild species (*L. habrochaites* f.

*typicum* accession LA 1777 referred to as LA 1777, and *L. habrochaites* f. *glabratum* accession PI 134417 referred to as PI 134417). Seeds of these accessions were planted in styrofoam seedling trays in potting soil (potting mix, Culterra (Pty) Ltd., Muldersdrift). Seedlings were transplanted into 4 L pots, 30 days after planting and maintained in an enclosed facility at ambient temperature and humidity. The seedlings were fertilised one week after emergence and again three weeks later with a multi-nutrient solution (Nutrifeed, Stark Ayres (Pty) Ltd., Benoni).

### **2.3.1 Larval feeding choice-tests**

To determine the feeding preferences of *T. absoluta* L3 larvae for each of the seven tomato accessions, tests were conducted by providing larvae with leaf material of either one (no-choice), two- (two-choice) or three accessions (multi-choice). The accessions, as well as the combinations used in the respective tests, are provided in table 2.1. Leaf discs (2 cm diameter) were punched from each of the seven tomato accessions. The bottoms of Petri dishes (9 cm (d) × 1.5 cm (h)) were covered with a 1.5% agar-agar layer to a depth of ±3 mm to prevent rapid desiccation of leaf-discs.

**Table 2.1:** Tomato accessions and combinations used in the choice tests with *Tuta absoluta* third-instar larvae.

| Accession and combinations used in choice-tests |                        |                                                                                                                                                                               |
|-------------------------------------------------|------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| One accession                                   | Two accessions         | Three accessions                                                                                                                                                              |
| Moneymaker                                      | Moneymaker × Commander | Moneymaker × Commander × Emerald<br>Moneymaker × Commander × Esty<br>Moneymaker × Commander × Cross<br>Moneymaker × Commander × LA 1777<br>Moneymaker × Commander × PI 134417 |
| Commander                                       | Moneymaker × Emerald   |                                                                                                                                                                               |
| Emerald                                         | Moneymaker × Esty      | Moneymaker × Emerald × Esty<br>Moneymaker × Emerald × Cross<br>Moneymaker × Emerald × LA 1777<br>Moneymaker × Emerald × PI 134417                                             |
| Esty                                            | Moneymaker × Cross     | Moneymaker × Esty × Cross<br>Moneymaker × Esty × LA 1777<br>Moneymaker × Esty × PI 134417                                                                                     |
| Cross                                           | Moneymaker × PI 134417 | Moneymaker × Cross × PI 134417<br>Moneymaker × Cross × LA 1777                                                                                                                |

### 2.3.1.1 No-choice test

For the no-choice test, a single disc of leaf material from an accession was placed in the centre of a Petri dish onto the agar layer (Fig. 2.1A). Two *T. absoluta* L3 larvae were placed onto the agar on one side of the Petri dish (Fig. 2.1A). The test consisted of 30 replicates per tomato accession. The Petri dishes were placed in a growth chamber at  $26 \pm 1$  °C,  $65 \pm 5\%$  RH and 14L: 10D photoperiod. The position of the larvae was recorded after 6 and 24 hours. Larval feeding preference was categorised as: (1) preference, if feeding damage was observed and at least one larva was present on the leaf disc, 2) no-choice (NC), if a larva was not on the leaf disc and no feeding damage was observed, or a leaf disc was damaged but no larva was present on the leaf disc, and/or the larva was found on the leaf-disc but no feeding damage was observed.

### **2.3.1.2 Two-choice test**

In the feeding choice test with two accessions, Moneymaker was used as a standard commercial accession. Larval feeding preference for Moneymaker and the other tomato accessions were therefore investigated. Two leaf discs of one of the tomato accessions (each 2 cm in diameter) and two Moneymaker leaf discs were placed in an alternate order on the agar layer along the edges of the Petri dish (Fig. 2.1B) (hereafter referred to as the two-choice tests, for ease of reading). Using the alternate arrangement increased the probability that a larva leaving a disc would encounter the other disc before possibly returning to the first disc (Henniges-Janssen *et al.*, 2014). Two *T. absoluta* L3 larvae were placed in the centre of each Petri dish. There were 20 replicates for each combination. The data were collected according to the methods described in 2.2.1.1 (No-choice test).

### **2.3.1.3 Multi-choice test**

Leaf discs from three different tomato accessions were exposed simultaneously to two *T. absoluta* L3 larvae. Moneymaker was included as a standard commercial accession in all the multi-choice tests. The three leaf discs, one from each accession, were arranged along the edges of a Petri dish, forming a triangle shape (Fig. 2.1C). Each combination was replicated 20 times. The data were collected according to the methods described in 2.2.1.1 (No-choice test).

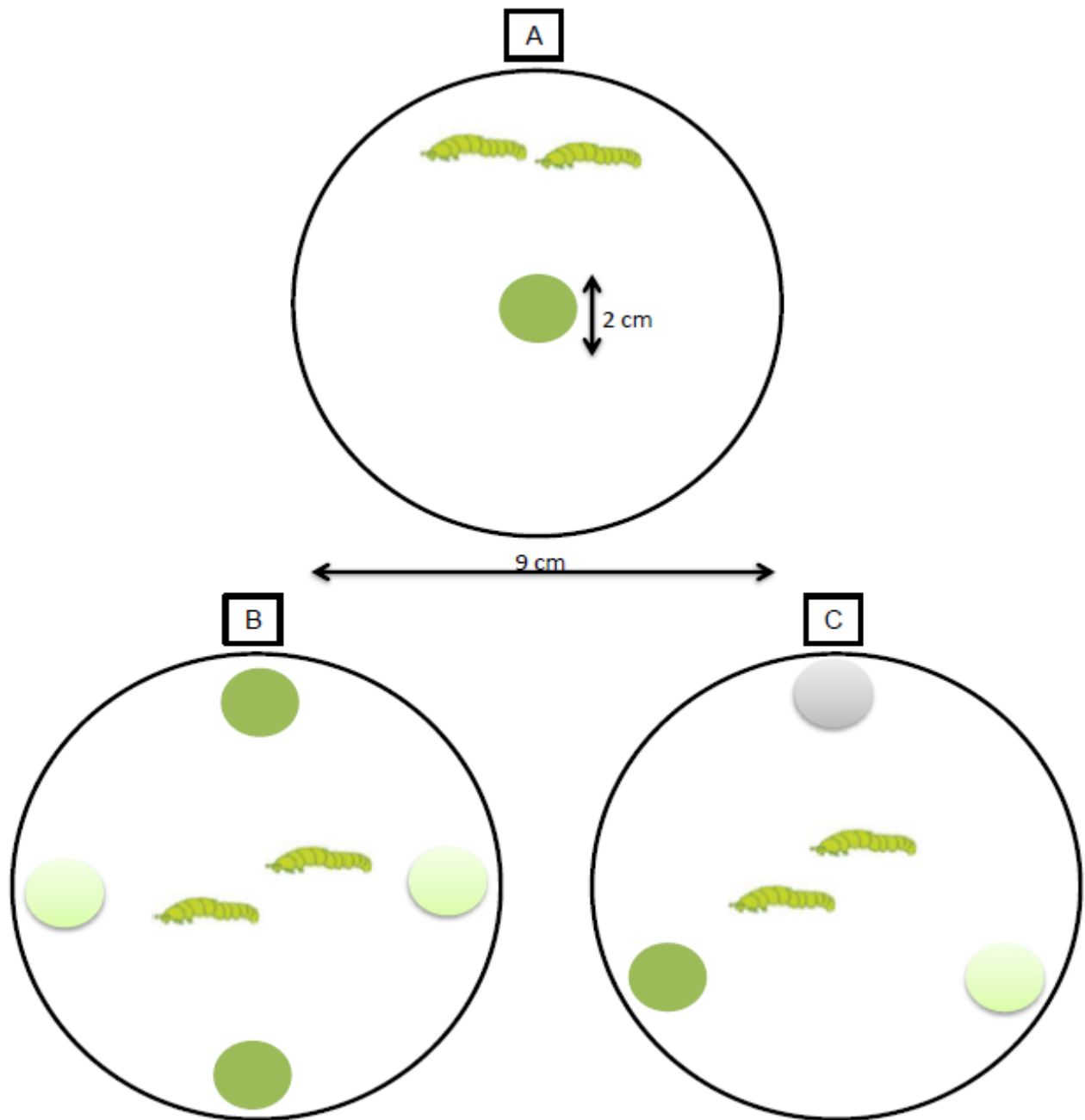


**Figure 2.1:** Experimental set-up for the respective choice tests with tomato accessions presented to two *Tuta absoluta* third-instar larvae: **A** - a single leaf disc (2 cm diameter) was placed in the centre of a Petri dish (9 cm diameter) with two larvae placed on one side of the Petri dish for no-choice tests, **B** - feeding choice test with two tomato accessions: leaf discs were presented in an alternate order along the edge of the Petri dish and larvae were placed in the centre (two-choice test), **C** - multiple-choice test with one leaf disc of three different accessions placed at the edges of the Petri dish and larvae were placed in the centre.

### **2.3.2 Consumption of leaf discs**

The percentage leaf disc area consumed in the respective choice tests was calculated after 24 hours. This was done by means of a leaf doctor smartphone application developed for quantifying plant disease severity (Pethybridge and Nelson, 2015). A colour image of each leaf disc was taken. The application's display screen was touched to select six different colours that represented healthy tissues. A threshold slider was then moved over the leaf disc until only the consumed area was transformed into a blue hue. The pixelated image was then analysed to obtain the percentage consumed area (Pethybridge and Nelson, 2015).

### **2.3.3 *Tuta absoluta* development on tomato accessions**

The same tomato accessions used in the feeding choice tests (Table 2.1) were used for the larval development bioassay. Petri dishes were prepared for the bioassay by covering the bottoms with a  $\pm 3$  mm thick layer of agar-agar (1.5%) to prevent rapid desiccation of the tomato leaflets. Eggs were removed from plants in the oviposition cage using a fine artist brush, and placed in the Petri dish. Hatching neonates were sampled from this Petri dish for use in the bioassay.

For the development bioassay, one leaflet of a single accession was placed per Petri dish and one neonate *T. absoluta* larva was placed on the leaflet. There were 15 replicates for each of the seven accessions. The Petri dishes were sealed with Parafilm® (Bemis Company, Inc., Neenah, Wisconsin United States) and kept in a growth chamber at  $26 \pm 2$  °C,  $65 \pm 5\%$  RH and 14L: 10D photoperiod. The leaflets were replaced every 48 hours until pupation of the larvae. Larval development time, pupal mass (48 hours after pupation), and survival of the immature stages were recorded.

### **2.3.4 Trichome types and densities on tomato accessions**

The second and third fully expanded leaflets from the terminal part of each of the seven accessions were removed according to methods described by Gurr and McGrath (2001). From each leaflet, a  $1 \text{ cm}^2$  piece was cut from the mid-part, between the main vein and leaflet edge. While cutting the leaf pieces, the surface of the leaf (adaxial and abaxial) that was to be examined always faced upwards to

prevent damage to the trichomes. The leaf pieces were picked up on their edges by means of a forceps and placed on the stage of a Nikon AZ100M microscope. A 0.87 mm<sup>2</sup> area of each leaf piece was demarcated and examined. There were 12 replicates for each of the adaxial and abaxial sides of the leaves of each accession. The five different types of trichomes (1, 3, 4 5 and 6) present, as well as the density of each type of trichome per demarcated area were identified and counted at 2x zoom magnification through a high resolution camera (Nikon Fiber Illuminator, Nikon, Japan). The camera was attached to a desktop computer with Nikon NIS Elements imaging software. Trichome morphology was classified based on the presence or absence of glandular heads as described in Channarayappa *et al.* (1992).

## **2.4 Data analyses**

The proportion of *T. absoluta* larvae that made a choice in the no-choice, two-choice and multi-choice tests was calculated, and data for each test were analysed 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 accessions per recording time (6h and 24h). Data on percentage leaf disc consumption, larval and pupal development, pupal mass, as well as trichome types and density data, were tested for homogeneity of variance (Levene's test) and normality (Shapiro-Wilk test). The percentage consumption of leaf discs in the no-choice and two-choice tests met the assumptions of homogeneity of variance and normality, and was analysed using One-way analysis of variance (ANOVA), followed by Tukey's HSD post hoc test to compare the means (no-choice) and for the two-choice test, a paired samples t-test was conducted. Percentage consumption of leaf discs in the multi-choice experiments, did not meet the assumptions of homogeneity of variance and normality, and was analysed using Friedman-ANOVA followed by Bonferroni-corrected Wilcoxon-matched-pairs test. Data on larval development time and pupal mass met the assumptions of normality and homogeneity of variance, and were analysed by means of one-way ANOVA followed by Tukey's unequal N HSD post hoc test. Data on pupal development times on the respective accessions, type and density of trichomes, did not meet the assumptions of normality and homogeneity of variance

and were analysed by means of the non-parametric, Kruskal-Wallis test followed by Dunn's multiple comparison post hoc test. All statistical analyses were performed with TIBCO® Statistica™ software version 13.5 (TIBCO software Inc., 2018).

## **2.5 Results**

### **2.5.1 Larval feeding choice-tests**

#### **2.5.1.1 *No choice test***

Significantly fewer larvae preferred to feed on the wild accession, PI 134417 compared to the Cross and three of the commercial accessions viz. Moneymaker, Commander and Emerald, six hours after they were provided with the plant material (Fig. 2.2). There was, however, no significant difference in feeding preference between PI 134417 and Esty at this time. Preference for the two wild accessions (LA 1777 and PI 134417) did also not differ significantly after six hours. However, after 24 hours, no significant preference could be detected for any of the accessions and feeding occurred on all accessions (Fig. 2.2).

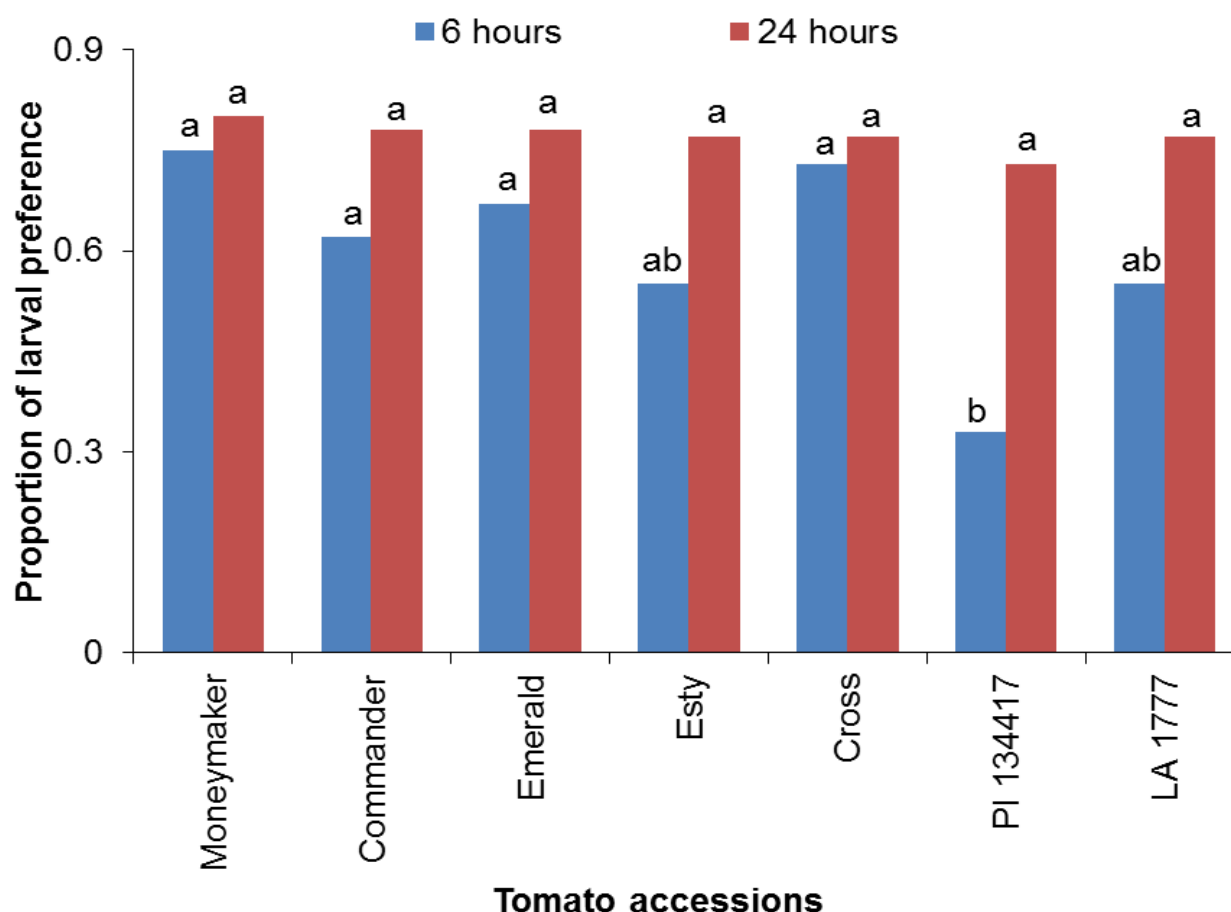


**Figure 2.2:** Preference of *Tuta absoluta* third-instar larvae in a no-choice test with tomato accessions after 6 and 24 hours. Preference by larvae for the respective tomato accessions was compared per recording time and analysed with binomial distribution tests followed by Bonferroni correction.

### 2.5.1.2 Two-choice test

In the two-choice tests, six hours after being provided with the leaf discs, more than a third of the larvae did not choose (NC) between the two accessions, in four out of the six combinations (Fig. 2.3A). Significantly more larvae chose to feed on MoneyMaker compared to Commander and the wild accession PI 134417, six hours after these accessions were provided. No significant preference was exhibited by the larvae for any of the other accessions, viz. cultivated accessions Emerald and Esty, Cross and the wild accession LA 1777. After 24 hours, most of the larvae chose between MoneyMaker and the respective accessions provided. The wild accession (PI 134417) was significantly less preferred for feeding compared to MoneyMaker. Larvae did, however, exhibit no significant feeding preference between MoneyMaker and any of the other accessions in the two- and multi-choice experiments (Fig. 2.3B).

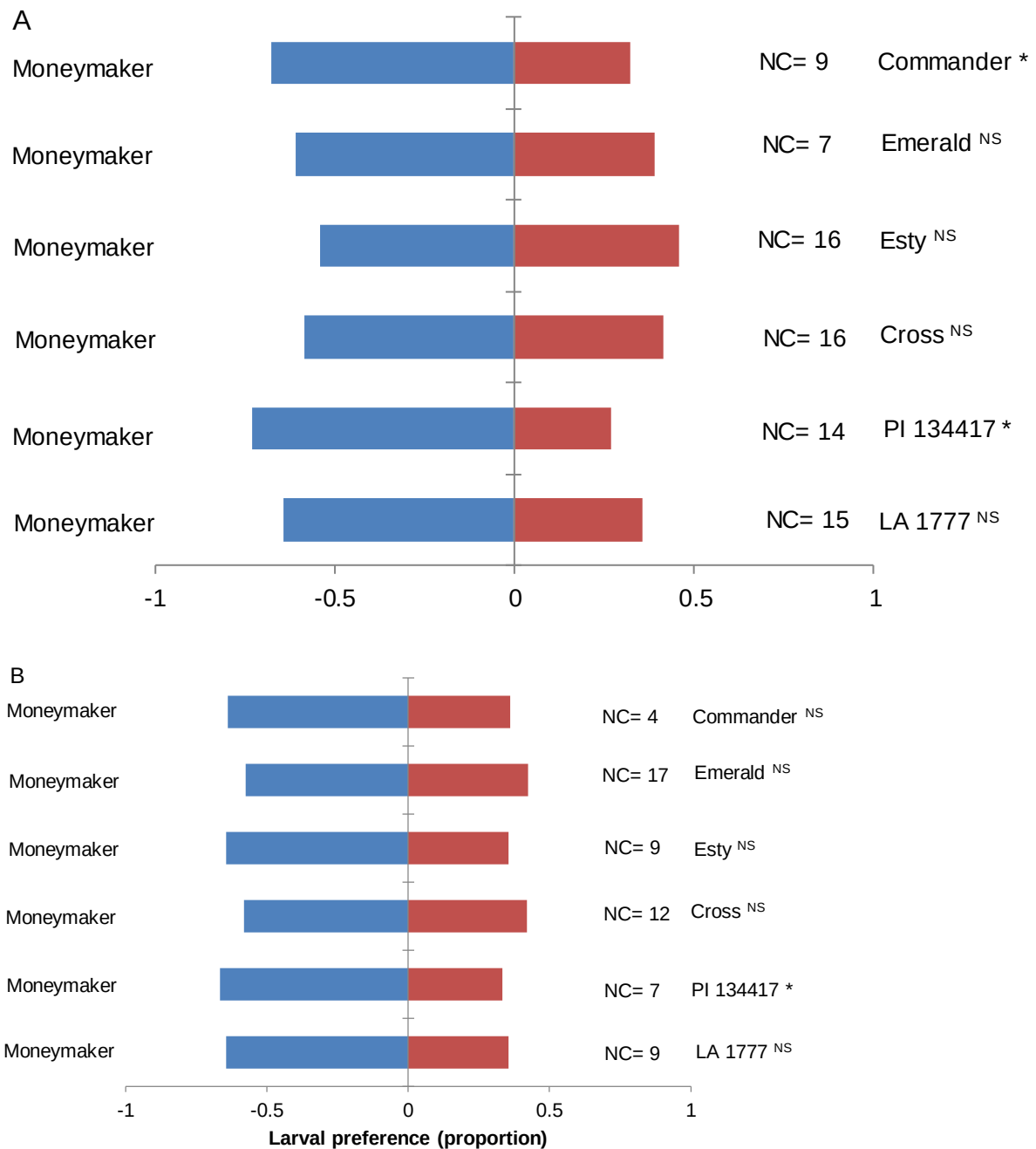


**Figure 2.3:** Preference of *Tuta absoluta* third-instar larvae in two-choice tests after **A.** 6 hours and **B.** 24 hours. \* indicates a significant difference in preference at  $P < 0.05$ , <sup>NS</sup> indicates no significant difference in preference between the two accessions and NC = the number of larvae (out of 40 per combination) that did not make a choice. (Binomial distribution tests followed by Bonferroni correction at  $P < 0.05$ ).

### **2.5.1.3 Multi-choice test**

Many larvae were wondering around inside the Petri dishes and did not make a choice between the three accessions, six hours after they were provided with leaf material (Fig. 2.4). No significant feeding preference was recorded for any of the accessions at this time, except in the combination with MoneyMaker x Esty x PI 134417 (wild accession), where the latter was significantly more preferred than MoneyMaker (Fig. 2.4). After 24 hours, most of the larvae had chosen between the accessions and settled on the leaves (Fig. 2.5). In the combination of MoneyMaker, Emerald and Cross, significantly more larvae preferred Emerald compared to MoneyMaker. The number of larvae that chose Emerald and the Cross, did, however, not differ significantly (Table 2.2). In the combination of MoneyMaker, Esty and PI 134417, a significant preference was shown for Esty (Table 2.2). *Tuta absoluta* larvae preferred MoneyMaker significantly more than PI 134417 when these two accessions were provided in a combination with LA 1777 (Fig. 2.5). There was, however, no significant difference in preference between PI 13447 and LA 1777 when this combination was provided (Table 2.2).

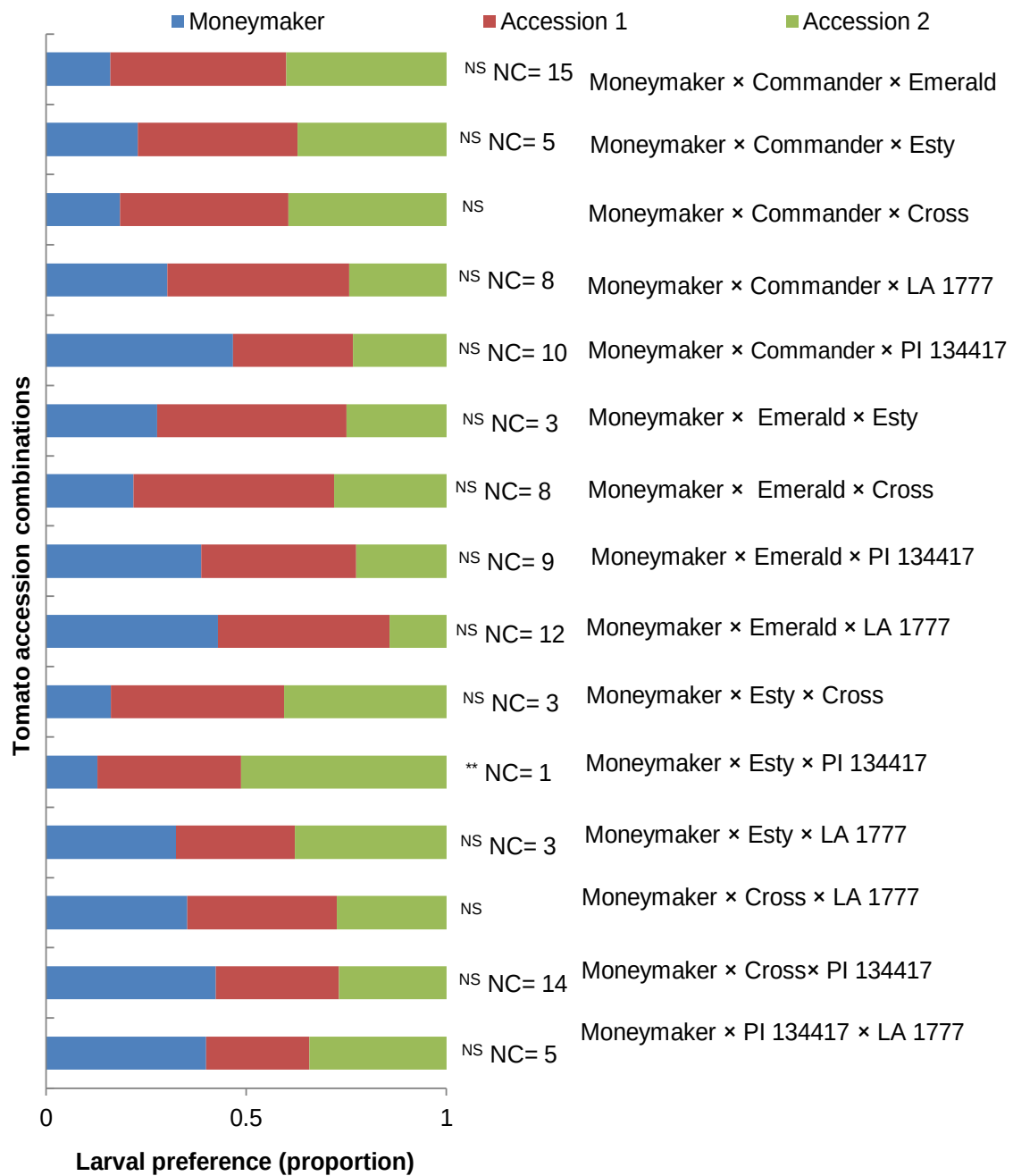


**Figure 2.4:** Preference of *Tuta absoluta* third-instar larvae for different tomato accessions in multi-choice tests after 6 hours. . 24 hours. \*\* indicates a significant difference in preference at  $P < 0.01$ , <sup>NS</sup> = no significant difference in preference between the three accessions and NC = the number of larvae (out of 40 per combination) that did not make a choice. Where NC value is not given, NC = 0. (Binomial distribution tests followed by Bonferroni correction at  $P < 0.05$ ).

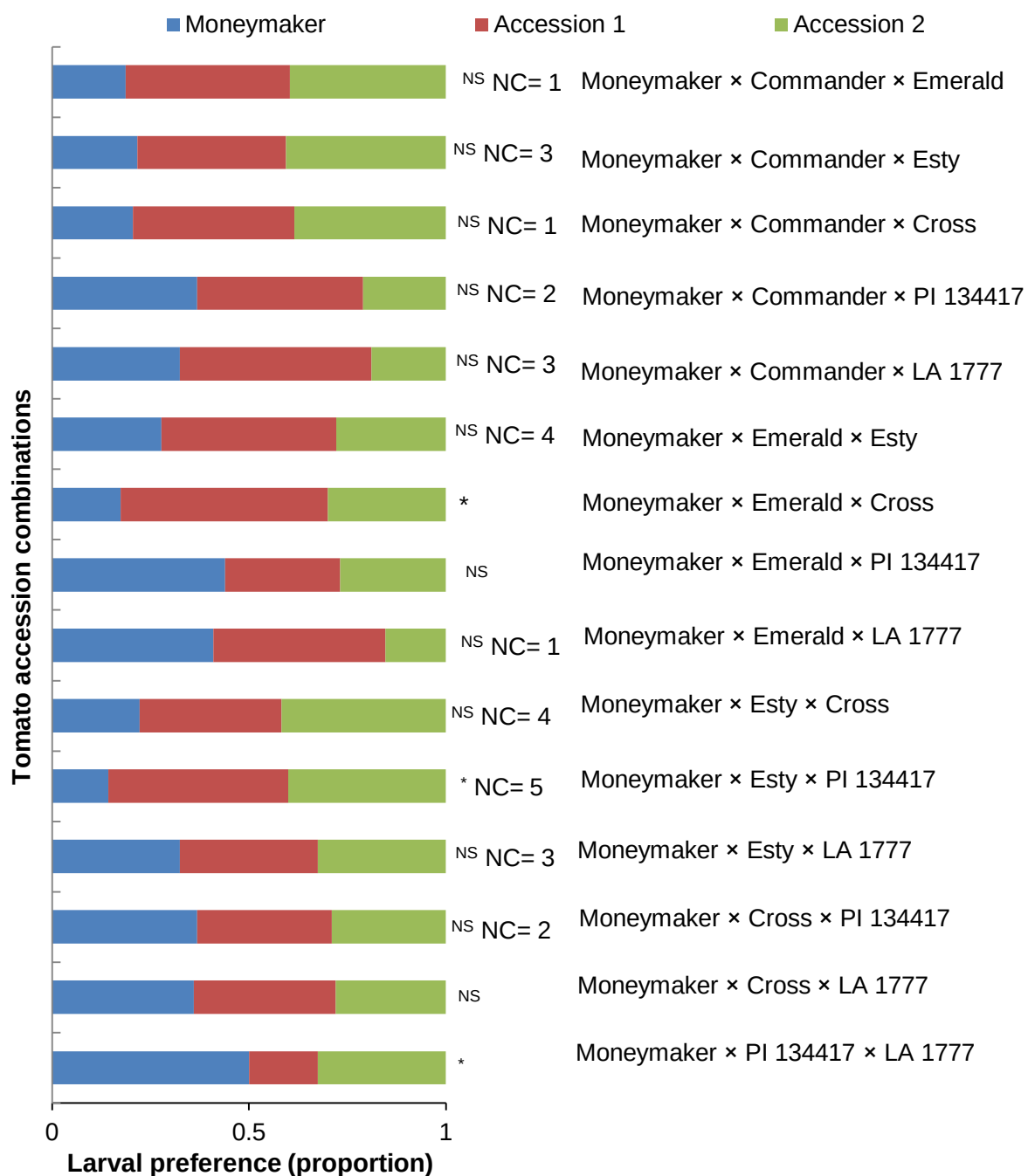


**Figure 2.5:** Preference of *Tuta absoluta* third-instar larvae for different tomato accessions in multi-choice tests after 24 hours, \* indicates a significant difference in preference at  $P < 0.05$  (see Table 2.2), <sup>NS</sup> = no significant difference in preference between the three accessions and NC = the number of larvae (out of 40 per combination) that did not make a choice. Where NC value is not given, NC = 0. (Binomial distribution tests followed by Bonferroni correction at  $P < 0.05$ ).

**Table 2.2:** Binomial distribution test result of the *Tuta absoluta* larval multi-choice preference tests with tomato accessions after 6 and 24 hours.

| Tomato accession combinations      | Specific combinations  | 6 hours<br>P - value | 24 hours<br>P - value |
|------------------------------------|------------------------|----------------------|-----------------------|
| Moneymaker × Commander × Emerald   | Moneymaker × Commander | NS                   | NS                    |
|                                    | Moneymaker × Emerald   | NS                   | NS                    |
|                                    | Commander × Emerald    | NS                   | NS                    |
| Moneymaker × Commander × Esty      | Moneymaker × Commander | NS                   | NS                    |
|                                    | Moneymaker × Esty      | NS                   | NS                    |
|                                    | Commander × Esty       | NS                   | NS                    |
| Moneymaker × Commander × Cross     | Moneymaker × Commander | NS                   | NS                    |
|                                    | Moneymaker × Cross     | NS                   | NS                    |
|                                    | Commander × Cross      | NS                   | NS                    |
| Moneymaker × Commander × PI 134417 | Moneymaker × Commander | NS                   | NS                    |
|                                    | Moneymaker × PI 134417 | NS                   | NS                    |
|                                    | Commander × PI 134417  | NS                   | NS                    |
| Moneymaker × Commander × LA 1777   | Moneymaker × Commander | NS                   | NS                    |
|                                    | Moneymaker × LA 1777   | NS                   | NS                    |
|                                    | Commander × LA 1777    | NS                   | NS                    |
| Moneymaker × Emerald × Esty        | Moneymaker × Emerald   | NS                   | NS                    |
|                                    | Moneymaker × Esty      | NS                   | NS                    |
|                                    | Emerald × Esty         | NS                   | NS                    |
| Moneymaker × Emerald × Cross       | Moneymaker × Emerald   | NS                   | *                     |
|                                    | Moneymaker × Cross     | NS                   | NS                    |
|                                    | Emerald × Cross        | NS                   | NS                    |
| Moneymaker × Emerald × PI 134417   | Moneymaker × Emerald   | NS                   | NS                    |
|                                    | Moneymaker × PI 134417 | NS                   | NS                    |
|                                    | Emerald × PI 134417    | NS                   | NS                    |
| Moneymaker × Emerald × LA 1777     | Moneymaker × Emerald   | NS                   | NS                    |
|                                    | Moneymaker × LA 1777   | NS                   | NS                    |
|                                    | Emerald × LA 1777      | NS                   | NS                    |
| Moneymaker × Esty × Cross          | Moneymaker × Esty      | NS                   | NS                    |
|                                    | Moneymaker × Cross     | NS                   | NS                    |
|                                    | Esty × Cross           | NS                   | NS                    |
| Moneymaker × Esty × PI 134417      | Moneymaker × Esty      | NS                   | *                     |

Table continues to the next page...

| Table 2.2 continues              |                        |    |    |
|----------------------------------|------------------------|----|----|
| Moneymaker × Esty × LA 1777      | Moneymaker × PI 134417 | ** | NS |
|                                  | Esty × PI 134417       | NS | NS |
|                                  | Moneymaker × Esty      | NS | NS |
|                                  | Moneymaker × LA 1777   | NS | NS |
|                                  | Esty × LA 1777         | NS | NS |
| Moneymaker × Cross × PI 134417   | Moneymaker × Cross     | NS | NS |
|                                  | Moneymaker × PI 134417 | NS | NS |
|                                  | Cross × PI 134417      | NS | NS |
| Moneymaker × Cross × LA 1777     | Moneymaker × Cross     | NS | NS |
|                                  | Moneymaker × LA 1777   | NS | NS |
|                                  | Cross × LA 1777        | NS | NS |
| Moneymaker × PI 134417 × LA 1777 | Moneymaker × PI 134417 | NS | *  |
|                                  | Moneymaker × LA 1777   | NS | NS |
|                                  | PI 134417 × LA 1777    | NS | NS |

Significance indicated by \* at  $P < 0.05$ ; \*\* at  $P < 0.01$  and NS = No significant difference

### 2.5.2 Consumption of leaf material

In the no-choice experiment during which a leaf disc of a single tomato accession was provided to larvae as food, a significantly larger area of Moneymaker leaves was consumed compared to the wild accession PI 134417 ( $F_{6, 195} = 2.13$ ,  $P = 0.05$ ). The consumed area of the leaves of the wild accession PI 134417 did, however, not differ from that of the other commercial accessions, viz. Commander, Emerald and Esty as well as from the Cross (Fig. 2.6). The percentage leaf areas consumed from the commercial accessions and wild accession LA 1777 also did not differ significantly.

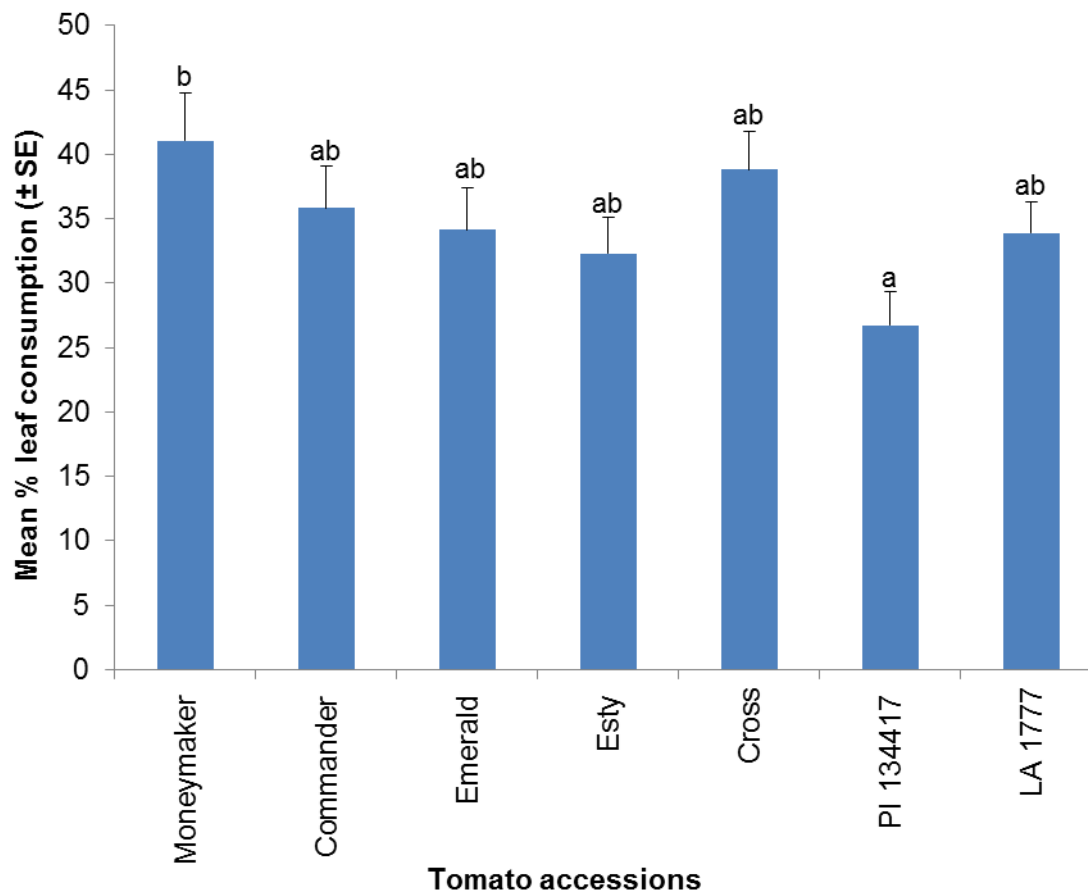


**Figure 2.6:** Consumption (mean % area) of leaf disc areas of different tomato accessions by *Tuta absoluta* third-instar larvae in a no-choice test, after 24 hours. Bars capped with the same letter do not differ significantly at  $P < 0.05$  (Tukey's HSD).

In the two-choice test (where Moneymaker was provided to the larvae together with another accession), a significantly larger leaf area was consumed from Moneymaker compared to Emerald, Esty, PI 1344417 and LA 1777 (Fig. 2.7). The area of the leaf discs consumed in the Moneymaker/Cross and Moneymaker/Commander combinations did not differ between the respective accessions ( $P > 0.05$ ) (Fig. 2.7).

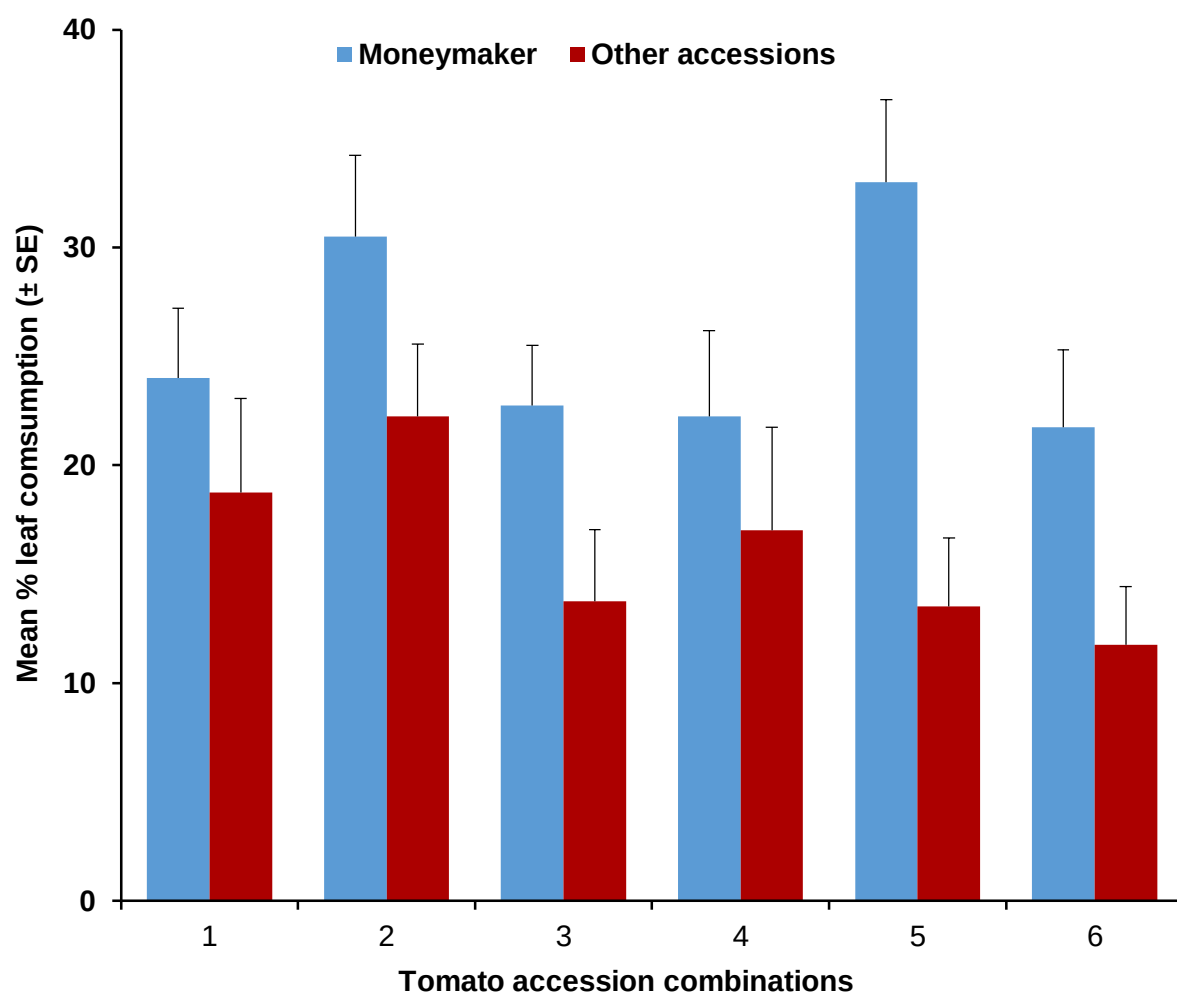


**Figure 2.7:** Consumption (mean % area) of leaf disc areas by *Tuta absoluta* third-instar larvae in choice tests with two tomato accessions after 24 hours. The accessions tested in combination with Moneymaker were: **1.** Commander; **2.** Emerald; **3.** Esty; **4.** Cross; **5.** PI 134417; **6.** LA 1777 (Paired samples t-test).

In the multi-choice tests, the percentage leaf material consumed by *T. absoluta* larvae did not differ significantly between three accessions provided in combinations where two of these accessions were Moneymaker and Commander, regardless if the third accession was one of the commercial accessions, Emerald or Esty, the Cross, or a wild accession PI 134417 or LA 1777 ( $P > 0.05$ ) (Fig. 2.8; Table 2.3). Significantly larger disc areas of the two commercial accessions, Moneymaker and Emerald were consumed when it was provided in combination with either one of the wild accessions, PI 134417 or LA 1777 (Fig. 2.8; Table 2.3). The percentage leaf disc consumed did not differ significantly in combinations including Moneymaker, Esty and any other accession. The percentage area consumed also did not differ

significantly between Moneymaker, Cross and the wild accession PI 134417. Larvae consumed significantly more of the Moneymaker leaf disc compared to the Cross and wild accession LA 1777 in their combination (Fig. 2.8). Significantly larger areas of the Moneymaker discs were consumed compared to those of the wild accessions (Fig. 2.8).

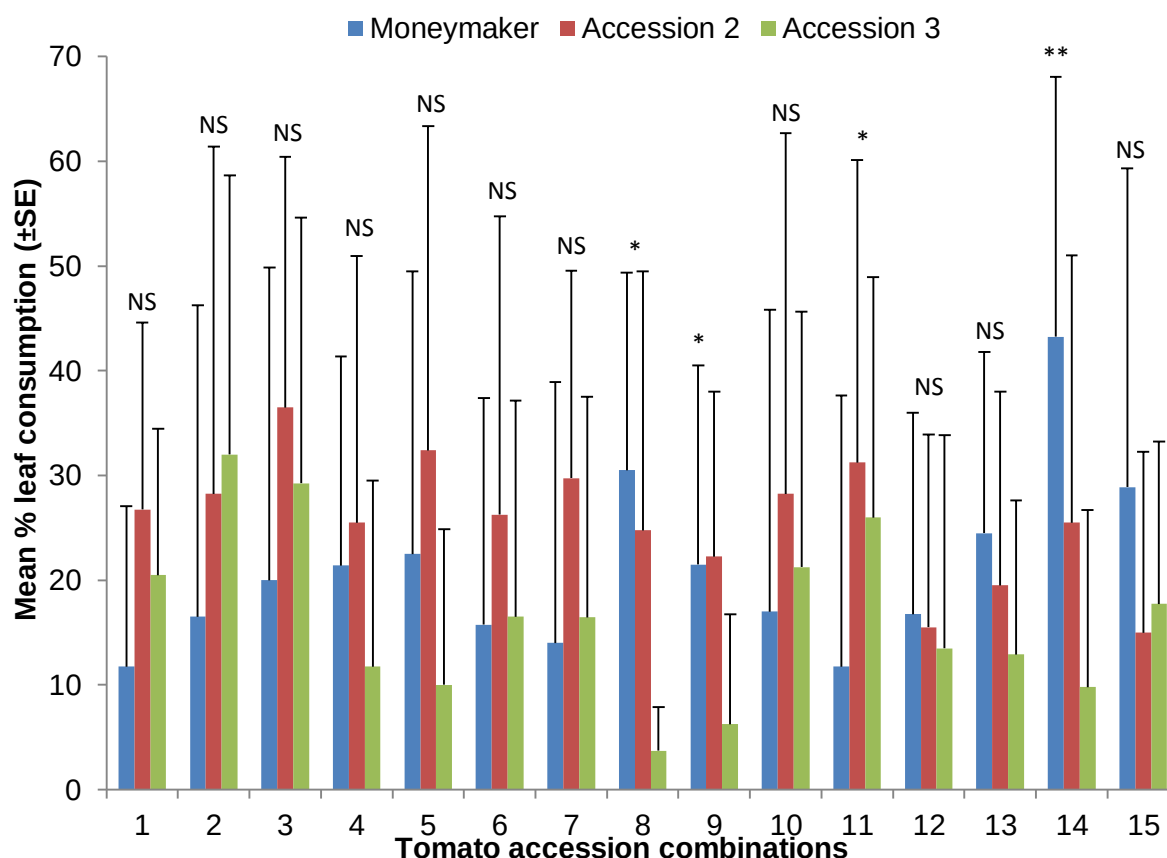


**Figure 2.8:** Consumption of leaf discs (mean  $\pm$  SE) by *Tuta absoluta* larvae (L3) in multi-choice tests with three tomato accessions after 24 hours. The accession tested in combination with Moneymaker were: **1.** Commander + Emerald; **2.** Commander + Esty; **3.** Commander + Cross; **4.** Commander + PI 134417; **5.** Commander + LA 1777; **6.** Emerald + Esty; **7.** Emerald + Cross; **8.** Emerald + PI 134417; **9.** Emerald + LA 1777; **10.** Esty + Cross; **11.** Esty + PI 134417; **12.** Esty + LA 1777; **13.** Cross + PI 134417; **14.** Cross + LA 1777 and **15.** PI 134417 + LA 1777. Significance determined with Friedman ANOVA is indicated by \*  $P < 0.05$ , \*\*  $P < 0.01$  and NS = No significant difference, ( $n = 20$  for all combinations).

**Table 2.3:** Results from the Friedman analyses of variance and Bonferroni-corrected Wilcoxon matched-pair test for percentage leaf disc areas consumed by *Tuta absoluta* third-instar larvae in multi-choice tests after 24 hours.

| nr | Tomato accession combinations                                                                                                                                         | Friedman ANOVA                    | Bonferroni-corrected Wilcoxon matched-pair test |
|----|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------|-------------------------------------------------|
| 1  | Money maker × Commander × Emerald                                                                                                                                     | $F_r = 5.00$ , 2 df, $P = 0.82$   | NS                                              |
| 2  | Money maker × Commander × Esty                                                                                                                                        | $F_r = 2.90$ , 2 df, $P = 0.35$   | NS                                              |
| 3  | Money maker × Commander × Cross                                                                                                                                       | $F_r = 3.97$ , 2 df, $P = 0.14$   | NS                                              |
| 4  | Money maker × Commander × PI 134417                                                                                                                                   | $F_r = 3.48$ , 2 df, $P = 0.17$   | NS                                              |
| 5  | Money maker × Commander × LA 1777                                                                                                                                     | $F_r = 5.38$ , 2 df, $P = 0.07$   | NS                                              |
| 6  | Money maker × Emerald × Esty                                                                                                                                          | $F_r = 1.10$ , 2 df, $P = 0.58$   | NS                                              |
| 7  | Money maker × Emerald × Cross                                                                                                                                         | $F_r = 3.39$ , 2 df, $P = 0.18$   | NS                                              |
| 8  | Money maker × Emerald × PI 134417                                                                                                                                     | $F_r = 0.83$ , 2 df, $P = 0.02$   |                                                 |
|    | <ul style="list-style-type: none"> <li>Money maker × Emerald</li> <li>Money maker × PI 134417</li> <li>Emerald × PI 134417</li> </ul>                                 |                                   | NS<br>***<br>**                                 |
| 9  | Money maker × Emerald × LA 1777                                                                                                                                       | $F_r = 8.20$ , 2 df, $P = 0.02$   |                                                 |
|    | <ul style="list-style-type: none"> <li>Money maker × Emerald</li> <li>Money maker × LA 1777</li> <li>Emerald × LA 1777</li> </ul>                                     |                                   | NS<br>NS<br>**                                  |
| 10 | Money maker × Esty × Cross                                                                                                                                            | $F_r = 1.32$ , 2 df, $P = 0.52$   | NS                                              |
| 11 | Money maker × Esty × PI 134417                                                                                                                                        | $F_r = 6.19$ , 2 df, $P = 0.04$   |                                                 |
|    | <ul style="list-style-type: none"> <li>Money maker × Esty</li> <li>Money maker × PI 134417</li> <li>Esty × PI 134417</li> <li>Money maker × Esty × LA 1777</li> </ul> | $F_r = 0.79$ , 2 df, $P = 0.67$   | NS<br>NS<br>*<br>NS                             |
| 12 | Money maker × Cross × PI 134417                                                                                                                                       | $F_r = 3.04$ , 2 df, $P = 0.22$   | NS                                              |
| 14 | Money maker × Cross × LA 1777                                                                                                                                         | $F_r = 12.33$ , 2 df, $P = 0.002$ |                                                 |
|    | <ul style="list-style-type: none"> <li>Money maker × Cross</li> <li>Money maker × LA 1777</li> <li>Cross × LA 1777</li> </ul>                                         |                                   | NS<br>**<br>*                                   |
| 15 | Money maker × PI 134417 × LA 1777                                                                                                                                     | $F_r = 2.03$ , 2 df, $P = 0.36$   | NS                                              |

Significance indicated by \*  $P < 0.05$ , \*\*  $P < 0.01$ , and NS = No significant difference.

### **2.5.3 *Tuta absoluta* development on tomato accessions**

Larval development times on the wild accession PI 134417 and the Cross were omitted from the analysis of data since larvae were unable to complete their development on these two accessions. Larval development was slow on these accessions, and the last surviving larvae died after 13 and 14 days on accession PI 134417 and the Cross, respectively. Larval mortality on LA 1777 (wild accession) was also high (Table 2.4), but some larvae completed their development on LA 1777. Larval development times on the four commercial accessions were significantly shorter than on LA 1777 ( $F_{4, 57} = 3.77$ ,  $P < 0.01$ ), for all four commercial accessions development times were similar. Pupal mass of larvae that were reared on the wild accession LA 1777 was also lower, albeit not significantly different from the pupal mass of larvae that fed on the four cultivated accessions, which also did not differ significantly ( $F_{4, 44} = 0.74$ ,  $P = 0.57$ ). Pupae reared from larvae that fed on the wild accession LA 1777 were not included in the analyses for development times because too few pupae were obtained. Development times of pupae from larvae that fed on the cultivated accessions were similar ( $H(3, N = 46) = 3.96$ ,  $P = 0.27$ ) and total development time (larvae-pupae) on the commercial accessions did not differ significantly ( $F_{3, 42} = 0.93$ ,  $P = 0.44$ ).

**Table 2.4:** Development time of *Tuta absoluta* larvae and pupae as well as pupal mass of larvae that fed on the respective tomato accessions.

| Tomato accessions | Larval mortality (%) | Larval development time (days) $\pm$ SE | Pupal mass (mcg) $\pm$ SE | Pupal development time (days) $\pm$ SE | Total development time (days) $\pm$ SE |
|-------------------|----------------------|-----------------------------------------|---------------------------|----------------------------------------|----------------------------------------|
| Moneymaker        | 13.3                 | 10.87 $\pm$ 1.25a                       | 4.21 $\pm$ 0.24a          | 7.08 $\pm$ 0.37a                       | 18.91 $\pm$ 0.53a                      |
| Commander         | 26.7                 | 10.86 $\pm$ 1.46a                       | 4.40 $\pm$ 0.40a          | 7.91 $\pm$ 0.40a                       | 18.91 $\pm$ 0.53a                      |
| Emerald           | 20.0                 | 10.36 $\pm$ 1.34a                       | 4.14 $\pm$ 0.24a          | 7.64 $\pm$ 0.40a                       | 18.00 $\pm$ 0.49a                      |
| Esty              | 26.7                 | 10.67 $\pm$ 1.84a                       | 4.13 $\pm$ 0.23a          | 7.09 $\pm$ 0.40a                       | 17.73 $\pm$ 0.53a                      |
| LA 1777           | 73.3                 | 13.85 $\pm$ 0.69b                       | 3.30 $\pm$ 0.52a          | <sup>#</sup> 7.33 $\pm$ 0.77           | <sup>#</sup> 21.00 $\pm$ 1.01          |
| Cross             | 100                  | -                                       | -                         | -                                      | -                                      |
| PI                | 100                  | -                                       | -                         | -                                      | -                                      |

Means followed by the same letter in a column are not significantly different at  $P < 0.05$  (Tukey's HSD). <sup>#</sup>Means not included in the analyses.

#### 2.5.4 Trichome types and densities on tomato accessions

The number of trichomes on the adaxial- (Table 2.5) and abaxial (Table 2.6) surface of leaves differed significantly between the respective accessions. No type 1 trichomes occurred on the adaxial leaf surface of the commercial accession (Esty), while significantly higher numbers of this trichome type was found on the adaxial leaf surface of Moneymaker than on Commander and Emerald. Both the accessions Cross and PI 134417 did not have any type 3 trichomes in the demarcated areas on the adaxial side of the leaves. Significantly higher numbers of these trichomes were found on Esty than on these two accessions. No type 5 trichomes occurred on the adaxial leaf surface of the Cross, PI 134417 and LA 1777, while significantly more of these trichomes were present on Esty than on Emerald. There was, however, no significant difference in the number of type 5 trichomes in the demarcated areas on the adaxial leaf surfaces of Moneymaker, Commander and Emerald. The number of

type 5 trichomes did also not differ between Moneymaker, Commander and Esty. Significantly fewer type 6 trichomes occurred on the adaxial leaf surface of Moneymaker compared to all other accessions, except PI 134417. Significantly fewer type 6 trichomes were also present on the adaxial leaf surface of PI 134417 than on the other wild accession, LA 1777.

**Table 2.5:** Trichome density as mean numbers ( $\pm$  SE) of different types of leaf trichomes in a demarcated area ( $0.87 \text{ mm}^2$ ), on the adaxial surface of wild and cultivated tomato accessions.

| Tomato accessions              | Leaf trichome types                   |                                      |                                      |                                       |                                       |
|--------------------------------|---------------------------------------|--------------------------------------|--------------------------------------|---------------------------------------|---------------------------------------|
|                                | 1                                     | 3                                    | 4                                    | 5                                     | 6                                     |
| Moneymaker                     | $3.08 \pm 0.58a$                      | $1.17 \pm 0.59ab$                    | $0.00 \pm 0.00a$                     | $1.08 \pm 0.36abc$                    | $0.50 \pm 0.19a$                      |
| Commander                      | $0.25 \pm 0.13bc$                     | $0.67 \pm 0.22ab$                    | $0.00 \pm 0.00a$                     | $2.33 \pm 0.68bc$                     | $5.00 \pm 0.93bc$                     |
| Emerald                        | $0.42 \pm 0.15bc$                     | $0.83 \pm 0.42ab$                    | $0.00 \pm 0.00a$                     | $0.25 \pm 0.13ab$                     | $3.67 \pm 0.56bc$                     |
| Esty                           | $0.00 \pm 0.00b$                      | $1.25 \pm 0.33b$                     | $0.00 \pm 0.00a$                     | $2.92 \pm 0.40c$                      | $4.17 \pm 0.68bc$                     |
| Cross                          | $1.67 \pm 0.45ac$                     | $0.00 \pm 0.00a$                     | $1.42 \pm 0.76ab$                    | $0.00 \pm 0.00a$                      | $4.58 \pm 0.51bc$                     |
| PI 134417                      | $0.92 \pm 0.23ab$                     | $0.00 \pm 0.00a$                     | $9.33 \pm 0.76c$                     | $0.00 \pm 0.00a$                      | $1.50 \pm 0.44ab$                     |
| LA 1777                        | $1.83 \pm 0.41ac$                     | $0.25 \pm 0.13ab$                    | $4.50 \pm 0.76bc$                    | $0.00 \pm 0.00a$                      | $5.83 \pm 0.04c$                      |
| Kruskal-Wallis test statistics | H (6, N = 84)<br>= 43.94<br>P < 0.001 | H (6, N = 84)<br>= 20.53<br>P < 0.01 | H (6, N = 84)<br>= 7.46<br>P < 0.001 | H (6, N = 84)<br>= 53.92<br>P < 0.001 | H (6, N = 84)<br>= 34.75<br>P < 0.001 |

Significantly higher numbers of type 1 trichomes occurred on the abaxial surface of Moneymaker leaves compared to that of the Cross and the wild accession, LA 1777 (Table 2.6). Although very few type 1 trichomes occurred on the cultivated accession Commander, it did not differ significantly from the number of type 1 trichomes on any of the other accessions. Significantly more type 3 trichomes were present on the abaxial leaf area of the Moneymaker and Emerald leaves, compared to the Cross, on which no type 3 trichomes were found. None of the cultivated accessions had any type 4 trichomes on the abaxial surfaces of the leaf. Significantly fewer type 4 trichomes were present on the abaxial side of the Cross compared to the wild accession, PI 134417. The number of type 4 trichomes on the abaxial leaf surfaces of the two wild accessions, PI 134417 and LA 1777, did however not differ significantly. Type 5 trichomes were, however, absent on the abaxial surface areas of these two wild accessions, as well as that of the Cross, while the number of these

trichomes on the abaxial leaf surface areas did not differ significantly between any of the commercial accessions. Significantly more type 6 trichomes were present in the demarcated areas on the adaxial leaf surface of the wild accession, LA 1777 compared to the commercial accessions Commander, Emerald and Esty, as well as the wild accession PI 134417. The number of type 6 trichomes on the abaxial leaf surface of the Moneymaker, Cross and LA 1777 did not differ significantly. The type 6 trichome on the abaxial leaf surfaces of the Cross was also significantly more than those present on Esty.

**Table 2.6:** Trichome density as mean number ( $\pm$  SE) of leaf trichome types in a demarcated area ( $0.87 \text{ mm}^2$ ), on the abaxial surface of wild and cultivated tomato accessions.

| Tomato accessions              | Leaf trichome types               |                                    |                                    |                                    |                                    |
|--------------------------------|-----------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
|                                | 1                                 | 3                                  | 4                                  | 5                                  | 6                                  |
| Moneymaker                     | $3.75 \pm 0.71a$                  | $1.7 \pm 0.71ab$                   | $0.0 \pm 0.00a$                    | $1.16 \pm 0.52ab$                  | $2.75 \pm 0.35abc$                 |
| Commander                      | $0.83 \pm 0.21ab$                 | $0.17 \pm 0.11ab$                  | $0.0 \pm 0.00a$                    | $4.00 \pm 0.51a$                   | $1.58 \pm 0.36ab$                  |
| Emerald                        | $2.42 \pm 0.68ab$                 | $1.50 \pm 0.42a$                   | $0.0 \pm 0.00a$                    | $2.67 \pm 0.50a$                   | $1.75 \pm 0.84ab$                  |
| Esty                           | $2.42 \pm 0.70ab$                 | $0.08 \pm 0.08ab$                  | $0.0 \pm 0.00a$                    | $2.67 \pm 1.00a$                   | $1.00 \pm 0.35a$                   |
| Cross                          | $0.67 \pm 0.28b$                  | $0.0 \pm 0.00b$                    | $3.00 \pm 0.49ab$                  | $0.0 \pm 0.00b$                    | $3.58 \pm 0.47bc$                  |
| PI 134417                      | $2.83 \pm 0.75ab$                 | $0.17 \pm 0.11ab$                  | $13.17 \pm 0.83b$                  | $0.0 \pm 0.00b$                    | $1.25 \pm 0.39ab$                  |
| LA 1777                        | $0.67 \pm 0.36b$                  | $0.33 \pm 0.14ab$                  | $5.67 \pm 1.51ab$                  | $0.0 \pm 0.00b$                    | $5.67 \pm 0.66c$                   |
| Kruskal-Wallis test statistics | H (6, N = 84) = 20.95<br>P < 0.05 | H (6, N = 84) = 25.24<br>P < 0.001 | H (6, N = 84) = 63.38<br>P < 0.001 | H (6, N = 84) = 49.68<br>P < 0.001 | H (6, N = 84) = 37.39<br>P < 0.001 |

Means followed by the same letter in a column are not significantly different at  $P < 0.05$  (Dunn's multiple comparison post hoc test).

Images of the adaxial leaf surfaces of Moneymaker and the wild accession PI 134417 taken with a Nikon AZ100M microscope are shown in Figure 2.9.

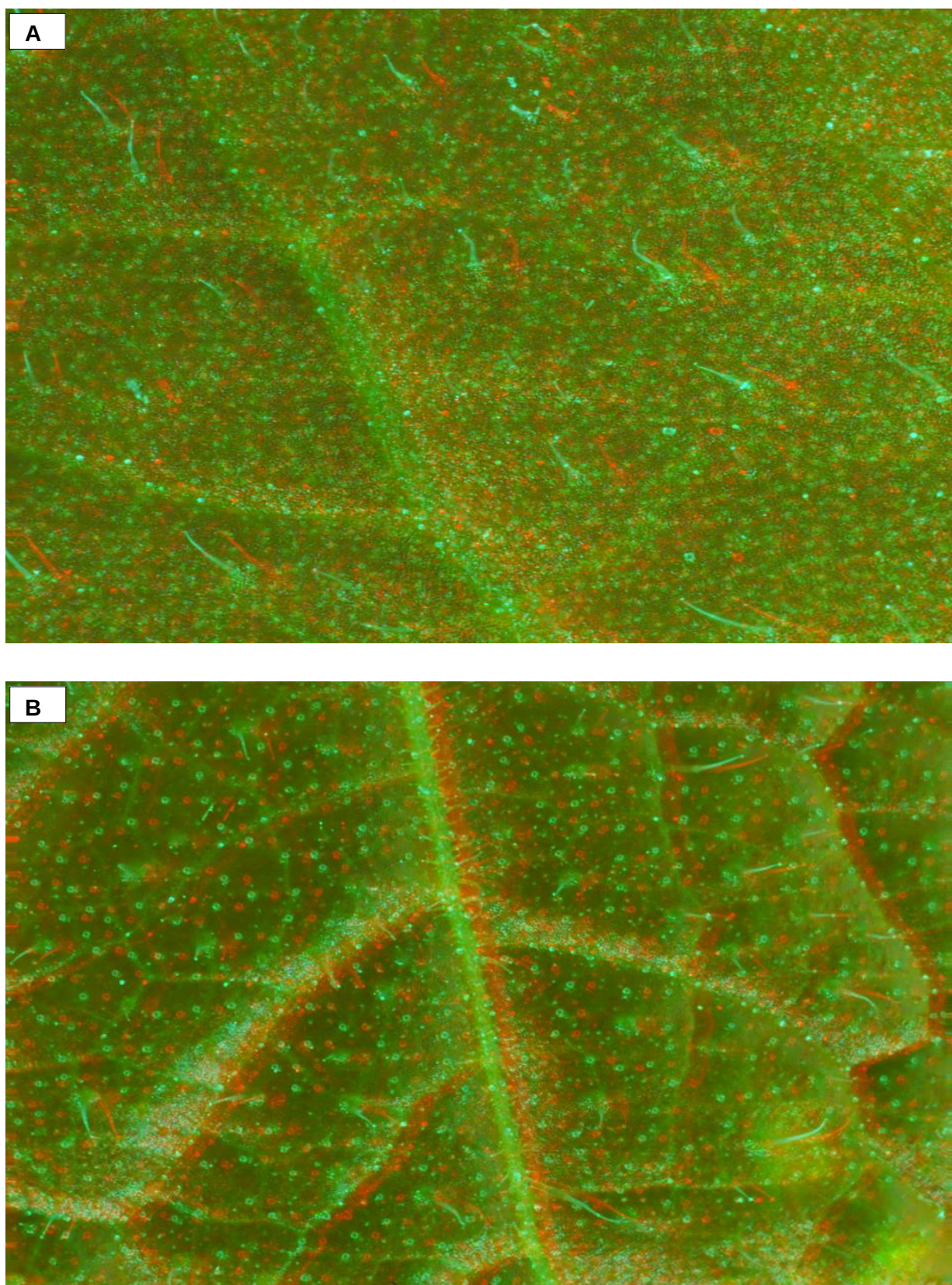


**Figure 2.9:** Adaxial leaf surfaces of two tomato accessions. **A** - commercial accession Moneymaker and **B** - wild accession PI 134417 (Note: these images should be viewed with 3D-glasses).

## 2.6 Discussion

Results confirmed the four commercial tomato accessions, viz. Moneymaker, Commander, Emerald and Esty to be highly suited for *T. absoluta* development. This explains the many complaints from farmers regarding infestations of their tomato crops by this insect pest in South Africa. Since biological parameters of insects reared on different host plants vary significantly, it also determines the expected pest load on the crop. Variations arise from the differences in the quality and quantity of nutrients as well as trichome type and density of the host plants (Nethononda *et al.* 2016; Ghaderi *et al.* 2017; Shahbaz *et al.*, 2019). The ingestion of trichomes by larvae affects their growth and development (Weinhold and Baldwin 2011).

Although larvae developed on leaf tissue of wild accession PI 134417 and the Cross, development was slow on these accessions and no larvae were able to survive and complete their life cycle. Larvae were able to complete their development on wild accession LA 1777; however, high larval mortality and slow larval development confirmed the unsuitability of this accession. These findings are consistent with the previous studies of Eigenbrode *et al.* (1996), Ecole *et al.* (2001), Leite *et al.* (2001), Vitta *et al.* (2016) and Oliveira *et al.* (2020), who reported these accessions to hamper the movement of *T. absoluta* larvae, as a result of the rupturing of glandular trichomes.

In tomatoes, trichomes are characterised into two distinct forms commonly known as glandular and non-glandular (Glas *et al.*, 2012). Trichomes of types 1, 4, and 6 are glandular while 3 and 5 are non-glandular (Channarayappa *et al.*, 1992; Glas *et al.*, 2012; Cho *et al.*, 2017). The presence of non-glandular trichomes is related to the greater susceptibility of insect pests (Gurr and McGrath, 2002), while glandular trichomes cause, on contact with the insect pest, physical entrapment with the secondary metabolites rupturing from their small membranous heads (Simmons *et al.*, 2003; Simmons and Gurr, 2004; Oliveira *et al.*, 2020). The presence of type 4 and 6 trichomes is associated with insect resistance in wild tomato accessions (Dimock and Kennedy, 1983; Chatzivasileiadis and Sabelis, 1997, Muigai *et al.*, 2002; Bawin *et al.*, 2016). Although chemical composition of these trichomes was not investigated in this study, three compounds which are known to occur in trichomes, i.e. acyl sugars, zingiberene, and 2-tridecanone, are known to be associated with

insect resistance (Vercosa de Magalhaes *et al.*, 2001; Antonious and Kochhar, 2003; Tissier, 2012; Oliveira *et al.*, 2012).

The different accessions showed significant variations in terms of morphological characteristics as well as trichome types and densities. Commercial accessions had no trichome type 4, but it occurred on the leaves of both wild accessions as well as the Cross. Many type 6 trichomes occurred on the leaves of LA 1777. The tips of type 6 trichomes in the commercial accessions are four-lobed structures, but those on the wild accessions are globular (Antonious, 2001). The high larval mortality found on PI 134417 could be ascribed to the predominant occurrence of type 4 trichomes. However, the low density of type 4 and 6 trichomes on the leaves of the Cross, which was less preferred by larvae contradict this observation. Wild accession LA 1777, which had greater densities of these trichome types, was found to partially support *T. absoluta* development. Thus, larval mortality on the Cross could therefore not be attributed to the high density of trichomes. Similar to these findings, *L. habrochaites* f. *glabratum* LA 1033 and LA 1777, were reported by Frelichowski and Juvik (2001) to have similar densities of type 4 trichomes, but their resistance to *Helicoverpa zea* (Hübner) (Lepidoptera: Noctuidae) and *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) differed. The presence of type 4 trichomes on LA 1033 had no effect on larval survival of *S. exigua* (89%) and its susceptibility was comparable to that of cultivated accessions. Nonetheless, although high larval mortality was found on LA 1777 in this study, Firdaus *et al.* (2012) stressed that its resistance to insect pests may deteriorate over time. Despite the occurrence of type 6 trichomes on leaves of commercial accessions, results from this study showed that commercial accessions did not have a negative effect on larval survival and development times of *T. absoluta*. This is in agreement with the high survival rate of *T. absoluta* larvae on commercial accessions reported by Bitew (2018). Although there was no significant difference, pupae reared on commercial accessions were heavier than those from larvae reared on the wild accession LA 1777. Since pupal mass is linearly related to the potential fecundity of an insect (Tammeru *et al.*, 2002; Pereyra and Sanchez, 2006), a higher level of infestation resulting from increased fecundity could be expected, following larval feeding on these accessions. The adult body size is significantly affected by feeding activities in the later stages of larval development while reproductive capacity increases with adult size (Berger *et al.*, 2006;

Gharekhani and Salek-Ebrahimi; 2014). Considering that insect development time is related to the nutritional quality of the host plant (Zalucki *et al.*, 2002; Ganbalani *et al.* 2016), shorter total development time for *T. absoluta* on commercial accessions was expected. Although longer pupa development times were recorded on commercial accessions Commander and Emerald when compared to LA 1777, Silva *et al.* (2015) suggests that such incompatibilities are common between development times predicted from constant development-temperature relationships. In addition, *T. absoluta* pupal development time is reported to last for 7-9 days (Pereyra and Sanchez, 2006; Erdogan and Babaroglu, 2014).

The high survival rate of *T. absoluta* on some of the accessions in this study, could not be ascribed to the presence or density of a particular trichome type only. However, glandular trichomes, particularly trichome type 4, did affect survival of *T. absoluta* larvae.

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## CHAPTER 3

### The suitability of selected tomato accessions for *T. absoluta* oviposition preference and larval host

#### 3.1 Abstract

Host plant resistance is an important component of integrated pest management strategies. Identification of accessions with insect resistance can be used in breeding programmes, and cultivation of such cultivars will contribute to improved pest management. The objectives of this study were to investigate the oviposition preference of *T. absoluta* moths for different tomato accessions and to evaluate these accessions for their suitability as larval hosts of this pest by rating the feeding damage caused by the larvae. The accessions included four commercially available cultivated accessions viz. Moneymaker, Commander, Emerald and Esty and two wild species viz. *Lycopersicon habrochaites* f. *typicum*, accession LA 1777 and *Lycopersicon habrochaites* f. *glabratum*, accession PI 134417. To determine *T. absoluta* oviposition preference, experiments were conducted under greenhouse conditions at  $24 \pm 0.5$  °C temperature and  $80 \pm 2\%$  relative humidity (RH), with no-choice and two-choice tests. In the no-choice test, moths oviposited significantly higher numbers of eggs on cultivated accessions compared to the wild accessions ( $F_{5, 66} = 13.55$ ,  $P < 0.001$ ). Similar observations were made when accessions were provided in two-choice tests with Moneymaker. To evaluate the tomato accessions for their suitability for larval hosts, experiment was conducted under semi-field condition in an insect-free screen-cage. The incidence of leaf mines caused by larval feeding was significantly lower in leaves of wild accessions, however, LA 1777 did not differ significantly from that of the cultivated accession, Esty. Moneymaker had significantly more active mines when compared to the other accessions. Wild accession PI 134417 had no active mines, 25 days after moths laid eggs on the plants. Cultivated accessions exhibited significantly higher degrees of plant damage compared to wild accessions. There were significant differences between the mass of pupae reared on Moneymaker and the other commercial accessions i.e. Commander, Emerald and Esty. The numbers of pupae reared on wild accession LA 1777 were very low and not included for analyses. No pupae were obtained from the wild accession PI 134417. The commercial accessions were therefore suitable for

development of *T. absoluta* larvae, in contrast to the wild accessions that were found not to be suitable for *T. absoluta* larval development.

**Key words:** Host suitability, *Lycopersicon*, oviposition preference, wild accessions

### 3.2 Introduction

The selection of host plants that are suitable for oviposition and larval development is a crucial step for herbivorous insects (Jaenike, 1990; Anderson *et al.*, 2011; Soler *et al.*, 2012; Batallas *et al.*, 2020). According to the oviposition-preference-offspring-performance hypothesis (preference-performance hypothesis) formulated by Jaenike (1978), female insects will choose a site on a host plant that will maximise the fitness and optimize the performance of the offspring. This suggests that female insects possess the ability to assess the quality of a host plant, since it affects the reproductive strategies of the insects and varies greatly between and within plant species (Buteler *et al.*, 2009; Molina and Trumper, 2012; König *et al.*, 2016; Varella *et al.*, 2017). However, the female oviposition preference and larval performance relationship might range from excellent to poor, especially in phytophagous insects (Thompson, 1988; Scheirs *et al.*, 2000). Female insects that oviposit on hosts that are less suitable for offspring performance, indicates a poor correlation between oviposition preference and host plant suitability (Jallow and Zalucki, 2003; Hufnagel *et al.*, 2017; Ali *et al.*, 2019). Biological parameters such as survival, reproduction, development time and longevity contribute to the propensity of the insect to persist, and are responsible for this discrepancy (Thompson 1988; Gripenberg *et al.* 2010).

Host plant quality, through physical and chemical resistance traits such as defensive compounds and trichomes, affects the behaviour and fitness of the herbivore insects (Awmack and Leather, 2002; Simmons and Gurr, 2005; Amin *et al.*, 2016; Meneses-Arias *et al.*, 2020). The characteristics of the plants are suggested to influence the decision by the female insect to select oviposition sites (Ramaswamy, 1988; Renwick and Chew, 1994) and, may interfere with the locomotion and feeding behaviour of the insect herbivore (Voigt *et al.*, 2007; Hagenbucher *et al.*, 2013). In tomato, the source of host plant resistance to herbivorous insects is associated with wild *Lycopersicon* accessions (Oliveira *et al.*, 2009; Antônio *et al.*, 2011; Lucini *et al.*, 2015; Sohrabi *et al.*, 2017). *Tuta absoluta* is a pest of solanaceous crops but feeds

mainly on tomato (Aynalem, 2018). Larvae cause damage to various plant parts, but mainly on leaves and fruit (Mohamed *et al.*, 2012). Host plant resistance is part of the fundamental pillars of IPM (Kogan, 1998). Identification of accessions with insect resistance for use in resistance breeding programmes, will contribute to improved pest management.

The objectives of this study were to determine oviposition preference of *T. absoluta* moths for six tomato accessions and screen their feeding preference for these accessions, under semi-field conditions.

### **3.3 Material and methods**

#### ***Tuta absoluta* rearing**

*Tuta absoluta* pupae were removed from the stock colony (see Chapter 2, section 2.2) and their sex was determined. The morphology of the genitalia visible on the last segments of the pupae were examined under a stereomicroscope (Nikon SMZ 1500), to determine the sex according to Genç (2016). The male and female pupae were placed separately in small plastic vials. On the day of eclosion, one male and one female moth were placed together in a vial for 24 hours to allow mating. Moths were provided with a piece of cotton wool, saturated with a 10% sugar water solution as a food source.

#### ***Tomato accessions***

Seed of six tomato accessions obtained from Sakata Vegetables Europe, Uchaud (France), and Sakata Seed Southern Africa (Pty) Ltd (Lanseria, Gauteng), were sown in styrofoam seedling trays in potting soil (potting mix, Culterra (Pty) Ltd., Muldersdrift). Plants were transplanted into 100 ml styrofoam cups. Plants for the semi-field trial were transplanted into 4L pots and were used 60 days after sowing (6-8 leaf stage). The tomato accessions were: two wild accessions *viz.* *L. habrochaites* f. *typicum*, accession LA1777 (hereafter referred to as LA 1777) and *L. habrochaites* f. *glabratum*, accession PI 134417 (referred to as PI 134417) and four commercially available cultivated accessions *viz.* Moneymaker, Commander, Emerald and Esty. All plants were watered three times a week and fertilised with a

multi-nutrient solution (Nutrifeed, Stark Ayres (Pty) Ltd., Benoni, Gauteng), one week after emergence and again three weeks later.

### 3.3.1 Oviposition preference tests

The oviposition preference of *T. absoluta* moths for the six tomato accessions was investigated in no-choice and two-choice tests. The choice tests were conducted using 60-day old potted plants. Plants were placed in plastic containers (37 × 25 × 24 cm) with ventilation openings, which were covered with a fine nylon mesh, on two opposite sides. The containers were kept in a greenhouse at  $24 \pm 0.5$  °C and  $80 \pm 2\%$  relative humidity (RH). Temperature and RH were recorded at 60-min intervals using iButtons® from ColdChain Thermo Dynamics (Fairbridge Technologies).

For the no-choice tests, a single potted plant was placed per container (Fig. 3.1A). In the two-choice test, Moneymaker was used as the standard accession. One plant of Moneymaker was placed per container in combination with one plant from another accession. Plants were approximately 15 cm apart (Fig. 3.1B). There were 12 replicates per accession in all the choice tests. For both tests, one male-female moth pair (placed together for 24 hours after eclosion), was released into each container. Plants were replaced every 24 hours, for a period of 72 hours, and the numbers of eggs per plant were recorded. A fine artist brush and handheld magnifying glass were used to remove the eggs from the plants.

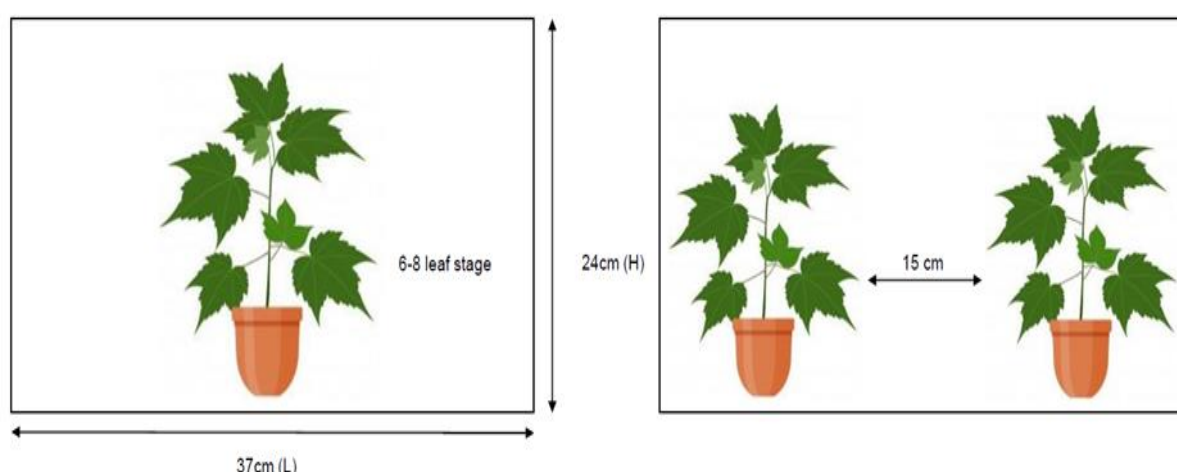


**Figure 0.1 A** - One plant per accession was placed per container in no-choice tests, and **B** - One seedling, from two different accessions, was placed per container in two-choice tests.

### **3.3.2 Semi-field screening of tomato accessions for susceptibility to *Tuta absoluta***

Potted plants were placed inside an insect-free screen-cage (6 m (l) × 4 m (w) × 2.7 m (h)) under prevailing environmental conditions. The experimental layout was a completely randomized block design with four blocks. Each block consisted of one row (four potted plants) of each of the six tomato accessions. The spacing between the pots within a row was 45 cm and 60 cm between the rows.

A total of 200 - 250 mated *T. absoluta* female moths were released into the cage. Feeding damage caused by larvae was rated. Three fully expanded leaves from the distal end of each plant were sampled 25 days after release of the moths. The total number of mines and active mines (with a larva inside) were counted and the lengths of the mines were measured as indication of larval feeding. The percentage of damaged leaves was assessed as follows: the number of infested leaves per plant/the-total number of leaves per plant) × 100. The length of the mines was classified into four categories, viz. < 1, 1-1.5, 1.6-2.0 and > 2.0 cm. After sampling, infested plant material was placed into containers that were kept in a rearing room at 26 ± 2°C, 65 ± 5% RH and 14L: 10D photoperiod. Daily collection of pupae was done from these containers and the pupae recovered from the plant material of each accession were weighed when they were 24 hours old.

### **3.4 Data analysis**

Data sets were analysed for normality (Shapiro-Wilks test) and homoscedasticity (Levene's test). To meet the assumptions, data from the no-choice oviposition experiments was square root transformed prior to analysis by means of one-way analysis of variance (ANOVA), but non-transformed data are presented in the results. Data from the two-choice oviposition tests did not meet the assumptions for normality and homoscedasticity, and were therefore analysed with the Wilcoxon signed-rank test. For the number of mines, percentage-damaged leaves and mines of sizes < 1 cm, 1-1.5 cm and 1.6-2, the data sets met the assumptions of normality (Shapiro-Wilks test) and homoscedasticity, and were analysed by means of one-way ANOVA, followed by Tukey's unequal N HSD post hoc test to compare the means. The number of active mines and mines greater than 2 cm did not meet the

assumptions of normality and homoscedasticity, and data were analysed by means of the non-parametric Kruskal-Wallis test followed by Dunn's multiple comparison post hoc test. Data on the percentage-damaged leaves was not normally distributed, but met the assumptions for homoscedasticity, and were analysed by means of Welch's ANOVA followed by the Bonferroni post hoc test. The level of significance was set at 5% and analyses were performed on TIBCO® Statistica™ software version 13.5, 2018 (Palo Alto, CA, USA).

### 3.5 Results

#### 3.5.1 Oviposition preference tests

*Tuta absoluta* moths oviposited on all six tomato accessions in the no-choice test (Table 3.1). Significantly fewer eggs were laid on the two wild accessions compared to the four commercial accessions ( $F_{5, 66} = 13.55$ ,  $P < 0.001$ ). There were no significant differences between the mean number of eggs laid on the four commercial accessions, viz. Moneymaker, Commander, Emerald and Esty.

**Table 0.1:** Mean number of eggs ( $\pm$ SE) laid by a single *Tuta absoluta* moth on tomato accessions in no-choice tests over a 72 hour period.

| Tomato accessions | Mean number of eggs $\pm$ SE |
|-------------------|------------------------------|
| Moneymaker        | 23.67 $\pm$ 1.83a            |
| Commander         | 22.25 $\pm$ 1.82a            |
| Emerald           | 21.58 $\pm$ 1.10a            |
| Esty              | 19.17 $\pm$ 2.15a            |
| LA 1777           | 9.83 $\pm$ 1.50b             |
| PI 134417         | 10.16 $\pm$ 1.51b            |

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

In the two-choice tests, Moneymaker was not significantly preferred for oviposition by *T. absoluta* moths to any of the three commercial accessions viz. Commander, Emerald or Esty. Moneymaker was, however, significantly preferred to the two wild accessions by *T. absoluta* moths for oviposition, with significantly more eggs laid on Moneymaker compared to the wild accessions, LA 1777 and PI 134417 in the respective two-choice tests (Fig. 3.2).

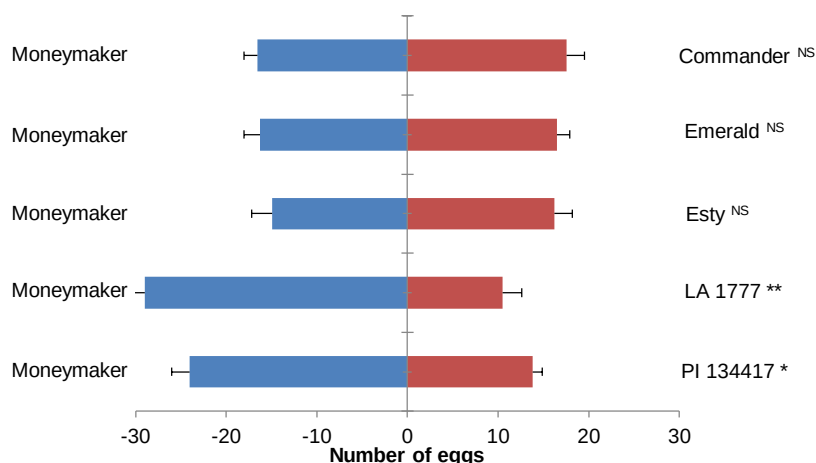


**Figure 0.2:** Mean number of *Tuta absoluta* eggs ( $\pm$ SE) laid in two choice tests with the respective accessions compared to MoneyMaker. The accessions tested in combination with MoneyMaker were Commander, Emerald, Esty, PI 134417 and LA 1777. (Wilcoxon signed-rank test). Significance indicated by \* at  $P < 0.05$  and \*\* at  $P < 0.01$ .

### 3.5.2 Semi-field screening of tomato accessions for susceptibility to *Tuta absoluta*

In the semi-field screening trial, the total number of mines differed significantly between the respective accessions ( $F_{5, 15} = 20.08$ ,  $P < 0.0001$ ) (Table 3.2). There was, however no significant difference in number of mines in the leaves of the two wild accessions, PI 134417 and LA 1777. Both the wild accessions, had significantly fewer mines compared to the commercial accessions, MoneyMaker, Commander, Esty and Emerald. MoneyMaker had a significantly higher number of *T. absoluta* mines in the leaves than any of the other commercial accession ( $P < 0.05$ ).

Examples of the damage to plants from the respective accessions are shown in figure 3.3. The active mines in MoneyMaker were significantly more than in any of the other accessions (Table 3.2). No active mines were found in the wild accession PI 134417, 25 days after release of the moths. The number of active mines in both wild accessions and the commercial accessions, Commander, Esty and Emerald, did

not differ significantly from each other. There was significantly less leaf damage on the wild accessions compared to the commercial accessions, with no significant difference in damage between the two wild accessions. The percentage of leaves damaged by *T. absoluta* on the four commercial accessions was similar.

**Table 0.2:** Mean number of mines ( $\pm$  SE) and active mines ( $\pm$  SE), 25 days after moth release, in three leaves from each of six tomato accessions and percentage damaged leaves caused by *Tuta absoluta*.

| Tomato accessions | Number of mines $\pm$ SE                                             | Active mines $\pm$ SE                                                    | Percentage damaged leaves (%)                               |
|-------------------|----------------------------------------------------------------------|--------------------------------------------------------------------------|-------------------------------------------------------------|
| Moneymaker        | 146.33 $\pm$ 8.11a                                                   | 92.33 $\pm$ 9.91 a                                                       | 95.43 $\pm$ 2.32a                                           |
| Commander         | 85.75 $\pm$ 14.53bc                                                  | 31.50 $\pm$ 06.79 b                                                      | 87.62 $\pm$ 2.33a                                           |
| Emerald           | 91.25 $\pm$ 6.30c                                                    | 46.25 $\pm$ 07.32 b                                                      | 89.32 $\pm$ 3.72a                                           |
| Esty              | 66.00 $\pm$ 15.31bc                                                  | 31.33 $\pm$ 14.9 b                                                       | 89.70 $\pm$ 3.60a                                           |
| PI 134417         | 14.75 $\pm$ 4.19d                                                    | 0.00 $\pm$ 0.00 b                                                        | 33.68 $\pm$ 2.00b                                           |
| LA 1777           | 37.00 $\pm$ 7.23d                                                    | 9.33 $\pm$ 03.76 b                                                       | 64.62 $\pm$ 4.09b                                           |
| Statistics        | F <sub>5,15</sub> = 20.08;<br>P < 0.0001<br>Tukey's unequal N<br>HSD | H(5, N = 21) =<br>17.40; P < 0.01)<br>Dunn's multiple<br>comparison test | F <sub>5,17</sub> = 62.53;<br>P < 0.0001<br>Bonferroni test |

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

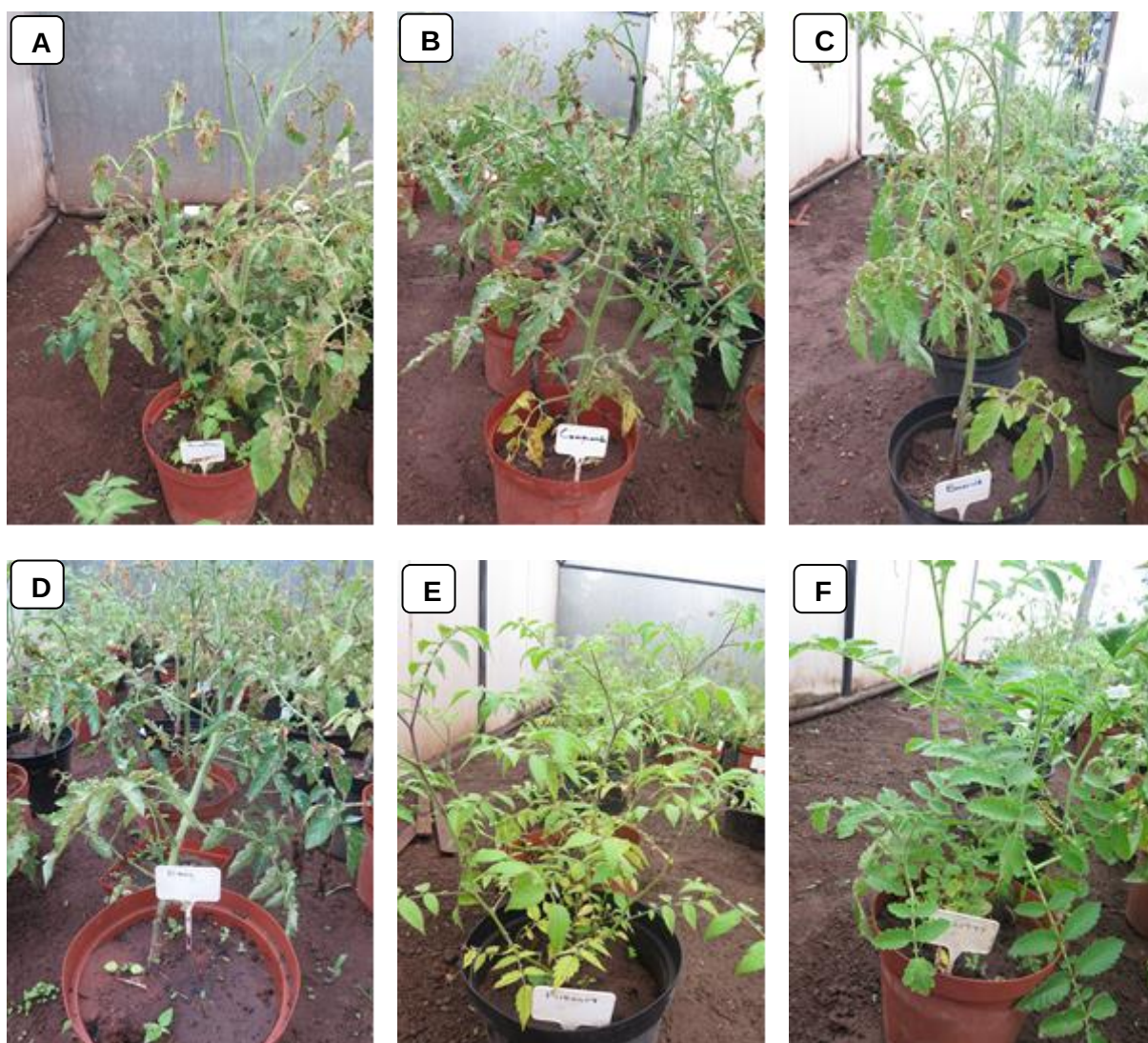


**Figure 0.3:** *Tuta absoluta* semi-field experiment. Damage inflicted to the respective accession: **A** - Moneymaker, **B** - Commander, **C** - Emerald, **D** - Esty, **E** - PI 134417 and **F** - LA 1777.

The number of mines per size category differed significantly within as well as between the respective accessions (Table 3.3). Significantly, more mines, that were less than 1 cm in length occurred on Moneymaker compared to all the other accessions ( $F_{5, 15} = 9.25$ ,  $P < 0.001$ ), with no significant difference between the other accessions. There was significantly fewer mines, 1-1.5 cm in length, on the wild accession, PI 134417, compared to Moneymaker, Emerald and Esty ( $F_{5,15} = 7.54$ ,  $P = 0.001$ ). The number of mines in the category, 1 - 1.5 cm did not differ between PI 134417 and the commercial accession Commander, but significantly, more mines in this category occurred on Moneymaker than on Esty. The cultivated accessions Moneymaker, Commander and Emerald had significantly more mines, 1.6-2 cm in

length, than the wild accessions ( $F_{5, 15} = 13.20$ ,  $P = 0.00005$ ). There was no significant difference in the number of mines (1.6 - 2cm) in Esty and LA 1777 leaves. The number of mines greater than 2 cm were significantly more on Moneymaker, compared to PI 134417, that did not have any mines in this category ( $H(5, N = 21) = 16.22$ ,  $P < 0.01$ ). Moneymaker had significantly more mines of less than 1 cm than 1-1.5, 1.6-2 and 2 cm ( $F_{3, 8} = 9.61$ ,  $P < 0.01$ ). Mines of all categories appeared in numbers that did not differ significantly, in Commander ( $F_{3, 12} = 2.61$ ,  $P > 0.05$ ) as well as Esty ( $F_{3, 8} = 0.31$ ,  $P > 0.05$ ). The number of mines greater than 2 cm on Emerald were significantly less compared to those shorter than 1 cm and between 1.6-2 cm. ( $F_{3,12} = 6.01$ ,  $P = 0.01$ ). Very few mines occurred in PI 134417, regardless of the mine lengths. However, the number of mines shorter than 1 cm in length, were significantly more than those longer than 2 cm, since no mines exceeding 2 cm, occurred in PI 134417 ( $F_{3,12} = 3.73$ ,  $P < 0.05$ ). The number of short mines in the wild accession LA 1777 (<1 and 1–1.5 cm) was significantly more than mines exceeding 1.5 cm ( $F_{3, 8} = 7.43$ ,  $P = 0.01$ ).

**Table 0.3:** Means ( $\pm$ SE) of different mine sizes obtained on different wild and cultivated tomato accessions

| Tomato accessions | < 1 cm             | 1-1.5 cm             | 1.6-2 cm            | > 2 cm              |
|-------------------|--------------------|----------------------|---------------------|---------------------|
| Moneymaker        | 57.00 $\pm$ 5.69aA | 31.00 $\pm$ 1.73aB   | 29.00 $\pm$ 2.65aB  | 29.33 $\pm$ 5.93aB  |
| Commander         | 25.75 $\pm$ 6.25bA | 15.75 $\pm$ 4.61abcA | 29.50 $\pm$ 3.84aA  | 14.75 $\pm$ 2.66abA |
| Emerald           | 29.25 $\pm$ 3.38bA | 20.25 $\pm$ 1.89abAB | 28.25 $\pm$ 4.48aA  | 13.50 $\pm$ 1.19abB |
| Esty              | 18.00 $\pm$ 9.07bA | 13.33 $\pm$ 5.24bcA  | 20.67 $\pm$ 3.71acA | 14.00 $\pm$ 5.51abA |
| PI 134417         | 6.75 $\pm$ 1.49bA  | 4.75 $\pm$ 1.49cAB   | 3.25 $\pm$ 1.49bcAB | 0.00 $\pm$ 0.00bB   |
| LA 1777           | 21.67 $\pm$ 5.24bA | 8.33 $\pm$ 3.33bcAB  | 6.00 $\pm$ 1.73abB  | 1.00 $\pm$ 0.58abB  |

Means within the column followed by the same lower case letter, and within the row, followed by the same upper case letter, are not significantly different at  $P < 0.05$  (Tukey's HSD).

*Tuta absoluta* pupae reared from larvae that fed on Moneymaker were significantly heavier than those from larvae reared on Commander, Emerald and Esty ( $F_{3,28} = 18.503$ ,  $P < 0.0001$ ). Larvae did not develop to the pupal stage on PI 13447, and only a few pupated from LA 1777. These were, however, not enough to be included in this analysis.

**Table 0.4:** Mean mass ( $\pm$ SE) of *Tuta absoluta* pupae recovered from tomato genotypes grown under semi-field conditions.

| Tomato accessions | Pupae mass (mg)   |
|-------------------|-------------------|
| Moneymaker        | 27.60 $\pm$ 1.11b |
| Commander         | 21.20 $\pm$ 0.48a |
| Emerald           | 21.20 $\pm$ 0.65a |
| Esty              | 20.80 $\pm$ 0.55a |

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

### 3.6 Discussion

When given a choice, *T. absoluta* oviposited more on commercial than wild accessions. Moths preferred to lay eggs on the upper third of the commercial accessions while those found on the wild accessions were fewer and immensely scattered. Results from this study confirmed the high suitability of commercial tomato accessions, especially Moneymaker, for development of *T. absoluta* larvae. Given the low numbers of eggs and mines found on the wild accessions, antixenosis (non-preference) and antibiosis (performance) mechanisms may be present. For wild tomato accessions, antixenosis is associated with chemical compounds in the plant tissues (Vitta *et al.*, 2016). Tomato leaf volatiles fulfil a significant role in the host finding by mated *T. absoluta* females. Volatile cues contain certain information that indicates the suitability of the host for larvae (Proffit *et al.*, 2011). In agreement to the present study, Labory *et al.* (1999), Proffit *et al.* (2011), Vosman *et al.* (2018) and Savi *et al.* (2019) reported low numbers of eggs on, and damage inflicted to wild accessions when compared to commercial accessions. Allelochemicals in wild accessions have been reported to be associated with lower levels of insect oviposition (Maluf *et al.*, 2010; Dias *et al.*, 2013). More eggs were laid on the

commercial accessions in this study. It can be ascribed to the ability of *T. absoluta* to detect inter-accession variation (Proffit *et al.*, 2011).

The low incidence of mines, active mines and fewer large mines on the wild accessions by *T. absoluta* larvae indicate the avoidance of these accessions by this pest. *Tuta absoluta* larvae are deterred from feeding by the antibiotic effect of plants, as well as the toxic compounds omitted during leaf mesophyll feeding. Vitta *et al.* (2016) reported that the antibiotic effect of tomato plants on *T. absoluta* larvae might be acute on young larvae and result in mortality in older larvae. To evaluate the level of insect resistance, the number of small mines is taken into consideration, together with the numbers of larger mines (Suinaga *et al.*, 2004). In the present study, given the continuous oviposition period during the 25 days, a constant increase in mines of all lengths was realised on the commercial accessions. Moths continued to lay eggs on these accessions and increased the numbers of larvae that emerged over time. Consequently, more active mines, which increased in length over time, occurred on the commercial accessions. Most mines on the wild accessions were short, and inactive. Larvae that left their mines were assumed to have left due to not finding these accessions suitable for establishment (antixenosis) and/or development (antibiosis) (Ecole *et al.*, 1999).

In general, older larvae in Lepidoptera species migrate by walking or ballooning (Zalucki *et al.*, 2002). *Tuta absoluta* third-instar larvae exhibit good locomotion (Galdino *et al.*, 2011). Wild accession LA 1777 had very few larger mines on certain plants. Through closer inspection, it was detected that larvae migrated from cultivated accessions, which had very heavy infestations in search for food. The numbers of active mines observed in this accession had many late-instar larvae despite the continuous emergence of larvae. Although LA 1777 is reported to have resistance to herbivory, including to *T. absoluta* (Eigenbrode and Trumble, 1993; Ecole *et al.*, 2001; Frelichowski and Juvik, 2001; Gurr and McGrath, 2001; Muigai *et al.*, 2002; Bitew, 2018), this accession was able to maintain the pest in this study, albeit in very low numbers. The continued feeding of *T. absoluta* on this accession may therefore indicate moderate resistance towards this pest.

The commercial accessions investigated in this study were all susceptible to *T. absoluta* infestations and consequently suffered feeding damage by the pest. The

parameters studied as possible indicators of resistance, did however show slight variations. Moneymaker sustained high infestation levels, and pupae from larvae that fed on this accession, were heavier than those from the other commercial accessions in this study. Although not investigated in this study, these differences may be attributed to characteristics such as nutrients provided as well as primary and secondary compounds that may relate to insect resistance (Ghaderi *et al.*, 2017).

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## CHAPTER 4

### Conclusion and recommendations

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a key and devastating pest of tomato (*Solanum lycopersicum* L.), which damages plants through its leaf-mining activity (Biondi *et al.*, 2018; Mansour *et al.*, 2018). The endophytic feeding behaviour of larvae as well as the rapid evolution of insecticide resistance in field populations, challenges current management tactics which largely relies on application of insecticides (Lietti *et al.* 2005; Gebremariam, 2015; Mansour *et al.*, 2018; Biondi *et al.*, 2018).

The development of resistant accessions has over the years received much attention since host plant resistance is an invaluable component of integrated pest management systems (Berlinger, 1986; Guedes and Picanço 2012; Smith and Clement, 2012; Terzidis *et al.*, 2014). Host plant resistance influences the fitness of insect pests and subsequently their population dynamics, the quantification thereof may provide valuable insights into the development and use of resistant cultivars (Tsai, 1998; Tsai and Wang, 2001; Smith, 2005; Amin *et al.*, 2016). It affects most of the life history parameters viz. mating, oviposition, feeding, growth and development. In this study, the fitness of *T. absoluta* was evaluated on different tomato accessions characterised by different morphological characteristics.

Lepidoptera species, including *T. absoluta*, locate their host plants through semiochemicals emitted from leaves and glandular trichomes (Khederi *et al.*, 2014; Ataide *et al.*, 2017; Msisi *et al.*, 2020). In this study, *T. absoluta* moths oviposited on leaves of all the tested tomato accessions, but the number of eggs differed, which showed preference for certain accessions. The number of eggs laid is largely determined by the leaf contact perception of the female moth (Proffitt *et al.*, 2011). Renwick and Chew (1994) reported that physical and chemical characteristics of the leaf determine its suitability for oviposition. Despite the low oviposition preference for the wild accessions, female moths laid eggs on all of the accessions included in this study. The preference-performance hypothesis, which suggests that species evolve to oviposit on host plants on which their offspring will thrive (Gripenberg *et al.*, 2010), and was found in this study also. The first-instar larvae, which are small in size, were unable to locate the feeding site and develop on the leaf surfaces of wild accessions and the Cross (Chapter 2). Eigenbrode *et al.* (1996) reported that glandular trichomes rupture as first-instar larvae move over them in

search of feeding sites on *Solanum hirsutum* f. *typicum* accessions. These exudates from the glandular trichomes contribute to the physical and chemical mechanisms responsible for resistance to herbivorous insects (Tingey and Gibson, 1978; Goffreda *et al.*, 1988). Conversely, late-instar larvae during feeding preference and semi field experiments (Chapter 2 and 3) showed the capability to feed extensively on some of the accessions characterised by morphological appearance associated with resistance (Oliveira *et al.*, 2009). Galdino *et al.* (2015) reiterated that such behaviour is influenced by the tendency of larvae to develop tolerance to toxins and probably stronger mandibles that allow them to establish on well-defended leaves. This suggests that the preference for wild accession LA 1777 by late instar larvae to develop would as a result favour grazer-like behaviour (parasite/grazer hypothesis suggests that individuals will move between the plant species to complete development) of this pest. In addition, the findings of this study suggests that LA 1777 is a sub-optimal host for early instars, but more suitable for late instar larva. The behaviour by late-instar larvae (third and fourth) is therefore suggested to show partial support for the hierarchy threshold model, which suggests that each host plant has an acceptability level for the insect, based upon the balance of factors internal to the insect and sensory cues received from the host plant (Withers, 1999).

Considering the development times, survival rate and numbers and lengths of mines of *T. absoluta* on the accessions included in this study, it is reasonable to conclude that the difference in the morphological characteristics of the different accessions had a significant influence on the preferences of *T. absoluta*. Trichome types 4, which are not present in commercial accessions, and type 6, are the sources of some of the compounds associated with resistance to *T. absoluta* (Khederi *et al.*, 2014). Chatzivasileiadis *et al.* (1999) reported that trichome type 6 which occurs on leaves of Moneymaker exuded compounds of lower concentration and that the efficacy of these compounds depended on the density of the trichomes. In their study, these authors found that for these compounds to cause 50% mortality, *Tetranychus urticae* Koch (Acari: Tetranychidae) needs to come into contact with dense trichomes at least 88 times per day in the case of Moneymaker and only two times on PI 134417. In this study, Moneymaker was observed to have less-dense trichome type 6 on the leaves. A high density of glandular trichomes in general was observed to occur on the stems of all cultivated accessions, assuming that the level of glandular trichome density of this nature could be of assistance for insect resistance if accessions with more trichomes on the leaf surfaces could be achieved through plant breeding.

Presence of only non-glandular trichomes, especially trichome type 5, is associated with very low levels of pest resistance (Firdaus *et al.*, 2012; Rakha *et al.*, 2017). It was reported to provide a suitable microclimate for eggs and protection for eggs and larvae from natural enemies (Berlinger 1986; Butter and Vir, 1989). Gurr and McGrath (2002) reported a positive correlation between the incidence of type 5 trichomes and susceptibility of tomato cultivars to *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae).

Given the findings of this study, it is apparent that the characteristics responsible for insect resistance in wild accessions and the Cross, were not present in cultivated accessions. These characteristics are linked to the presence of glandular trichomes, particularly type 4. It is recommended that further studies be conducted to elucidate the causal mechanisms of resistance in these accessions. The observation that older instar larvae of *T. absoluta* were able to establish on leaves of wild accession LA 1777, shows that there are other mechanisms and/or factors involved that are not known. The threat of loss of resistance over time by this accession needs to be brought to attention, as it possesses at least one of the promising resistance traits.

It is recommended that further studies be done to understand the mechanisms of resistance that are present in the wild accessions and the Cross used in this study.

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## **Appendix A:** Language editing

### Declaration of language editing

#### Language editing statement

To whom this may concern,

I, Prof. J van den Berg, hereby declare that the thesis titled: “Evaluation of tomato cultivars for resistance to *Tuta absoluta* (Lepidoptera: Gelechiidae)” by RB Mphahlele 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

27 November 2020

Date

## Appendix B: Ethics letter



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### ETHICS APPROVAL LETTER OF STUDY

Based on approval by the **Faculty of Natural and Agricultural Sciences Ethics Committee (FNASREC)**, the Faculty of Natural and Agricultural Sciences Ethics Committee hereby **approves** your study as indicated below. This implies that the North-West University Senate Committee for Research Ethics (NWU-SCRE) grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

|                                                                                                                                                                                                                                                               |             |   |   |   |                       |         |   |   |   |      |   |   |        |   |   |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|---|---|---|-----------------------|---------|---|---|---|------|---|---|--------|---|---|
| <b>Study title: Evaluation of tomato cultivars for resistance against Tuta absoluta (Lepidoptera: Gelechiidae)</b>                                                                                                                                            |             |   |   |   |                       |         |   |   |   |      |   |   |        |   |   |
| <b>Study Leader/Supervisor: Prof MJ du Plessis</b>                                                                                                                                                                                                            |             |   |   |   |                       |         |   |   |   |      |   |   |        |   |   |
| <b>Student: RB Mphahlele</b>                                                                                                                                                                                                                                  |             |   |   |   |                       |         |   |   |   |      |   |   |        |   |   |
| <b>Ethics number:</b>                                                                                                                                                                                                                                         | N           | W | U | - | 0                     | 1       | 5 | 2 | 4 | -    | 2 | 0 | -      | A | 9 |
|                                                                                                                                                                                                                                                               | Institution |   |   |   | Study Number          |         |   |   |   | Year |   |   | Status |   |   |
| Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation                                                                                                                                                                   |             |   |   |   |                       |         |   |   |   |      |   |   |        |   |   |
| <b>Application type:</b>                                                                                                                                                                                                                                      | Single      |   |   |   | <b>Risk Category:</b> | Minimal |   |   |   |      |   |   |        |   |   |
| <b>Commencement date: 01/02/2020</b>                                                                                                                                                                                                                          |             |   |   |   |                       |         |   |   |   |      |   |   |        |   |   |
| <b>Expiry date: 01/04/2021</b>                                                                                                                                                                                                                                |             |   |   |   |                       |         |   |   |   |      |   |   |        |   |   |
| <b>Approval of the study is initially provided for a year, after which continuation of the study is dependent on receipt and review of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.</b> |             |   |   |   |                       |         |   |   |   |      |   |   |        |   |   |

Special in process conditions of the research for approval (if applicable):

- The following documentation are archived by FNASREC and should be complete and kept up to date:
  - Research proposal
  - Signed approval from the scientific committee indicating the proposed risk category
- All researchers involved in the study should submit signed NWU code of conduct statements annually.
- All researchers of low risk studies should submit proof of relevant ethics training every two years.
- All researchers that take part in activities that pose a safety and security threat to the researchers or the environment should submit a risk assessment form annually.
- All research involving human interaction should follow best ethical practise and keep documents as proof. This includes informed consent, questionnaires, incorporation of risk-benefit, and responsible data management.
- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the FNASREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

**General conditions:**

*While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:*

- *The study leader/supervisor (principle investigator)/researcher must report in the prescribed format to the FNASREC:*
  - *annually (or as otherwise requested) on the monitoring of the study, whereby a letter of continuation will be provided, and upon completion of the study; and*
  - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the study leader/researcher must apply for approval of these amendments at the FNASREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *Annually a number of studies may be randomly selected for an external audit.*
- *The date of approval indicates the first date that 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.*
- *FNAS-REC 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)