


# Distribution, abundance and natural enemies of the invasive tomato leafminer, *Tuta absoluta* (Meyrick) in Kenya

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## Research Paper

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### Abstract

*Tuta absoluta* (Meyrick) has become a serious menace to sustainable production of tomato in Kenya. A survey was conducted between April 2015 and June 2016 to determine its distribution, abundance, infestation, and damage levels on tomato, and associated natural enemies. Trap counts of *T. absoluta* moths were recorded in all surveyed 29 counties, which indicated its nationwide distribution irrespective of altitude. *Tuta absoluta* was present in both open fields and greenhouses. The highest moth/trap/day was  $115.38 \pm 15.90$ . Highest leaf infestation was 92.22% and the highest number of mines and larvae per leaf were  $3.71 \pm 0.28$  and  $2.16 \pm 0.45$ , respectively. Trap captures in terms of moth/trap/day were linearly and positively related to leaf infestations in open fields ( $R^2 = 0.81$ ) and greenhouses ( $R^2 = 0.61$ ). Highest fruits' infestation and damage were 60.00 and 59.61%, respectively, while the highest number of mines per fruit was  $7.50 \pm 0.50$ . *Nesidiocoris tenuis* (Reuter) and *Macrolophus pygmaeus* (Rambur) were identified as predators of *T. absoluta* larvae. Nine species of larval parasitoids were recovered from infested foliage, with a combined parasitism of  $7.26 \pm 0.65\%$ . *Hockeria* species was the most dominant (31.25%) and accounted for  $12.88 \pm 1.47\%$  parasitism. Two species of larval parasitoids, *Hockeria* and *Necremnus* were obtained from sentinel plants with an average parasitism of  $1.13 \pm 0.25$ . The overall abundance and parasitism rates of recovered natural enemies were low to effectively control the field populations of *T. absoluta*. These findings form the basis of researching and developing effective and sustainable management strategies for the pest.

## Introduction

Tomato (*Lycopersicon esculentum* (Mill.) (Solanaceae)) is among the most extensively cultivated and consumed vegetables in Kenya (MoALF, 2015; HCD, 2017, 2018). It has an annual production of 472,690 t cultivated in 21,718 ha (FAO, 2020). Its value is estimated at KShs 19.9 billion and contributes 38% of the total value of exotic vegetables in Kenya (HCD, 2018). Tomato is highly nutritious and provides good amounts of minerals, antioxidants and vitamins. Besides meeting the nutritional food requirement, tomato production serves as a reliable source of employment and income, thereby contributing to improving livelihoods, and economic growth of the country. Tomato is cultivated in all 47 counties in Kenya under both rain-fed and irrigation systems on open fields or under greenhouse technology (MoALF, 2015). This vegetable contributes significantly to enhancing food security and alleviating poverty. However, successful production is not often achieved due to numerous biotic constraints, among which arthropod pests rank high (Wafula *et al.*, 2018). The tomato leafminer, *Tuta absoluta* (Meyrick, 1917) has exacerbated this agricultural problem.

*Tuta absoluta*, a pest native to South America, was detected in eastern Spain in late 2006 and has since become a devastating tomato pest of worldwide significance (Desneux *et al.*, 2010; Biondi *et al.*, 2018). *Tuta absoluta* is presently spread across Africa and menacing sustainable production of tomato (Mansour *et al.*, 2018; CABI, 2020; EPPO, 2020). It was initially detected on Kenyan tomato in Mpeketoni and Witu fields in Lamu County in 2014 and subsequently reported in other counties such as Isiolo, Kirinyaga, Meru, Nairobi, Nakuru, Kakamega, Kajiado and other Rift Valley and Nyanza counties (KALRO, 2014; Mugo, 2014). This invasion poses an important threat to nutrition and food security in Kenya and would result in detrimental socioeconomic impact on livelihoods of small- and medium-holder farmers. Indeed, Pratt *et al.* (2017) estimated an annual monetary loss of KShs 5.98–6.65 billion caused by *T. absoluta* damages on tomato. Compounded efforts to manage the pest are also associated with increased costs of production and resultant high prices of tomato in the market (Desneux *et al.*, 2011; Pratt *et al.*, 2017).

Economic losses are derived from direct feeding effects of the larvae. In severe infestations, tomato leaves dry up and attack on other plant parts leads to crop malformation, particularly

the developing shoots, and consequent reduction of yield (Urbaneja *et al.*, 2013). Direct attack on fruits induces rotting that reduces both quality and marketability. Infestation levels of *T. absoluta* are determined using counts of trapped moths, and mines and larvae present on tomato leaves and fruits. Nevertheless, trap catches often provide a more reliable prediction of infestation levels and assists in making timely control decisions before the actual damage on foliage and fruits (Benvenega *et al.*, 2007). Research studies have revealed maximum fruit damage of 100% on open fields and 43.33% in greenhouses (Chermiti *et al.*, 2009; Mohamed *et al.*, 2012).

The current control practices for *T. absoluta* in tomato production systems in Kenya are limited to routine application of synthetic insecticides just like in the native and other invaded regions (Zappalà *et al.*, 2013; Mansour *et al.*, 2018; Nderitu *et al.*, 2018). The larvae, however, escape this approach due to their leaf-mining behaviour (Luna *et al.*, 2012). Consequently, farmers resort to unorthodox control measures such as increasing dosages and frequency of applications, as well as application of pesticide cocktails resulting in reduced efficacy, and potentially more damaging to the environment including ecosystem service providers such as pollinators and other beneficial fauna (Luna *et al.*, 2012; Biondi *et al.*, 2018). This misuse and overuse of chemical insecticides could also lead to fast development of pesticide resistant strains as have been observed in many populations worldwide (Han *et al.*, 2018). Toxic pesticide residues may also persist on harvested fruits, leading to contamination and most notably, food safety concerns for consumers. For these reasons, alternative eco-friendly control strategies are warranted.

An integrated pest management (IPM) strategy based on biological control as the key component is considered as the most viable approach to address *T. absoluta*. However, crucial information regarding its biology and ecology that is required for the development of an effective, sustainable and environmentally friendly IPM package is scanty. Studies have shown that *T. absoluta* has diverse species of spontaneous natural enemies (Mansour *et al.*, 2018; Ferracini *et al.*, 2019). Therefore, generalist natural enemies in tomato production systems in Kenya could apparently suppress the populations of *T. absoluta*. However, their field performance is majorly limited by extensive use of pesticides (Nderitu *et al.*, 2018). More effort is thus needed to identify and conserve the indigenous natural enemies adapting to *T. absoluta* to guide on suitable biological control programs and increase their impact in pest management. Studies on geographical distribution and abundance as well as infestation and damage levels on tomato will also be crucial in evaluating its pest status and potential economic risks (Allen and Humble, 2002; Benvenega *et al.*, 2007; Urbaneja *et al.*, 2013). In this regard, the objectives of our study were to (i) determine the abundance of *T. absoluta* and levels of infestation and damage on tomato in different localities in Kenya, (ii) identify the indigenous natural enemies associated with *T. absoluta* in Kenya. Our findings would form the basis upon which suitable pest management programs with emphasis on biological control could be developed.

## Materials and methods

### Field survey

A field survey was conducted in 39 localities in Kenya, from April 2015 to June 2016 to determine the distribution and abundance of *T. absoluta*, infestation and damage levels on tomato, and

associated natural enemies. These localities represent three altitudes commonly found in Kenya: high-, mid- and lowlands. Elevations above 1800 m above sea level (a.s.l) are the highlands; while midlands occupy elevations between 900 and 1800 m a.s.l, and the lowlands are elevations below 900 m a.s.l (Otolo and Wakhungu, 2013). The survey involved open fields in smallholder farms of <0.5 acres and greenhouses. Selection of a sampling site depended on the availability of tomato at the required phenological stages. These included plants at or nearly flowering stage for sampling of leaves and plants at flowering/fruitlet stage for sampling of fruits. Farmers' practices such as pesticides usage and tomato cultivars planted were not taken into consideration during the study.

### Sampling methods

Adult populations of *T. absoluta* were sampled using delta traps baited with *T. absoluta* sex pheromone. For each locality, three study sites with tomato plants at or nearly flowering stage were sampled and only one delta trap was used per site. Traps were loaded with removable sticky inserts and sex pheromone lure TUA-Optima PH-937-OPTI (Russell IPM, UK). They were hung at a height corresponding to the upper canopy of the plant (Megido *et al.*, 2013), and data were recorded weekly for four consecutive weeks.

Sampling of tomato leaves was carried out in a transversal zig-zag sampling pattern at the same sites of pheromone trapping. Thirty plants were randomly selected and assigned labels. Two leaves were picked at random from the middle stratum of each plant (Gomide *et al.*, 2001). Sixty leaves were thus sampled per site. They were examined and the number of infested leaves was recorded. They were kept in plastic containers (20 × 13 × 8 cm) containing damp paper towels and covered with lids containing fine muslin cloth (16 × 9 cm). The leaves were labelled per site and transported to the laboratory at the International Centre of Insect Physiology and Ecology (*icipe*). They were checked under a stereomicroscope (Leica Microsystems Limited, Switzerland) at 20× magnification to ascertain infestation and counts of mines and larvae per leaf were also recorded. Tomato fruits were sampled from plants at flowering/fruitlet stage. Two study sites were selected per locality and 20 fruits were collected randomly per site. They were placed in plastic containers (20 × 13 × 15 cm) and labelled per site. In the laboratory, all fruits were weighed and the number of mines per fruit and total number of infested fruits were recorded. Global positioning system (GPS) readings and altitudes were recorded for all the sampling sites (table 1).

### Sampling of natural enemies of *T. absoluta*

Predators of *T. absoluta* were sampled through active searching on tomato foliage and collected using an adapted aspirator. The aspirator had a plastic collecting vial, and a cap with two rubber tubes running through it. The tube to suck insects into the vial was relatively long, and the tube to draw air was fitted with a fine netting. Parasitoids were targeted from field-collected and infested foliage and fruits. Following the tally, infested leaves were placed in Perspex cages (30 × 30 × 30 cm) fitted with fine netting on opposite sides for ventilation. The cages had a round opening (14 cm diameter) at the front side fixed with a fine netting. The leaves were moistened regularly and developing larvae of *T. absoluta* were provided with fresh foliage as needed. Infested fruits were placed singly in small containers (6.5 cm height × 11 cm

**Table 1.** Sampling sites for *Tuta absoluta* and associated indigenous natural enemies from different localities in Kenya between April 2015 and June 2016

Site	Locality	County	GPS coordinates	Elevation (m)	No. of parasitoids	No. of predators
<i>Open fields</i>						
1	Mwea	Kirinyaga	S00°36'24.8" E037°22'30.0"	1217	<sup>a</sup>	4 Nt
2	Mwea	Kirinyaga	S00°37'23.7" E037°21'39.3"	1213	1 Di, 3 Go, 2 Br	11 Nt
3	Mwea	Kirinyaga	S00°36'24.2" E037°22'27.0"	1212	<sup>a</sup>	2 Nt
1	Gichugu	Kirinyaga	S00°27'55.1" E037°18'36.8"	1634	4 Di, 4 Nf, 3 Br	7 Nt, 1 Mp
2	Gichugu	Kirinyaga	S00°28'02.9" E037°18'56.0"	1624	3 Di, 2 Br, 1 Nf	<sup>a</sup>
3	Gichugu	Kirinyaga	S00°27'52.7" E037°18'49.4"	1650	<sup>a</sup>	<sup>a</sup>
1	Kisii	Kisii	S00°40'41.8" E035°03'14.4"	1838	1 Di	2 Nt
2	Kisii	Kisii	S00°40'16.6" E035°02'24.9"	1899	<sup>a</sup>	1 Nt
3	Kisii	Kisii	S00°46'54.8" E035°03'06.5"	1878	<sup>a</sup>	<sup>a</sup>
1	Taveta	Taita Taveta	S03°26'55.4" E037°39'22.9"	732	7 By, 14 Ho, 3 Ne	6 Nt
2	Taveta	Taita Taveta	S03°22'53.3" E037°43'11.5"	767	3 Ne, 5 By, 9 Ho	2 Nt
3	Taveta	Taita Taveta	S03°25'53.5" E037°43'57.6"	737	9 Ho, 7 By, 5 Ne	1 Nt
1	Loitoktok	Kajiado	S02°50'57.9" E037°32'15.9"	1427	5 By, 7 Ho, 3 Ne	<sup>a</sup>
2	Loitoktok	Kajiado	S02°50'29.7" E037°32'20.2"	1410	1 By	2 Nt
3	Loitoktok	Kajiado	S02°55'48.1" E037°30'27.9"	1775	3 Ho, 2 By	<sup>a</sup>
1	Isiolo	Isiolo	N00°19'33.0" E037°33'14.1"	1160	<sup>a</sup>	2 Nt
2	Isiolo	Isiolo	N00°19'54.7" E037°33'16.4"	1165	<sup>a</sup>	<sup>a</sup>
3	Isiolo	Isiolo	N00°19'45.6" E037°33'23.5"	1160	<sup>a</sup>	<sup>a</sup>
1	Meru	Meru	N00°00'21.6" E037°51'03.9"	920	<sup>a</sup>	2 Nt
2	Meru	Meru	S00°00'43.1" E037°49'16.1"	976	2 Go, 3 Sr, 1 Di, 9 Cb	7 Nt
3	Meru	Meru	N00°00'34.6" E037°51'35.6"	907	3 Ne, 4 Cb, 1 Di	4 Nt
1	Nakuru	Nakuru	N00°03'02.9" E036°13'42.6"	1955	<sup>a</sup>	<sup>a</sup>
2	Nakuru	Nakuru	N00°03'22.9" E036°13'44.7"	1956	<sup>a</sup>	<sup>a</sup>
3	Nakuru	Nakuru	S00°09'05.8" E036°08'41.6"	2057	<sup>a</sup>	<sup>a</sup>
1	Nyahururu	Laikipia	N00°03'32.6" E036°21'41.4"	2382	<sup>a</sup>	<sup>a</sup>
2	Nyahururu	Laikipia	S00°02'32.4" E036°19'14.6"	2426	<sup>a</sup>	1 Nt
3	Nyahururu	Laikipia	N00°03'20.4" E036°21'48.2"	2380	<sup>a</sup>	3 Nt
1	Nyeri	Nyeri	S00°27'44.0" E036°55'48.5"	1854	<sup>a</sup>	<sup>a</sup>
2	Nyeri	Nyeri	S00°27'59.8" E036°56'22.1"	1860	1 Di,	<sup>a</sup>
3	Nyeri	Nyeri	S00°27'28.5" E036°57'01.4"	1804	1 Nf	<sup>a</sup>
1	Kwale	Kwale	S04°23'26.0" E039°29'43.6"	25	1 Ne, 3 Ho	<sup>a</sup>
2	Kwale	Kwale	S04°19'16.4" E039°31'14.2"	47	7 By, 5 Ho, 1 Ne	3 Nt
3	Kwale	Kwale	S04°19'27.1" E039°31'13.0"	48	<sup>a</sup>	<sup>a</sup>
1	Malindi	Kilifi	S03°05'04.8" E040°06'14.5"	46	<sup>a</sup>	<sup>a</sup>
2	Malindi	Kilifi	S03°01'00.1" E040°00'44.7"	123	<sup>a</sup>	<sup>a</sup>
3	Malindi	Kilifi	S03°08'36.0" E040°06'26.6"	6	<sup>a</sup>	<sup>a</sup>
1	Kilifi	Kilifi	S03°36'35.2" E039°49'57.2"	10	<sup>a</sup>	<sup>a</sup>
2	Kilifi	Kilifi	S03°35'58.1" E039°49'08.3"	48	<sup>a</sup>	<sup>a</sup>
3	Kilifi	Kilifi	S03°34'24.4" E039°49'10.4"	103	<sup>a</sup>	<sup>a</sup>
1	Murang'a	Murang'a	S00°45'20.9" E037°08'45.4"	1292	<sup>a</sup>	<sup>a</sup>
2	Murang'a	Murang'a	S00°45'29.3" E037°08'48.1"	1297	<sup>a</sup>	<sup>a</sup>
3	Murang'a	Murang'a	S00°45'08.6" E037°08'00.6"	1331	<sup>a</sup>	<sup>a</sup>

(Continued)

Table 1. (Continued.)

Site	Locality	County	GPS coordinates	Elevation (m)	No. of parasitoids	No. of predators
1	Limuru	Kiambu	S01°05'01.7" E036°37'30.6"	2356	a	2 Nt
2	Limuru	Kiambu	S01°05'00.8" E036°37'21.7"	2366	a	a
3	Limuru	Kiambu	S01°04'07.9" E036°37'58.4"	2377	a	a
1	Kiambu	Kiambu	S01°05'17.9" E036°49'09.2"	1871	a	a
2	Kiambu	Kiambu	S01°03'42.0" E036°47'15.4"	1855	a	a
3	Kiambu	Kiambu	S00°54'46.7" E037°04'51.8"	1850	a	a
1	Machakos	Machakos	S01°09'47.4" E037°31'52.6"	1270	a	a
2	Machakos	Machakos	S01°13'56.0" E037°27'31.5"	1195	a	a
3	Machakos	Machakos	S01°10'01.5" E037°31'45.5"	1276	a	a
1	Kitui	Kitui	S00°56'02.9" E038°03'20.9"	1008	a	a
2	Kitui	Kitui	S00°50'56.5" E038°00'21.4"	871	a	a
3	Kitui	Kitui	S00°50'48.9" E038°00'23.6"	881	a	a
1	Thika	Kiambu	S01°03'31.0" E037°07'27.0"	1478	a	a
2	Thika	Kiambu	S01°03'49.2" E037°10'45.8"	1455	a	a
3	Thika	Kiambu	S01°03'41.8" E037°10'49.4"	1452	a	a
1	Sergoit	Elgeyo-Marakwet	N00°41'18.2" E035°25'49.7"	2206	a	a
2	Sergoit	Elgeyo-Marakwet	N00°41'18.8" E035°25'35.1"	2173	a	a
3	Sergoit	Elgeyo-Marakwet	N00°41'17.9" E035°25'50.2"	2182	a	a
1	Eldoret	Uasin Gishu	N00°52'21.6" E035°21'53.1"	1940	a	a
2	Eldoret	Uasin Gishu	N00°52'19.7" E035°21'59.4"	1934	a	a
3	Eldoret	Uasin Gishu	N00°52'21.4" E035°21'54.7"	1940	a	a
1	Embu	Embu	S00°32'33.9" E037°28'20.8"	1295	a	a
2	Embu	Embu	S00°32'24.2" E037°28'39.6"	1359	a	a
3	Embu	Embu	S00°28'28.3" E037°34'53.0"	1294	a	a
1	Migori	Migori	S01°03'47.0" E034°28'09.3"	1358	a	2 Nt
2	Migori	Migori	S01°05'35.3" E034°25'52.1"	1401	a	a
3	Migori	Migori	S01°05'16.1" E034°25'58.6"	1387	a	4 Nt
1	Homa Bay	Homa Bay	S00°28'23.1" E034°33'45.5"	1155	a	a
2	Homa Bay	Homa Bay	S00°27'29.6" E034°33'35.5"	1152	a	a
3	Homa Bay	Homa Bay	S00°27'31.4" E034°38'08.6"	1150	a	a
1	Kakamega	Kakamega	N00°45'22.5" E035°08'06.4"	1918	a	2 Nt
2	Kakamega	Kakamega	N00°41'00.8" E035°09'12.9"	1961	a	a
3	Kakamega	Kakamega	N00°40'45.5" E035°08'03.8"	1954	3 Di	4 Nt
1	Bungoma	Bungoma	N00°48'47.1" E034°29'15.3"	1671	a	a
2	Bungoma	Bungoma	N00°47'45.5" E034°30'39.1"	1638	1 Go	a
3	Bungoma	Bungoma	N00°47'02.1" E034°30'48.9"	1650	a	a
1	Nanyuki	Laikipia	N00°04'39.9" E037°07'00.0"	1885	5 Go	1 Mp
2	Nanyuki	Laikipia	N00°04'34.5" E037°07'07.2"	1862	2 Go	3 Nt
3	Nanyuki	Laikipia	N00°04'07.5" E037°06'45.2"	1851	a	a
1	Makueni	Makueni	S02°23'18.1" E038°00'03.0"	849	a	a
2	Makueni	Makueni	S02°23'06.6" E038°00'09.5"	835	a	a
3	Makueni	Makueni	S00°23'00.5" E038°00'16.8"	828	a	a
1	Kilgoris	Narok	S00°54'57.0" E034°56'55.6"	1946	a	a

(Continued)

Table 1. (Continued.)

Site	Locality	County	GPS coordinates	Elevation (m)	No. of parasitoids	No. of predators
2	Kilgoris	Narok	S00°54'56.7" E034°57'00.6"	1946	<sup>a</sup>	<sup>a</sup>
3	Kilgoris	Narok	S00°54'56.3" E034°57'08.0"	1959	<sup>a</sup>	<sup>a</sup>
1	Narok	Narok	S00°58'35.2" E034°58'20.9"	1765	<sup>a</sup>	<sup>a</sup>
2	Narok	Narok	S00°59'33.8" E034°58'58.9"	1754	<sup>a</sup>	<sup>a</sup>
3	Narok	Narok	S00°06'13.3" E035°51'55.8"	1848	<sup>a</sup>	<sup>a</sup>
1	Ol Kalou	Nyandarua	S00°15'17.9" E036°23'03.2"	1771	<sup>a</sup>	<sup>a</sup>
2	Ol Kalou	Nyandarua	S00°11'47.7" E036°28'47.8"	2356	<sup>a</sup>	<sup>a</sup>
3	Ol Kalou	Nyandarua	S00°11'47.9" E036°28'48.5"	2350	<sup>a</sup>	2 Nt
1	Wiyumirie	Laikipia	S00°04'09.9" E036°34'27.8"	2236	<sup>a</sup>	<sup>a</sup>
2	Wiyumirie	Laikipia	S00°04'16.9" E036°34'29.1"	2240	<sup>a</sup>	<sup>a</sup>
3	Wiyumirie	Laikipia	S00°04'04.7" E036°32'06.9"	2366	<sup>a</sup>	<sup>a</sup>
1	Lamu	Lamu	S02°23'09.8" E040°26'58.2"	16	<sup>a</sup>	<sup>a</sup>
1	Trans Nzoia	Trans Nzoia	N00°58'47.9" E035°07'14.6"	1919	<sup>a</sup>	<sup>a</sup>
2	Trans Nzoia	Trans Nzoia	N00°58'59.5" E035°07'13.7"	1924	<sup>a</sup>	<sup>a</sup>
3	Trans Nzoia	Trans Nzoia	N00°58'59.7" E035°05'53.4"	1822	<sup>a</sup>	<sup>a</sup>
<i>Greenhouses</i>						
1	Kisii	Kisii	S00°40'43.4" E035°03'15.2"	1842	<sup>a</sup>	<sup>a</sup>
2	Kisii	Kisii	S00°46'55.1" E035°03'05.5"	1828	<sup>a</sup>	<sup>a</sup>
3	Kisii	Kisii	S00°45'57.1" E034°59'22.3"	2070	<sup>a</sup>	<sup>a</sup>
1	Taveta	Taita Taveta	S03°22'52.1" E037°43'10.7"	763	<sup>a</sup>	<sup>a</sup>
2	Taveta	Taita Taveta	S03°36'33.7" E038°22'45.0"	734	<sup>a</sup>	<sup>a</sup>
3	Taveta	Taita Taveta	S03°25'53.5" E037°43'57.6"	737	<sup>a</sup>	<sup>a</sup>
1	Mwatate	Taita Taveta	S03°36'36.7" E038°22'20.2"	866	<sup>a</sup>	<sup>a</sup>
2	Mwatate	Taita Taveta	S03°36'36.8" E038°22'25.5"	859	<sup>a</sup>	<sup>a</sup>
3	Mwatate	Taita Taveta	S03°33'32.7" E038°22'39.5"	872	<sup>a</sup>	<sup>a</sup>
1	Nyahururu	Laikipia	N00°02'21.1" E036°21'43.0"	2372	<sup>a</sup>	<sup>a</sup>
2	Nyahururu	Laikipia	N00°03'20.4" E036°21'48.2"	2380	<sup>a</sup>	<sup>a</sup>
3	Nyahururu	Laikipia	N00°03'13.8" E036°21'51.3"	2389	<sup>a</sup>	<sup>a</sup>
1	Kilifi	Kilifi	S03°33'35.7" E039°50'32.4"	43	<sup>a</sup>	<sup>a</sup>
2	Kilifi	Kilifi	S03°42'05.1" E039°49'53.0"	35	<sup>a</sup>	<sup>a</sup>
3	Kilifi	Kilifi	S03°41'11.5" E039°51'21.2"	18	<sup>a</sup>	<sup>a</sup>
1	Kabete	Kiambu	S01°14'24.5" E036°43'53.6"	1812	<sup>a</sup>	<sup>a</sup>
2	Kabete	Kiambu	S01°14'16.8" E036°42'56.2"	1871	<sup>a</sup>	<sup>a</sup>
3	Kabete	Kiambu	S01°14'28.7" E036°44'47.8"	1804	<sup>a</sup>	<sup>a</sup>
1	Limuru	Kiambu	S01°09'33.1" E036°38'35.2"	2251	<sup>a</sup>	<sup>a</sup>
2	Limuru	Kiambu	S01°05'01.3" E036°37'29.1"	2357	<sup>a</sup>	<sup>a</sup>
3	Limuru	Kiambu	S01°05'21.1" E036°38'37.9"	2332	<sup>a</sup>	<sup>a</sup>
1	Machakos	Machakos	S01°10'12.2" E037°31'40.8"	1279	<sup>a</sup>	<sup>a</sup>
2	Machakos	Machakos	S01°09'47.4" E037°31'52.6"	1270	<sup>a</sup>	<sup>a</sup>
3	Machakos	Machakos	S01°06'52.4" E037°22'24.5"	1336	<sup>a</sup>	<sup>a</sup>
1	Migori	Migori	S01°06'21.2" E034°28'18.2"	1380	<sup>a</sup>	<sup>a</sup>
2	Migori	Migori	S01°01'27.5" E034°28'51.0"	1538	<sup>a</sup>	<sup>a</sup>
3	Migori	Migori	S01°03'30.9" E034°27'56.1"	1358	<sup>a</sup>	<sup>a</sup>

(Continued)

**Table 1.** (Continued.)

Site	Locality	County	GPS coordinates	Elevation (m)	No. of parasitoids	No. of predators
1	Kakamega	Kakamega	N00°46'29.1" E035°04'37.5"	1794	a	a
2	Kakamega	Kakamega	N00°44'53.0" E035°07'00.3"	1913	a	a
3	Kakamega	Kakamega	N00°42'05.4" E035°09'15.1"	1965	a	a
1	Busia	Busia	N00°26'53.2" E034°06'38.5"	1213	a	a
2	Busia	Busia	N00°23'13.3" E034°14'25.5"	1229	a	a
3	Busia	Busia	N00°24'55.7" E034°06'18.4"	1189	a	a
1	Nairobi	Nairobi	S01°13'26.6" E036°53'49.1"	1608	a	a
2	Nairobi	Nairobi	S01°16'30.7" E036°42'59.5"	1856	a	a
3	Nairobi	Nairobi	S01°16'21.5" E036°43'06.4"	1840	a	a
1	Naivasha	Nakuru	S00°49'28.4" E036°32'42.9"	2515	a	a
2	Naivasha	Nakuru	S00°40'05.6" E036°23'09.1"	1900	a	a
3	Naivasha	Nakuru	S00°37'35.7" E036°22'49.5"	1903	a	a

Natural enemies associated with *T. absoluta*: Di, *Diglyphus isaea* (Walker); Nf, *Neochrysocharis formosa* (Westwood); Br, *Bracon* sp.; Ho, *Hockeria* sp.; By, *Brachymeria* sp.; Ne, *Necremnus* sp.; Go, *Goniozus* sp.; Cb, *Chelonus blackburni* (Cameron); Sr, *Stenomiesius rufescens* (Retzius); Nt, *Nesidiocoris tenuis* (Reuter); Mp, *Macrolophus pygmaeus* (Rambur).

<sup>a</sup>Parasitoids and predators not found.

top diameter and 9 cm bottom diameter) containing sterilized sand as a medium for pupation and to absorb soggy of the ripening tomatoes. They were covered with a fine muslin cloth using rubber bands. All emerging parasitoids and *T. absoluta* moths were collected and recorded daily.

### Sentinel plants

Sentinel plants were used to search for parasitoids in a procedure slightly modified from Abbes *et al.* (2014). Tomato seeds (var. Simlaw Rio Grande) were sown in plastic germination trays (51 × 32 × 6.5 cm height) in a screen house at the *icipé*. Seedlings with at least three leaves were transplanted (2 plants per pot) into 2-liter plastic pots (16 cm height × 15 cm top diameter and 8.5 cm bottom diameter) containing soil supplemented with farmyard manure. The plants were maintained under standard agronomic practices. Plants of ~25–30 cm were used for rearing purposes.

A colony of *T. absoluta* was established from moths emerging from infested foliage and fruits. They were aspirated into Perspex cages (65 × 45 × 45 cm) fitted with a fine netting on opposite sides and had a round opening (20 cm diameter) at the front side to which a fine net sleeve was fixed. The moths were provided with streaks of undiluted honey on the upper wall of cages and moistened cotton wool were placed at the bottom of the cages. The insects were maintained under laboratory conditions of 25 ± 1 °C, 60 ± 10% RH and L16:D8 photoperiod (Abbes *et al.*, 2014). Three potted tomato plants were introduced into the cages for oviposition. They were removed after 2 days and placed on the laboratory bench awaiting hatching of eggs. Foliage with developing larvae was cut and placed in separate cages. The larvae were provided with fresh leaves for food until pupal formation. Emerging moths were aspirated daily into the rearing cages. This procedure was repeated severally to maintain the colony of *T. absoluta*.

Sentinel plants were prepared by infesting healthy potted tomato plants with eggs and three larval instars of *T. absoluta* (Abbes *et al.*, 2014). This procedure was aimed at finding egg and larval parasitoids. Four plants were separately infested with

50 eggs, first, second and third instar larvae using a soft camel hair-brush. Larvae were allowed to establish mines for 1 h and the plants were placed beside open field tomato crops at the *icipé*. Plants were watered regularly and after 7 days, foliage was cut and placed in Perspex cages (30 × 30 × 30 cm) for the emergence of moths and/or parasitoids. Fourth instars were not included in the study due to their high mobility and tendencies of falling off from the leaves. Pupal parasitoids were targeted by placing 50 green pupae in a glass Petri dish (9.2 cm diameter × 1.7 cm height) and placing them in open field tomato crops for 1 week. They were placed on raised ground free from ants and other crawling insects. One thousand individuals of each developmental stage were exposed. All collected natural enemies were preserved in 70% ethanol for morphological identification and 95% ethanol for molecular identification. They were stored at –20 °C.

### Morphological identification of *T. absoluta* and natural enemies

Adult specimens were identified based on their morphological characteristics by Dr Robert Copeland of biosystematics support unit (BSU), *icipé*. Molecular identification was done to confirm the species identity of *T. absoluta* (Kinyanjui *et al.*, 2019), and associated natural enemies.

### DNA extraction

For the natural enemies, two adults were randomly selected per species. All samples were surface sterilized using 3% sodium hypochlorite and rinsed with distilled water. They were then put in sterile 1.5 ml Eppendorf tubes. Genomic DNA was extracted from individual insect using Isolate II Genomic DNA Kit (Bioline, UK) following the manufacturer's instructions.

### Polymerase chain reaction (PCR) and sequencing

PCR was carried out to amplify a fragment of mitochondrial cytochrome *c* oxidase subunit I (COI) gene using Folmer primers

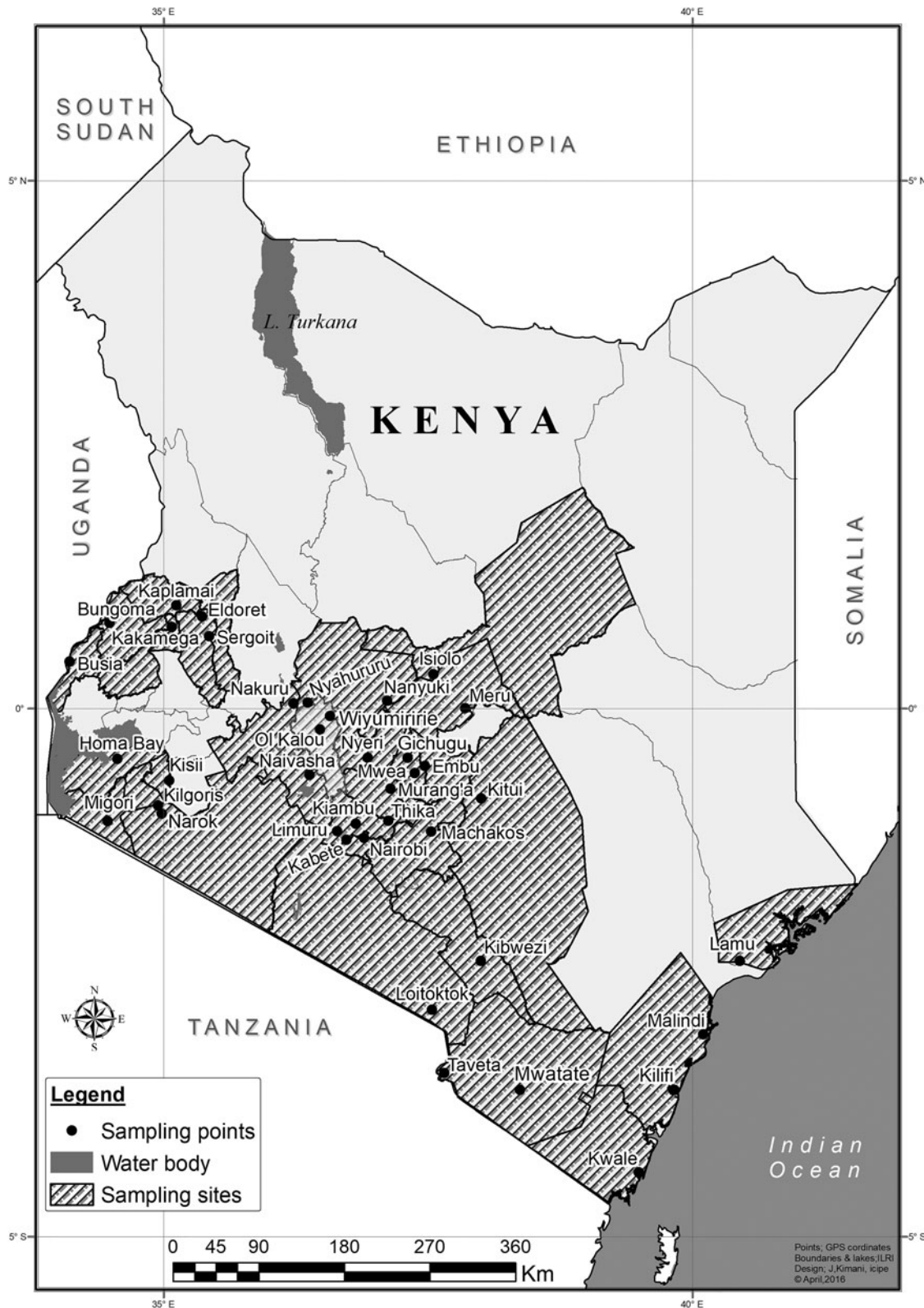


Figure 1. Map of Kenya showing the sampling sites for *Tuta absoluta*.

(LCO 1490 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO 2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') and Lep primers (LepF1 5'-ATTCAACCAATCATAAAGATATTGG-3' and LepR1 5'-TAAACTTCTGGATGTCCAAAAATCA-3')

(Folmer *et al.*, 1994; Smith *et al.*, 2007). The Lep primers were used in cases of poor amplification of the COI gene region by the Folmer primers. PCR was carried out in a 20 µl volume containing 5× *MyTaq* reaction buffer (Bioline; 5 mM dNTPs, 15 mM

MgCl<sub>2</sub>, stabilizers and enhancers), 0.5 pmol μl<sup>-1</sup> of each primer, 0.5 mM MgCl<sub>2</sub>, 0.0625 U μl<sup>-1</sup> *MyTaq* DNA polymerase (Bioline) and 15 ng μl<sup>-1</sup> of DNA template. Standard cycling conditions of 2 min at 95 °C, then 35 cycles of 30 s at 95 °C, 40 s at 54.1 °C (Folmer primers) and 48.1 °C (Lep primers) and 1 min at 72 °C, followed by a final elongation step of 10 min at 72 °C were used. Reactions were set up in a Mastercycler Nexus thermal cycler (Eppendorf, Germany). PCR products of ~700 bp were resolved through a 1.5% agarose gel and purified using Isolate II PCR and Gel Kit (Bioline, UK) as per the manufacturer's instructions. Purified samples were sent to a commercial sequencing facility (Macrogen Inc., Europe) for bidirectional sequencing using ABI 3700 sequencer. Voucher specimens were stored at the BSU and Molecular Pathology Laboratory, *icip*.

### Data analysis

Data on weekly trap catches of *T. absoluta* moths were analyzed using a generalized linear mixed model (GLMM) with random intercept and slope to assess the linear effect of time on the abundance of *T. absoluta*. GLMM was carried out using the *lmer* function of the *lme4* package (Bates *et al.*, 2015), and the overall factor effect was assessed using analysis of deviance with Wald  $\chi^2$  as the test statistic. Data were compared between open fields and greenhouses, and different altitudes and localities. Infestation levels were calculated as percentage of the number of infested leaves or fruits to the total number of leaves or fruits sampled per locality. Data were subjected to a generalized linear model assuming a quasi-binomial distribution error and logit link. Statistical differences in the weekly trap catches and infestation levels were compared using an adjusted Tukey's test.

Data on trap catches per day were averaged per locality and regressed against leaf infestations to test for a positive correlation between the two variables. Abundance of *T. absoluta* larvae on tomato was assessed by calculating the number of mines and larvae present on infested leaves or fruits per locality. Data were log-transformed ( $x+1$ ) to comply with normality assumptions and homogeneity of variance and subjected to analysis of variance (ANOVA). Fruit damage was expressed as a percentage of the weight of infested fruits divided by the total weight of all fruits sampled per locality. All percentages were transformed by arcsine square root and analyzed using ANOVA. Comparisons were made between open fields and greenhouses, and different altitudes and localities.

Relative abundance of predators was expressed as a percentage of counts of a single species in a locality over all sampled predators. Parasitism of the solitary parasitoids was calculated as a percentage of emerged parasitoid species divided by the total number of emerged parasitoids and *T. absoluta* moths per locality. Data were subjected to ANOVA after an arcsine square root transformation and comparisons were made between localities. When ANOVAs were significantly different, Tukey's HSD test was used to separate the means. Data on percentage parasitism obtained from sentinel tomato plants were first transformed by arcsine square root and subjected to a two-sample *t*-test. All analyses were carried out in R v3.2.3 software (R Development Core Team, 2015).

For molecular identification, COI sequences generated from both Folmer and Lep primers were assembled and edited using Chromas v2.1.1 (Technelysium Pty Ltd, Queensland, Australia). Sequence identities were determined using basic local alignment search tool (BLAST) (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) (Altschul *et al.*, 1990). The sequences were deposited in GenBank and assigned accession numbers MT916726 to MT916739.

**Table 2.** Overall abundance (mean  $\pm$  SE) of trapped *Tuta absoluta* moths across different altitudes and cultivation areas

<i>Tuta absoluta</i> moths			
Altitudes		Cultivation areas	
High	1588.48 $\pm$ 90.52a	Open fields	1736.52 $\pm$ 76.91a
Mid	1513.00 $\pm$ 109.22a	Greenhouses	1265.67 $\pm$ 108.97b
Low	1670.56 $\pm$ 131.49a	–	

Within columns, means followed by the same lowercase are not significantly different ( $P < 0.05$ , adjusted Tukey test).

## Results

### Distribution and abundance of *T. absoluta*

*Tuta absoluta* was present in all the sampled 39 localities representing 29 counties in Kenya (fig. 1). Overall abundance of trapped moths was significantly higher in open fields (1736.52  $\pm$  76.91) than in greenhouses (1265.67  $\pm$  108.97) (table 2;  $\chi^2 = 8.99$ ,  $df = 1$ ,  $P < 0.001$ ). However, abundance of *T. absoluta* in high (1588.48  $\pm$  90.52), mid (1670.56  $\pm$  131.49) and low altitudes (1513.00  $\pm$  109.22) did not differ significantly (table 2;  $\chi^2 = 0.62$ ,  $df = 2$ ,  $P = 0.730$ ). Analysis of linear effect of time revealed a highly significant difference between the weekly trap data ( $\chi^2 = 1119.02$ ,  $df = 3$ ;  $P < 0.001$ ). In week 1, 758.62  $\pm$  32.70 moths were recorded, whereas 218.40  $\pm$  10.45 moths were recorded in week 4 (fig. 2).

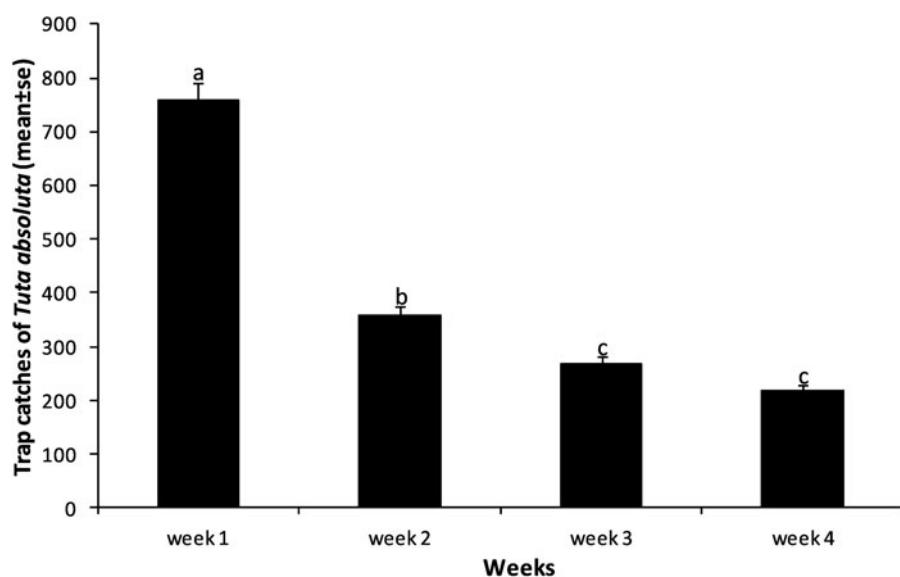
Average counts of moths per trap per day ranged from 7.75  $\pm$  4.37 to 115.38  $\pm$  15.90 and were significantly higher in open fields (62.02  $\pm$  2.75) than greenhouses (45.20  $\pm$  3.89) (table 3;  $\chi^2 = 8.96$ ,  $df = 1$ ,  $P < 0.001$ ). Within the greenhouses, significant differences were also observed in the abundance of *T. absoluta* recorded in different localities (table 3;  $F_{12,143} = 2.76$ ,  $P = 0.002$ ). Trapped moths were significantly higher in Kisii (77.08  $\pm$  19.39) and Kakamega (67.73  $\pm$  2.03) than in Nairobi (36.35  $\pm$  16.82), Kabete (19.35  $\pm$  9.98) and Busia (15.67  $\pm$  3.25). Similarly, open fields in Loitokitok (115.38  $\pm$  15.90), Mwea (98.00  $\pm$  14.27) and Meru (95.35  $\pm$  5.19) recorded significantly higher abundance than in Makueni (26.81  $\pm$  6.83), Kilgoris (22.98  $\pm$  5.54), Narok (18.25  $\pm$  4.14) and Thika (7.75  $\pm$  4.37) (table 3;  $F_{32,363} = 4.12$ ,  $P < 0.001$ ).

### Infestation and damage levels of *T. absoluta*

Leaf infestations were significantly higher in open fields (52.07  $\pm$  2.47%) than in greenhouses (37.99  $\pm$  3.69%) (table 3;  $F = 9.45$ ,  $df = 1$ ,  $P = 0.003$ ). Mean infestations in open fields ranged from 3.89  $\pm$  2.42 to 86.67  $\pm$  2.55% and were significantly lower in Thika (3.89  $\pm$  2.42%) than Taveta (86.67  $\pm$  0.00%) and Loitokitok (86.67  $\pm$  2.55%) (table 3;  $F_{32, 66} = 8.20$ ,  $P < 0.001$ ). In the greenhouses, leaf infestations ranged from 13.33  $\pm$  4.41% to 92.22  $\pm$  3.38% and were relatively high in Kisii (table 3;  $F_{12, 26} = 9.51$ ,  $P < 0.001$ ). No significant differences were found in leaf infestations across altitudes ( $F = 0.31$ ,  $df = 2$ ,  $P = 0.730$ ). Regression analysis yielded a significant linear and positive relationship between trapped *T. absoluta* moths and leaf infestations in both open fields ( $F_{1,31} = 132.02$ ,  $P < 0.001$ ) and greenhouses ( $F_{1,11} = 17.01$ ,  $P = 0.002$ ) (fig. 3).

There were no significant differences in the abundance of mines per leaf between greenhouses and open fields ( $F_{1,136} = 0.73$ ,  $P = 0.390$ ), hence data were pooled together. Mean counts of mines per leaf, however, differed significantly between localities





**Figure 2.** Weekly captures (mean  $\pm$  SE) of *Tuta absoluta* moths using delta traps baited with sex pheromone. Bars with same lowercase letters are not significantly different ( $P < 0.05$ , adjusted Tukey test).

( $F_{37,100} = 2.19$ ,  $P = 0.001$ ). An average count of 3 to 4 mines per leaf recorded in nine localities was significantly higher than  $1.10 \pm 0.56$  mines recorded in Thika (table 3). The maximum and minimum average counts of mines per leaf were  $3.71 \pm 0.28$  and  $1.10 \pm 0.56$ , respectively (table 3). No significant differences were observed in the mean count of mines per leaf across altitudes ( $F_{2,135} = 0.24$ ,  $P = 0.780$ ). The abundance of larvae per leaf ranged from  $0.42 \pm 0.42$  to  $2.16 \pm 0.45$  and did not differ significantly between open fields and greenhouses ( $F_{1,136} = 1.43$ ,  $P = 0.230$ ), and across altitudes ( $F_{2,135} = 0.63$ ,  $P = 0.540$ ) and localities ( $F_{37,100} = 1.34$ ,  $P = 0.130$ ) (table 3).

The levels of *T. absoluta* infestations on tomato fruits were  $17.14 \pm 5.81\%$  in the greenhouses and  $19.61 \pm 2.34\%$  on open fields, hence no significant differences were observed (table 4;  $F = 0.19$ ,  $df = 1$ ,  $P = 0.660$ ). Similarly, there were no significant differences in fruits' infestation across altitudes ( $F = 0.92$ ,  $df = 2$ ,  $P = 0.410$ ). Data were thus pooled together. Among localities, however, infested fruits in Kisii ( $60.00 \pm 15.00\%$ ) were significantly higher than in Naivasha ( $2.50 \pm 2.50$ ), Meru ( $0.00 \pm 0.00$ ) and Migori ( $0.00 \pm 0.00$ ) ( $F = 1.64$ ,  $df = 25$ ,  $P = 0.050$ ). The maximum average count of *T. absoluta* mines per fruit was  $7.50 \pm 0.50$ . There were no significant differences in the number of mines per fruit between open fields and greenhouses (table 4;  $F_{1,50} = 2.07$ ,  $P = 0.160$ ), and across localities ( $F_{25, 26} = 1.22$ ,  $P = 0.300$ ) and altitudes ( $F_{2,49} = 3.86$ ,  $P = 0.280$ ).

The damage levels on fruits in greenhouses ( $17.64 \pm 5.78\%$ ) and open fields ( $19.02 \pm 2.49\%$ ) did not differ significantly (table 4;  $F_{1,50} = 0.42$ ,  $P = 0.520$ ). Similarly, no significant differences were observed on the levels of fruits' damage across altitudes ( $F_{2,49} = 3.80$ ,  $P = 0.260$ ). For the localities, the damage of *T. absoluta* on tomato fruits was significantly higher in Kisii ( $59.61 \pm 12.13\%$ ) than in Naivasha ( $0.97 \pm 0.97$ ), Meru ( $0.00 \pm 0.00$ ) and Migori ( $0.00 \pm 0.00$ ) (table 4;  $F_{25, 26} = 1.44$ ,  $P = 0.045$ ).

### Natural enemies of *T. absoluta*

A total of 81 mirid bugs (Hemiptera: Miridae) comprising two species were recorded. *Nesidiocoris tenuis* (Reuter) was the most abundant predator (97.50%), and was recorded in 14 localities, while *Macrolophus pygmaeus* (Rambur) (2.45%) was sampled in

two localities (fig. 4). *Nesidiocoris tenuis* were assigned GenBank accession numbers MT916736 and MT916737, while no molecular analysis was conducted on *M. pygmaeus* due to limited samples. A total of 160 hymenopterans representing nine species emerged from infested leaves collected from 11 localities, and some could only be identified to the genus level (table 5). These larval parasitoids included four families, Chalcididae (*Brachymeria* and *Hockeria* species), Bethyridae (*Goniozus* sp.), Braconidae (*Chelonus blackburni* (Cameron) and *Bracon* sp.) and Eulophidae (*Diglyphus isaea* (Walker)), *Neochrysocharis formosa* (Westwood), *Stenomomesius rufescens* (Retzius) and *Necremnus* species. The overall parasitism was  $7.26 \pm 0.65\%$ . *Hockeria* species was the most abundant (31.25%) and accounted for the highest parasitism of  $12.88 \pm 1.47\%$  (table 5). Parasitism rates exhibited by individual species did not differ significantly when compared between localities ( $F = 1.86$ ,  $df = 10$ ,  $P = 0.120$ ). *Diglyphus isaea* was the most widely distributed parasitoid and was recorded in six localities (table 5). No natural enemies were recorded from infested leaves and fruits sampled in the greenhouses.

Nineteen parasitoids representing two species, *Hockeria* and *Necremnus* were recovered from sentinel plants infested with second and third larval instars of *T. absoluta*. The overall parasitism rate was  $1.13 \pm 0.25\%$  (table 6). Parasitism rates varied significantly between species ( $t = 3.50$ ,  $df = 78$ ,  $P < 0.001$ ), and *Hockeria* species was the most abundant (84.21%), with an average parasitism of  $1.94 \pm 0.42\%$  (table 6). Parasitism of individual species did not differ significantly between the second and third instars of *T. absoluta* larvae (table 6). In addition, no parasitoids were recorded from the eggs, first instar larvae and pupae of *T. absoluta*.

### Discussion

Our trap data indicated a widespread distribution of *T. absoluta* across Kenya. This could be mainly attributed to a year-round cropping and countrywide production of tomato, as well as favourable national agro-ecological conditions (MoALF, 2015; Tonnang *et al.*, 2015), hence ensuring an uninterrupted supply of host. Regional trade of tomato fruits and seedlings from probably infested to non-infested areas may also have played a role in

**Table 3.** Distribution, abundance and leaf infestation (mean  $\pm$  SE) for *Tuta absoluta* in greenhouse- and open field-cultivated tomato in Kenya

Locality	Trap catches/day	% of infested leaves	Mines/leaf <sup>1</sup>	Larvae/leaf <sup>2</sup>
<i>Greenhouses</i>				
Kisii	77.08 $\pm$ 19.39a	92.22 $\pm$ 3.38a	3.71 $\pm$ 0.28a	1.63 $\pm$ 0.06
Kakamega	67.73 $\pm$ 2.03a	51.11 $\pm$ 5.47bc	2.67 $\pm$ 0.45ab	1.23 $\pm$ 0.14
Naivasha	54.49 $\pm$ 0.70ab	13.33 $\pm$ 4.41de	1.79 $\pm$ 0.26ab	0.66 $\pm$ 0.11
Taveta	54.48 $\pm$ 14.67abc	56.11 $\pm$ 2.22b	3.01 $\pm$ 0.52ab	0.79 $\pm$ 0.18
Nyahururu	46.86 $\pm$ 15.98abc	33.33 $\pm$ 10.18bdce	3.48 $\pm$ 0.74a	0.91 $\pm$ 0.47
Kilifi	46.00 $\pm$ 3.72abc	40.56 $\pm$ 3.89bcd	2.64 $\pm$ 0.34ab	1.45 $\pm$ 0.40
Machakos	45.38 $\pm$ 13.65abc	50.00 $\pm$ 8.82bc	3.08 $\pm$ 0.21a	1.42 $\pm$ 0.40
Migori	42.24 $\pm$ 19.03abc	33.89 $\pm$ 8.18bcde	1.94 $\pm$ 0.37ab	1.06 $\pm$ 0.34
Limuru	41.21 $\pm$ 9.10abc	41.67 $\pm$ 1.92bcd	2.49 $\pm$ 0.37ab	1.18 $\pm$ 0.24
Mwatate	40.81 $\pm$ 13.03abcd	25.56 $\pm$ 5.30bcde	2.06 $\pm$ 0.43ab	1.36 $\pm$ 0.48
Nairobi	36.35 $\pm$ 16.82bcd	26.11 $\pm$ 9.64bcde	1.58 $\pm$ 0.12ab	0.69 $\pm$ 0.39
Kabete	19.35 $\pm$ 9.98bcd	13.33 $\pm$ 9.48e	1.69 $\pm$ 0.85ab	0.58 $\pm$ 0.48
Busia	15.67 $\pm$ 3.25d	16.67 $\pm$ 2.55cde	1.56 $\pm$ 0.43ab	0.66 $\pm$ 0.39
Total	45.20 $\pm$ 3.89A	37.99 $\pm$ 3.69A	2.45 $\pm$ 0.15A	1.05 $\pm$ 0.10A
<i>Open fields</i>				
Loitoktok	115.38 $\pm$ 15.90a	86.67 $\pm$ 2.55a	2.69 $\pm$ 0.17ab	1.14 $\pm$ 0.08
Mwea	98.00 $\pm$ 14.27ab	74.44 $\pm$ 2.42abc	2.94 $\pm$ 0.43ab	1.01 $\pm$ 0.06
Meru	95.35 $\pm$ 5.19ab	77.78 $\pm$ 7.29abc	2.89 $\pm$ 0.50ab	1.40 $\pm$ 0.25
Ol Kalou	89.50 $\pm$ 2.82ab	67.78 $\pm$ 8.18abcde	1.87 $\pm$ 0.21ab	0.52 $\pm$ 0.32
Nyahururu	85.57 $\pm$ 9.75abc	65.00 $\pm$ 6.01abcde	2.60 $\pm$ 0.41ab	1.12 $\pm$ 0.49
Bungoma	81.60 $\pm$ 1.89abc	70.56 $\pm$ 7.72abcde	2.37 $\pm$ 0.08ab	0.83 $\pm$ 0.13
Kakamega	81.17 $\pm$ 11.03abc	61.11 $\pm$ 2.22abcdef	3.67 $\pm$ 0.42a	1.28 $\pm$ 0.25
Kisii	78.87 $\pm$ 12.13abc	81.67 $\pm$ 10.84ab	2.67 $\pm$ 0.71ab	1.49 $\pm$ 0.46
Nakuru	83.05 $\pm$ 3.88abc	60.00 $\pm$ 4.41abcdef	2.72 $\pm$ 0.26ab	1.44 $\pm$ 0.15
Taveta	75.70 $\pm$ 6.75abc	86.67 $\pm$ 0.00a	3.06 $\pm$ 0.33ab	1.53 $\pm$ 0.03
Gichugu	70.14 $\pm$ 14.54abc	81.11 $\pm$ 8.18ab	3.38 $\pm$ 0.16ab	1.30 $\pm$ 0.07
Homa Bay	63.10 $\pm$ 7.13abc	67.22 $\pm$ 4.44abcde	2.95 $\pm$ 0.59ab	1.13 $\pm$ 0.20
Isiolo	68.75 $\pm$ 5.10abc	61.11 $\pm$ 15.94abcdef	2.37 $\pm$ 0.08ab	1.07 $\pm$ 0.09
Kilifi	70.42 $\pm$ 10.79abc	61.11 $\pm$ 7.78abcdef	1.95 $\pm$ 0.29ab	0.73 $\pm$ 0.37
Kitui	55.63 $\pm$ 12.11abcd	35.56 $\pm$ 2.00defgh	3.68 $\pm$ 0.28a	2.16 $\pm$ 0.45
Kwale	62.98 $\pm$ 6.93abc	59.44 $\pm$ 1.47abcdef	2.66 $\pm$ 0.52ab	1.18 $\pm$ 0.42
Limuru	52.88 $\pm$ 7.31abcd	39.44 $\pm$ 5.30cdefg	2.40 $\pm$ 0.17ab	1.02 $\pm$ 0.30
Machakos	68.39 $\pm$ 9.31abc	53.33 $\pm$ 12.06abcdefg	3.26 $\pm$ 0.39ab	1.65 $\pm$ 0.38
Malindi	53.50 $\pm$ 8.80abcd	33.33 $\pm$ 6.74efgh	2.10 $\pm$ 0.27ab	1.38 $\pm$ 0.15
Migori	63.25 $\pm$ 7.86abc	48.89 $\pm$ 8.18abcdefg	2.68 $\pm$ 0.50ab	1.06 $\pm$ 0.26
Murang'a	52.21 $\pm$ 4.42abcd	46.11 $\pm$ 4.34bcdefg	2.75 $\pm$ 0.31ab	1.08 $\pm$ 0.07
Nanyuki	71.58 $\pm$ 3.78abc	73.33 $\pm$ 8.39abcd	2.78 $\pm$ 0.48ab	1.56 $\pm$ 0.23
Nyeri	65.11 $\pm$ 6.68abc	58.33 $\pm$ 11.82abcdef	2.47 $\pm$ 0.28ab	1.28 $\pm$ 0.08
Sergoit	68.33 $\pm$ 12.42abc	47.78 $\pm$ 9.49abcdefg	2.88 $\pm$ 0.51ab	1.49 $\pm$ 0.68
Wiyumirie	59.39 $\pm$ 7.32abcd	52.78 $\pm$ 2.00abcdefg	2.44 $\pm$ 0.18ab	1.41 $\pm$ 0.03
Eldoret	38.74 $\pm$ 6.43cde	20.00 $\pm$ 4.19gh	2.10 $\pm$ 0.32ab	0.98 $\pm$ 0.43
Embu	39.46 $\pm$ 12.32bcde	20.00 $\pm$ 6.01gh	2.14 $\pm$ 0.39ab	1.42 $\pm$ 0.37

(Continued)

Table 3. (Continued.)

Locality	Trap catches/day	% of infested leaves	Mines/leaf <sup>1</sup>	Larvae/leaf <sup>2</sup>
Trans Nzoia	35.20 ± 6.20cde	24.44 ± 6.26fgh	2.40 ± 0.06ab	0.80 ± 0.50
Kiambu	27.57 ± 5.68de	25.00 ± 12.95fgh	1.52 ± 0.76ab	0.42 ± 0.42
Makueni	26.81 ± 6.83e	23.89 ± 9.09fgh	2.06 ± 0.19ab	1.05 ± 0.49
Kilgoris	22.98 ± 5.54e	25.00 ± 8.66fgh	2.33 ± 0.44ab	1.18 ± 0.45
Narok	18.25 ± 4.14e	25.56 ± 3.09fgh	2.08 ± 0.14ab	1.21 ± 0.12
Thika	7.75 ± 4.37f	3.89 ± 2.42h	1.10 ± 0.56b	0.43 ± 0.23
Total	62.02 ± 2.75B	52.07 ± 2.47B	2.54 ± 0.08A	1.18 ± 0.06A

Means followed by the same lowercase letters in a column within greenhouses and open fields are not significantly different. Means followed by the same uppercase letters for the mean totals in a column are not significantly different ( $P < 0.05$ , Tukey's HSD test).

<sup>1</sup>Pooled analysis for greenhouse and open field data.

<sup>2</sup>No significant differences across localities.

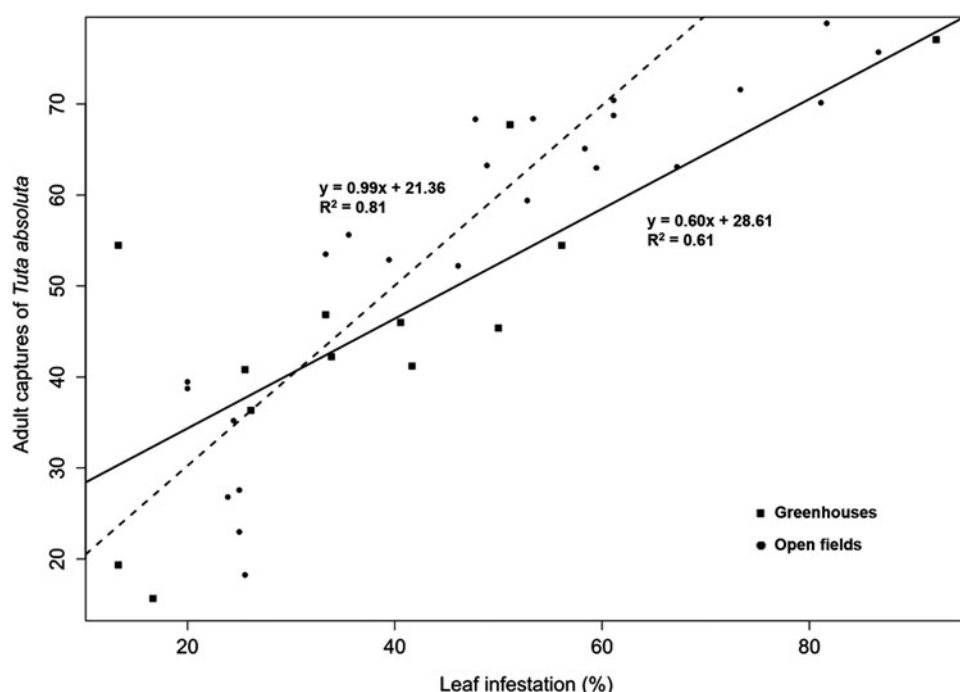


Figure 3. Linear regression of daily trap captures (mean ± SE) of *Tuta absoluta* moths vs. leaf infestation (mean ± SE) of tomato cultivated in different localities in Kenya. The black line and dotted lines represent greenhouses and open fields, respectively.

dispersing the pest nationwide (Tonnang *et al.*, 2015). *Tuta absoluta* was found in low altitudes of 6 m a.s.l and high altitudes of 2515 m a.s.l. Analyses of GLMM also showed that altitude did not significantly influence the abundance of the pest. These results agreed with Pratt *et al.* (2017) who reported that the geographical distribution of *T. absoluta* was unlikely to be determined by altitude. *Tuta absoluta* has been associated with altitudes <1000 m a.s.l. (Desneux *et al.*, 2010; Tonnang *et al.*, 2015). Our results, however, showed that the pest can also thrive in altitudes above 1000 m a.s.l. Previous results also confirmed the presence of *T. absoluta* in altitudes of 1235 m a.s.l in Tanzania and 1140 m a.s.l in Uganda (G. Kinyanjui *et al.*, unpublished data). These findings fitted a report in Colombia, where the pest was found in greenhouse and open field tomato crops at 2600 and 1900 m a.s.l, respectively (Desneux *et al.*, 2010).

The Kenyan highlands are generally characterized by cold climate. Therefore, the presence of *T. absoluta* in these areas in open fields rather than confined in greenhouses confirmed the species'

ability to survive colder conditions and cause damage at high altitudes (Tonnang *et al.*, 2015; Biondi *et al.*, 2018). Typically, altitude does not solely determine the distribution and abundance of a pest species but factors such as climatic and environmental conditions, and availability of suitable host plants play an important role (Geurts *et al.*, 2012). Therefore, besides high adaptability, the trap data of *T. absoluta* in Loitokitok and Mwea, which are among the major producers of tomato in Kenya (HCD, 2017), confirmed that availability of tomato seemed to have a decisive influence in the distribution and abundance of the pest.

Trap data corresponded to leaf infestations in open fields and greenhouses. This was expected since high adult populations reflect more oviposition and increased number of mines and larvae on foliage. Moreover, the phenological stage of nearly or at flowering, at which sampling was done, is usually characterized by high populations of *T. absoluta* eggs and first instar larvae, thus leading to increased levels of leaf infestations and high number of mines per leaf (Chermiti *et al.*, 2009). A positive correlation between

**Table 4.** Fruit infestation and damage (mean  $\pm$  SE) for *Tuta absoluta* in greenhouse- and open field-cultivated tomato in Kenya

Locality	% of fruit infested	Mines/fruit <sup>1</sup>	% Fruit damage
<i>Greenhouses</i>			
Kisii	60.00 $\pm$ 15.00a	4.13 $\pm$ 1.80	59.61 $\pm$ 12.13a
Mwatate	17.50 $\pm$ 7.50ab	2.85 $\pm$ 1.35	19.53 $\pm$ 9.05ab
Nyahururu	17.50 $\pm$ 7.50ab	3.50 $\pm$ 0.50	18.02 $\pm$ 8.19ab
Nairobi	15.00 $\pm$ 15.00ab	1.67 $\pm$ 1.67	14.88 $\pm$ 14.88ab
Kakamega	7.50 $\pm$ 7.50ab	1.50 $\pm$ 1.50	10.46 $\pm$ 10.46ab
Naivasha	2.50 $\pm$ 2.50b	1.50 $\pm$ 1.50	0.97 $\pm$ 0.97b
Migori	0.00 $\pm$ 0.00b	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00b
Total	17.14 $\pm$ 5.81A	2.16 $\pm$ 0.52A	17.64 $\pm$ 5.78A
<i>Open fields</i>			
Bungoma	10.00 $\pm$ 10.00ab	1.88 $\pm$ 1.88	10.88 $\pm$ 10.88ab
Embu	5.00 $\pm$ 5.00ab	1.50 $\pm$ 1.50	4.06 $\pm$ 4.06ab
Gichugu	35.00 $\pm$ 5.00ab	3.38 $\pm$ 0.88	31.90 $\pm$ 5.57ab
Homa Bay	7.50 $\pm$ 7.50ab	1.67 $\pm$ 1.67	6.03 $\pm$ 6.03ab
Isiolo	15.00 $\pm$ 15.00ab	3.08 $\pm$ 3.08	15.08 $\pm$ 15.08ab
Kilifi	25.00 $\pm$ 5.00ab	2.88 $\pm$ 0.13	23.92 $\pm$ 4.00ab
Kwale	7.50 $\pm$ 7.50ab	1.50 $\pm$ 1.50	5.56 $\pm$ 5.56ab
Loitoktok	30.00 $\pm$ 10.00ab	2.50 $\pm$ 0.25	31.75 $\pm$ 9.42ab
Machakos	17.50 $\pm$ 17.50ab	2.29 $\pm$ 2.29	16.47 $\pm$ 16.47ab
Makueni	22.50 $\pm$ 7.50ab	3.00 $\pm$ 0.33	20.24 $\pm$ 11.59ab
Meru	0.00 $\pm$ 0.00b	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00b
Murang'a	22.50 $\pm$ 12.50ab	4.04 $\pm$ 1.54	22.22 $\pm$ 15.25ab
Mwea	30.00 $\pm$ 5.00ab	2.64 $\pm$ 0.64	26.01 $\pm$ 14.57ab
Nanyuki	20.00 $\pm$ 5.00ab	7.50 $\pm$ 0.50	18.51 $\pm$ 5.63ab
Nyahururu	27.50 $\pm$ 17.50ab	4.19 $\pm$ 1.69	29.61 $\pm$ 21.89ab
Nyeri	20.00 $\pm$ 10.00ab	2.58 $\pm$ 1.08	18.69 $\pm$ 7.53ab
Ol Kalou	20.00 $\pm$ 10.00ab	6.25 $\pm$ 3.25	22.26 $\pm$ 8.57ab
Sergoit	25.00 $\pm$ 20.00ab	4.72 $\pm$ 0.72	24.88 $\pm$ 22.07ab
Taveta	32.50 $\pm$ 2.50ab	2.07 $\pm$ 0.07	33.39 $\pm$ 4.64ab
Total	19.61 $\pm$ 2.34A	3.03 $\pm$ 0.38A	19.02 $\pm$ 2.49A

Pooled analysis for greenhouse and open field data. Means followed by the same uppercase or lowercase letters in a column are not significantly different ( $P < 0.05$ , Tukey's HSD test).  
<sup>1</sup>No significant differences across localities.

trap data of *T. absoluta* and leaf infestations on tomato has also been reported (Benvenga *et al.*, 2007; Abbes and Chermiti, 2011; Assaf *et al.*, 2013). These two parameters are good indicators of damage levels (Benvenga *et al.*, 2007). Thus, based on our data of trapped moths and leaf infestations, *T. absoluta* could be among others, a contributing factor to economic damage of tomato in Kenya. The significant reduction of trap catches in week 4 could either be due to reduced pest populations because of pheromone trapping or reduced efficacy of pheromone lures (TUA-Optima) at the end of their shelf life (Megido *et al.*, 2013).

Significant differences were observed in leaf infestations across localities, which could be related to the differential abundance of

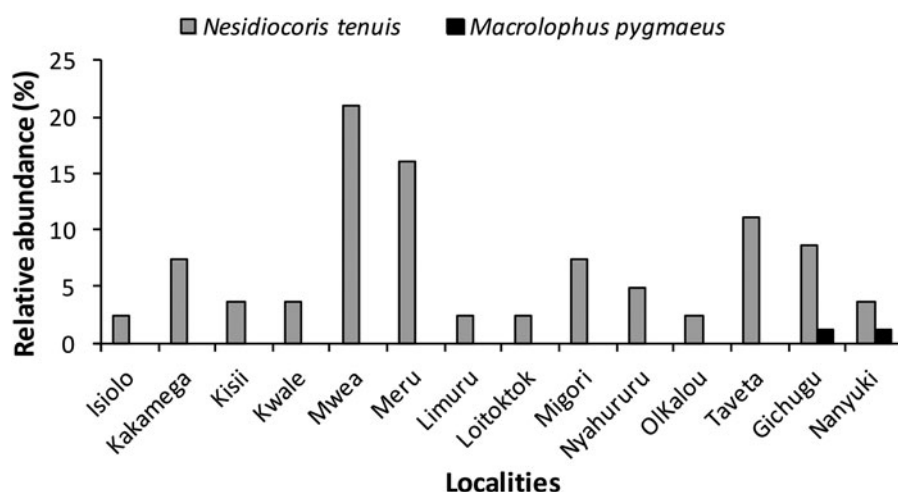
the pest as reported herein. In addition, different tomato cultivars were sampled, where some are relatively susceptible to *T. absoluta* attack (Gharekani and Salek-Ebrahimi, 2014). Generally, leaf infestations on greenhouse-protected tomato were lower than the vulnerable plants in the open fields. However, our results showed that once the pest enters the greenhouses, infestations could be severe reaching up to 92%. The observed differences in the abundance of mines and larvae on foliage could perhaps be due to migration of larvae to fresh mines or to pupation sites.

The levels of fruits' infestation and damage were generally low. The damage on open field tomato (19%) was lower than Sudan (80–100%) (Mohamed *et al.*, 2012). However, the overall fruit damage in greenhouses (18%) was close to the levels reported in protected cultivations in Tunisia (20%) (Chermiti *et al.*, 2009). Our results could be largely influenced by the phenological stage of the crop, since the sampled flowering/fruitlet stage is usually characterized by early or no fruits infestation (Chermiti *et al.*, 2009). At this crop stage, preferential fresh foliage is plenty for *T. absoluta* larvae (Galdino *et al.*, 2015), and as such, minimal or null infestations on fruits are expected. The relatively high values recorded in Kisii (59.6%) could be linked to high abundance of the pest, which corresponds to increased infestation densities, complete destruction of foliage and a consequent shift of feeding sites (Chermiti *et al.*, 2009; Galdino *et al.*, 2015). These results also confirmed the study of Cely *et al.* (2010) who reported that fruit damage was a function of density of *T. absoluta* populations. Maximum fruit damage is also likely to occur at senescence stage, and therefore, further studies are warranted to assess the population dynamics and damage caused by *T. absoluta* at different phenological stages of tomato crop.

Our data showed that indigenous natural enemies in Kenya are adapting to *T. absoluta* and could provide a solid foundation for sustainable management of the pest. However, the overall abundance was low. This could be explained by the short period of adaptation, considering that sampling of natural enemies was conducted between April 2015 and June 2016, and the first report of *T. absoluta* in Kenya was 2014. Nevertheless, most natural enemies of *T. absoluta* are generalists (Ferracini *et al.*, 2019). Thus, it is highly probable that species diversity will increase in future as more natural enemies adapt to the pest. Low abundance of natural enemies could also be due to adverse farming practices adopted by growers such as calendar-scheduled applications of pesticides to control *T. absoluta* and other tomato pests. Indeed, Nderitu *et al.* (2018) observed that farmers in Kirinyaga County sprayed up to 16 times per growing season, which had adverse effects on natural enemies. Awareness campaigns, therefore, on the need to conserve native natural enemies should be considered, because in addition to reducing environmental damage and human health risks, fortuitous biological control could provide some huge economic benefits to growers.

*Nesidiocoris tenuis* and *Macrolophus pygmaeus* were found preying on *T. absoluta*. These polyphagous predators' prey on a wide range of tomato pests and attack all the pre-imaginal stages of *T. absoluta* (Urbaneja *et al.*, 2009). Mollá *et al.* (2009) demonstrated that a good establishment of these two predators on tomato crop significantly reduced *T. absoluta* infestations on both leaves and fruits. Some authors, however, reported their failure to achieve acceptable levels and thus advocated for integration with other pest control alternatives (Mollá *et al.*, 2011; Abbes and Chermiti, 2012; Nannini *et al.*, 2012).

A record of nine species of larval parasitoids of *T. absoluta* indicated a higher diversity in Kenya than in Tunisia and



**Figure 4.** Relative abundance for predators of *Tuta absoluta* collected from different localities in Kenya.

**Table 5.** Species composition, abundance and parasitism of parasitoids associated with *Tuta absoluta* larvae in Kenya

Morphological identification	Corresponding taxon in GenBank and % identity	GenBank accessions	Sampled localities	Relative abundance (%)	Parasitism (%)
<i>Bracon</i> sp.	Opiinae (Braconidae sp.) (95%)	MT916727, MT916728	Gichugu, Mwea	7 (4.38%)	6.34 ± 1.12
<i>Diglyphus isaea</i>	<i>Diglyphus isaea</i> (98%)	MT916729, MT916730	Gichugu, Kakamega, Kisii, Meru, Mwea, Nyeri	15 (9.38%)	4.44 ± 1.17
<i>Neochrysocharis formosa</i>	<i>Neochrysocharis formosa</i> (99%)	MT916726	Gichugu, Nyeri	6 (3.75%)	5.35 ± 3.21
<i>Brachymeria</i> sp.	Chalcididae sp. (90%)	MT916731	Kwale, Loitokitok, Taveta	34 (21.25%)	9.89 ± 0.90
<i>Hockeria</i> sp.	Chalcididae sp. (90%)	MT916738, MT916739	Kwale, Loitokitok, Taveta	50 (31.25%)	12.88 ± 1.47
<i>Chelonus</i> sp.	<i>Chelonus blackburni</i> (93%)	MT916732, MT916733	Meru	13 (8.13%)	9.02 ± 2.67
<i>Necremnus</i> sp.	<sup>a</sup>	<sup>a</sup>	Kwale, Loitokitok, Meru, Taveta	19 (11.88%)	4.87 ± 0.80
<i>Goniozus</i> sp.	<i>Goniozus</i> sp. (91%)	MT916734, MT916735	Bungoma, Meru, Mwea, Nanyuki	13 (8.13%)	5.20 ± 1.11
<i>Stenomiesius rufescens</i>	<sup>b</sup>	<sup>b</sup>	Meru	3 (1.88%)	4.23 ± 0.00

Parasitoids were sampled from tomato crops. Percentage values accompanying species names in the column of the corresponding taxon represent the percentage similarity between study sequences and those from the NCBI GenBank database. Abundance is represented as counts of parasitoids (percentage composition).

<sup>a</sup>Samples did not match sequences present in the GenBank database.

<sup>b</sup>Molecular identification not done due to limited number of samples.

Algeria (Boualem *et al.*, 2012; Abbes *et al.*, 2014). However, parasitism rate of 12.9% is relatively lower than maximum parasitism rates reported in Tunisia (25.5%) and Turkey (37.0%) (Doğanlar and Yiğit, 2011; Abbes *et al.*, 2014). *Hockeria* species was the most promising in terms of abundance and parasitism rate. This finding differs from studies conducted in Tunisia, Spain and Italy, which reported *Necremnus* species as the most frequently encountered parasitoids (Ferracini *et al.*, 2012; Abbes *et al.*, 2014; Gabarra *et al.*, 2014).

In the study, different parasitoid species were found in different localities. This finding suggested that environmental and climatic conditions may have had an influence on diversity. Whilst *Hockeria* and *Brachymeria* species were the most abundant (52.5%), our results showed that they may be better adapted to mid and low altitudes. The widely distributed *Diglyphus isaea* also exhibited an adaptability to high and mid altitudes. Our results showed that parasitoids were mostly obtained from

localities that registered relatively high infestations and trap catches of *T. absoluta*. We hypothesize therefore that the occurrence and abundance of the pest may also have contributed to the overall abundance and diversity of the sampled parasitoids.

Species identifications of natural enemies concurred with reports in the native and invaded regions (Desneux *et al.*, 2010; Zappalà *et al.*, 2013; Ferracini *et al.*, 2019). *Hockeria unicolor* (Walker) and *Brachymeria secundaria* (Ruschka) have been reported in Spain and Turkey (Doğanlar and Yiğit, 2011; Gabarra *et al.*, 2014). Although these authors reported low frequency and parasitism, our study showed that *Hockeria* and *Brachymeria* species were the most important parasitoids of *T. absoluta* in Kenya. *Diglyphus isaea* (Walker) and *Neochrysocharis formosa* (Westwood) have been reported as parasitoids of *Liriomyza* species in vegetable systems in Kenya (Foba *et al.*, 2016). Our study, therefore, showed that these species have expanded their host range towards *T. absoluta* and

**Table 6.** Percentage parasitism (mean ± SE) of parasitoids obtained from sentinel tomato (var. Simlaw Rio Grande) between November 2015 and March 2016.

Species	Percentage parasitism (%)		t value	df	P	Overall parasitism (%)	Abundance
	2nd instars	3rd instars					
<i>Hockeria</i> sp.	1.76 ± 0.59a	2.11 ± 0.61a	-0.42	38	0.68	1.94 ± 0.42A	16 (84.21%)
<i>Necremnus</i> sp.	0.66 ± 0.36a	0.00 ± 0.00a	1.82	38	0.08	0.33 ± 0.19B	3 (15.79%)
Total	1.21 ± 0.35	1.06 ± 0.34				1.13 ± 0.25	19 (100%)

Means followed by the same lowercase letters in a row and same uppercase letters in a column are not significantly different ( $P < 0.05$ , two sample t-test).

corroborated reports in Algeria, France, Italy and Spain (Zappalà et al., 2013; Dehliz and Guénaoui, 2015). Although *D. isaea* reported a relatively wide distribution, parasitism on *T. absoluta* was lower than *Liriomyza* species (Foba et al., 2016).

To our knowledge, *Stenomomesius rufescens* (Retzius) is recorded for the first time as a larval parasitoid of *T. absoluta*. This finding agreed with studies from Spain, Algeria and France that have reported *Stenomomesius* species as parasitoids of *T. absoluta* larvae (Zappalà et al., 2013; Gabarra et al., 2014; Dehliz and Guénaoui, 2015). Our finding on *Necremnus* species fitted studies from various authors that have reported eight *Necremnus* species associated with *T. absoluta* larvae (Ferracini et al., 2019). Identification of *Bracon* and *Chelonus* species concurred with reports from native and several invaded regions (Ferracini et al., 2019). Molecular identification further identified *Chelonus* species as *Chelonus blackburni* (Cameron), and thus adds to the catalogue of *T. absoluta* parasitoids as a new record. The recovery of a *Goniozus* species could be supported by the studies that have reported *Goniozus nigrifemur* Ashmead as a larval parasitoid of *T. absoluta* (Ferracini et al., 2019). Despite the diverse species of parasitoid reported to form new association with *T. absoluta* in this study, the parasitism rate was quite low. This call for introduction of efficient co-evolved natural enemies from the pest aboriginal home for classical biological control of the pest in Kenya and Africa at large, an approach which is being explored (Aigbedion-Atalor, et al., 2020).

## Conclusion

Our study has shown that *T. absoluta* is widely distributed in Kenya and has attained significant levels of abundance and infestation on tomato in most production areas. The presence of *T. absoluta* at high-, mid- and low-elevation regions indicated that its nationwide distribution is not limited by altitude. The observed leaf infestation implied an important reduction in crop productivity, whereas damage on fruits reflected substantial financial losses and low returns. Our findings also indicated that several indigenous natural enemies have adapted to *T. absoluta*, thus the need to conserve them as a startup of biological control and exploitation in future IPM programs. More research, however, is required to evaluate their effectiveness as potential biocontrol agents of *T. absoluta*. Furthermore, the study revealed overall low abundance and parasitism rates which pave the way and call for introduction of efficient natural enemies as a potentially sustainable control alternative.

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