HOST-PLANT ACCEPTANCE, FECUNDITY AND LONGEVITY OF *Tetranychus evansi* (Acari: Tetranychidae) ON SELECTED TOMATO ACCESSIONS

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ABSTRACT

Seven tomato (*Lycopersicon* sp.) accessions were evaluated for their resistance to the tomato red spider mite, *Tetranychus evansi* Baker and Pritchard, in the laboratory. Data recorded was: (i) trichome density and (ii) fecundity and longevity. Whole potted intact tomato plants were used for the olfactometer bioassays, while leaf disk sections were used for the glass bridge bioassays. There was a significant ($P<0.05$) negative correlation between fecundity and longevity with the density of trichome types I and IV. Significantly more females chose the direction with 'Money Maker' (susceptible accession) when compared to other accessions. Thus, trichome density and plant-emitted volatiles seem to play an important role in host-plant selection of spider mites.

Key words: *Lycopersicon* sp., Trichomes, Tomato red spider mite, Volatiles

INTRODUCTION

The tomato red spider mite, *T. evansi* Baker and Pritchard, is an important pest of tomato in many parts of Africa (Knapp et al., 2003; Saunyama and Knapp, 2003; Duverney et al., 2005) and Europe (Escudero and Ferragut, 2005). Smallholder African farmers have mainly relied on frequent application of synthetic acaricides to control the mites (Smith Meyer, 1996; Saunyama and Knapp, 2003). However, the excessive and inappropriate use of acaricides has been associated with environmental and health problems, affecting non-target organisms, and promoting the rapid development of spider mite resistance to acaricides (Cranham and Helle, 1985; James and Price, 2002). For instance, soon after *T. evansi* was recorded as a new pest of tobacco in Zimbabwe, it developed resistance to thiophosphate acaricides due to frequent application of these acaricides (Blair, 1983).

Arthropod resistance in *Lycopersicon* has been associated with a diverse array of traits, including physical and chemical properties of trichomes as well as constitutively expressed and wound-induced chemical defenses associated with leaf lamellar exudates (Farrar and Kennedy 1991; Kennedy 2003). High densities of type VI trichomes (Luckwill, 1943) have been implicated in resistance of *L. hirsutum* var. *hirsutum* Dunal to two-spotted spider mite (*Tetranychus urticae* Koch) (Carter and Snyder, 1985, 1986; Weston et al*.,* 1989). Also resistance to *T. evansi* has been reported in *L. hirsutum* var. *hirsutum* and *L. pennellii* (Corr) D'Arcy (Maluf et al. 2001; Resende et al., 2002; Gonçalves et al., 2006). The host-plant range of spider mites is determined by adult females (Yano et al., 1998, 2001). The mites reach potential host-plants either by walking or through passive dispersal in wind and decide whether to lay eggs on the encountered host-plant or to leave as tested with *T. urticae* Koch females by Brandenburg and Kennedy (1982) and by Kennedy and Smitley (1985). Volatile plant kairomes could also play a role in host-plant selection (Dicke, 1986).

The objective of the present study was to establish the influence of trichomes of different wild and cultivated *Lycopersicon* accessions on oviposition and survival of *T. evansi* and to establish the response of the spider mites to odours associated with these tomato accessions, as a step to provide information for development of spider mite resistant cultivars.

MATERIALS AND METHODS
Plant Material and Mites
The investigations were performed on five accessions of cultivated tomato (*L. esculentum* Mill), namely varieties Money Maker and Marglobe (obtained from Simlaw Seeds, Nairobi, Kenya), landraces JKUAT 22/202183 (called JKUAT 22 hereinafter) and JKUAT 19 collected by the Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya staff, and LO 3279, as well as two accessions of wild origin, namely *L. hirsutum* var. *glabratum* C. H. Muller PI 134417, and *L. peruvianum* L. LA 2185 (obtained from the AVRDC, Shanhua Taiwan). Seeds were pre-germinated in soil enriched with compost in plastic seedling trays under ambient conditions in a screenhouse at the International Centre of Insect Physiology and Ecology (ICIPE) (S 01°13.140'; E 036°53.440') Nairobi, Kenya. Seedlings were transplanted after six weeks into pots filled with a mixture of red soil, compost and sand (3:2:1 v/v/v) and placed on benches in a screenhouse and arranged in a completely randomized design (CRD). Plants were watered daily and each pot was fertilized with 3 g Calcium

Ammonium Nitrate (CAN), containing 26% N and obtained from Jumbo Agrovet, Nairobi, Kenya, two weeks after transplanting.

The spider mites used in these experiments were sourced from a stock culture reared at ICIPE on 'Money Maker' plants at 25ºC and 50%-70% relative humidity (RH) under a photoperiod of 12 hours.

Trichome Type and Density
Three fully-expanded leaflets were collected at random from between the third and fifth leaf from the bottom of three randomly selected, 10-week-old plants of each accession. Classification and identification of trichome types was done according to Luckwill (1943) method, based on morphology and on the presence (types I, IV, VI, VII) or absence (types II, III and V) of glands. Ten squares (each 0.11 mm^2) were selected at random under a dissecting microscope (Leica MZ8, Leica Microsystems, Wetzlar, Germany), on the abaxial surface of each leaflet, and the trichomes counted. Trichome density per mm^2 was then calculated.

Mite Fecundity and Longevity
Tests were carried out on 20 leaf discs (25 mm diameter) taken from leaflets of similar age as those on which trichomes were counted. The leaf discs were placed individually on wet cotton wool in Petri dishes (60 mm diameter) with the abaxial surface facing up. A quiescent female deutonymph from the *T. evansi* culture was placed on each leaf disc. To ensure mating, two adult males were added to each leaf disc. The rearing units were placed in an incubator set at 25°C and 70%-80% RH. Males were removed one day after the female had emerged. Eggs were counted every 48 h with the help of a dissecting microscope until the mite died. Water was added each day on the cotton wool as was necessary to ensure adequate moisture for the leaf discs and to maintain a mite barrier. The leaf discs were changed every 4 days.

Olfactometer Bioassay
To bioassay the response of *T. evansi* females to odours from various tomato accessions, a modified Y-tube olfactometer similar to that described by Sabelis and Van de Baan (1983) was used. Before starting, the olfactometer was tested with blank cages to ascertain that mites displayed an equal response for the two arms of the olfactometer. Adult female mites were randomly collected from culture and used. The experiment was carried out at 23 ± 1°C, 50%-70% RH. A potted plant of the test accession was maintained in a glass chamber connected to one arm of the olfactometer and another chamber with a Money Maker plant. At the start of each bioassay, a female was placed on the wire at the entrance of the trunk on which the mites

walked on to either end of the olfactometer arms. A positive response was recorded when females oriented themselves towards the odour source from the test accession and reached the far end of the arm within 10 minutes from the start of the experiment. Mites that walked up and down without reaching one of the arms within 10 minutes were recorded as showing no response (0). Fourty replicates were used for each category on four different days.

Glass Slide Bioassay

The bioassay used was modified after van de Boom et al. (2003). Leaf sections were connected via glass slides, each measuring 5 cm x 2.5 cm. A permanent marker pen was used to mark a 1.0 cm x 1.5 cm area at both ends of the slide, and in the centre of the slide. The slides were then placed on wet cotton wool in a flat tray. Half leaf disks (12.5 mm diameter) for the two plant types were prepared from three randomly selected leaflets as described previously. The leaf portions were placed on the opposite marked ends of the glass slide. Ten female mites from the colony were transferred, using a camel-hair brush, to the centre of the slide and left to stand for 10 minutes. Thereafter, the number of mites that had settled on each leaf portion was recorded. Sixty replicates were used for each comparison.

Data Analysis

Data were subjected to analysis of variance (ANOVA) using Proc GLM (SAS Institute, 2000). Means were separated using the Fisher's Protected LSD test at P=0.05. Values of total fecundity and oviposition rate were logarithmically transformed before ANOVA. Correlation analysis was done using SAS PROC CORR. Data from the olfactometer and glass slide bioassays were analyzed using the Chi-square (χ^2) test (SAS Institute, 2000).

RESULTS

Leaflets differed significantly in the density of trichomes on the abaxial surface, except for trichomes types II (P= 0.55) and III (P = 0.43) (Table 1). The density of type IV trichomes was significantly (P<0.05) higher in the wild accessions PI 134417 (12.3) and LA 2185 (11.3) than in cultivated tomato accessions where they were absent in all accessions, except Marglobe. By contrast, type V density was significantly (P<0.05) higher on the cultivated accessions than on the wild tomatoes. Type III trichomes were only present in JKUAT 19 and type VII trichomes were only found in JKUAT 22 (Table 1).

Table 1. Trichome types and density per leaflet examined from seven different tomato accessions

Tomato accessions	Types and density (mm^2) of trichomes						
	I	II	III	IV	V	VI	VII
Money Maker	0.2 ± 0.2c	0.7 ± 0.4a	0.0 ± 0.0a	0.0 ± 0.0b	15.2 ± 1.4b	4.0 ± 0.7a	0.0 ± 0.0b
Marglobe	1.0 ± 0.5bc	0.0 ± 0.0a	0.0 ± 0.0a	0.7 ± 0.5b	7.6 ± 1.1c	3.3 ± 0.7ab	0.0 ± 0.0b
PI 134417	1.7 ± 0.6ab	0.2 ± 0.2a	0.0 ± 0.0a	12.3 ± 1.9a	1.2 ± 0.5d	5.2 ± 1.2a	0.0 ± 0.0b
JKUAT 22	0.2 ± 0.2c	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0b	16.6 ± 1.3b	0.0 ± 0.0c	3.3 ± 0.7a
JKUAT 19	0.5 ± 0.3c	0.0 ± 0.0a	0.2 ±0.2a	0.0 ± 0.0b	9.2 ± 1.3c	3.8 ± 0.8a	0.0 ± 0.0b
LA 2185	2.6 ± 0.6a	0.0 ± 0.0a	0.0 ± 0.0a	11.3 ± 1.4a	0.0 ± 0.0d	1.4 ± 0.5bc	0.0 ± 0.0b
LO 3279	0.0 ± 0.0c	2.2 ± 0.2a	0.0 ± 0.0a	0.0 ± 0.0b	22.3 ± 1.5a	4.0 ± 0.9a	0.0 ± 0.0b
F	5.5	0.8	1.0	38.8	51.4	5.3	25.4
df	6, 209	6, 209	6, 209	6, 209	6, 209	6, 209	6, 209
P-values	<0.0001	0.5454	0.4265	<0.0001	<0.0001	<0.0001	<0.0001

Means followed by the same letter within the same column are not significantly different at $P = 0.05$, according to Fisher's LSD test.

Females did not oviposit on the wild accessions PI 134417 and LA 2185. In addition, the lifespan was also shortest in these accessions. The highest fecundity and longevity was found in JKUAT 22 followed by Money Maker (Table 2). The biological parameters: fecundity and longevity were significantly ($P<0.05$) negatively correlated to the density of Type I, IV and VI trichomes (Table 3).

Significantly more spider mites chose the direction with Money Maker when comparisons were made against Marglobe, LO 3279, LA 2185 and PI 134417 in olfactometer and glass slide bioassays. Differences between Money Maker and JKUAT 19 and JKUAT 22/202183 were not significant ($P>0.05$) (Figures 1 and 2).

Table 2. Number of eggs/female, number of eggs per female per day and longevity (days) of *T. evansi* on tomato accessions

Tomato accessions	Number of eggs/female on life	Number of eggs/ female/day	Longevity (days)
Money Maker	32.4 ± 4.8a	4.1 ± 0.5a	8.3 ± 1.0ab
Marglobe	10.2 ± 2.4c	1.4 ± 0.3c	8.0 ± 1.3ab
PI 134417	0.0 ± 0.0d	0.0 ± 0.0d	4.3 ± 0.2c
JKUAT 22	48.8 ± 8.1a	5.1 ± 0.8a	9.2 ± 1.3a
JKUAT 19	15.0 ± 2.9bc	2.6 ± 0.4b	6.2 ± 0.6bc
LA 2185	0.0 ± 0.0d	0.0 ± 0.0d	4.4 ± 0.2c
LO 3279	18.5 ± 3.6b	2.5 ± 0.4b	8.1 ± 0.8ab
df	6, 139	6, 139	6, 139
P-values	<0.0001	<0.0001	0.0001

Means followed by the same letter within the same column are not significantly different at $P = 0.05$, according to Fisher's LSD test

Table 3. Correlation between trichome density and total fecundity and longevity of *Tetranychus evansi* on different tomato accessions

Trichome type	Correlation coefficient (r)	
	Fecundity	Longevity
I	-0.737*	-0.840**
II	0.093ns	0.311 ns
IV	-0.706*	-0.890***
V	0.744*	0.816**
VI	-0.493ns	-0.297ns

*, **, *** = significant; ns = not significant at *P* = 0.1, 0.05, 0.01, respectively
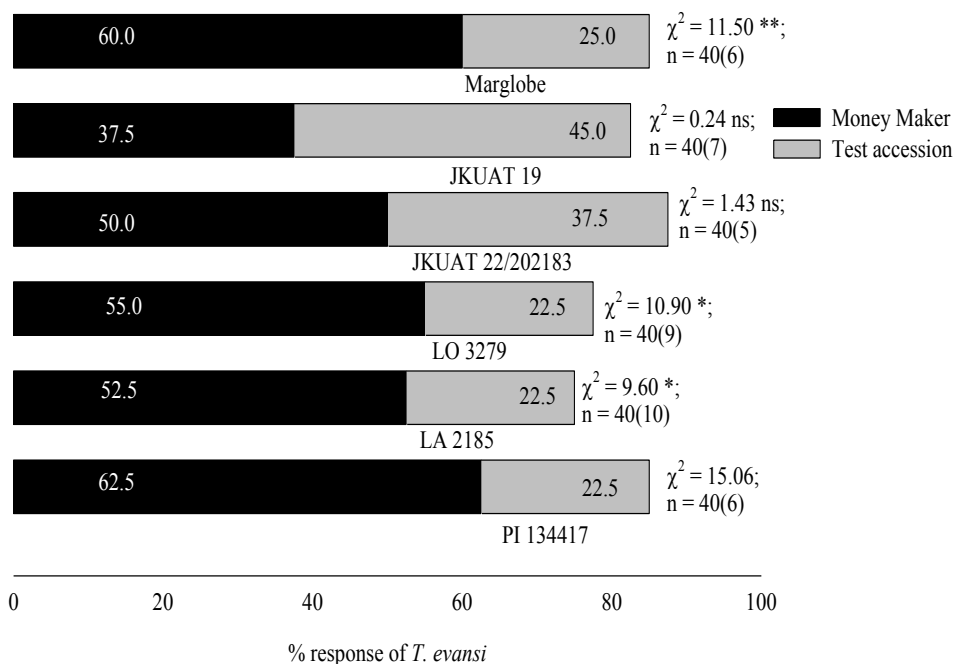
Figure 1. Response (mean ± S.E.) of *Tetranychus evansi* females to odor sources from intact plants of different tomato accessions in a Y-tube olfactometer. Asterisks (*) indicate significant differences between Money Maker and the test accession by Chi-square test (χ2) at *P = 0.05, **P = 0.01, ***P = 0.001; number of mites that did not make a choice is shown in brackets.
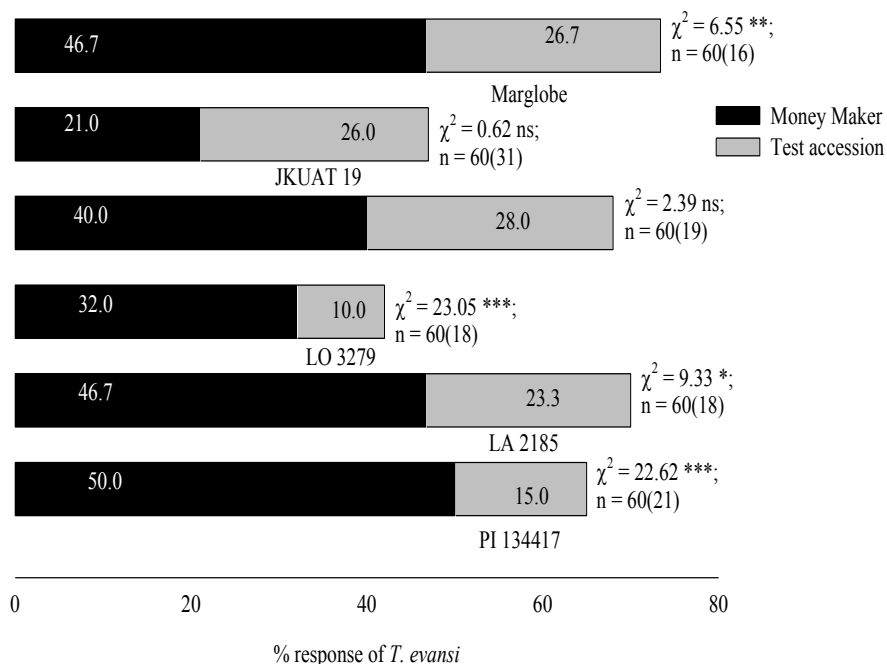
Figure 2. Response of *Tetranychus evansi* females to odor sources from leaf-disks of different tomato accessions on a glass slide. Asterisks (*) indicate significant differences between Money Maker and the test accession by Chi-square test (χ2) at *P = 0.05, **P = 0.01, ***P = 0.001; number of mites that did not make a choice is shown in brackets.

DISCUSSION

High densities of glandular type IV trichomes on *L. hirsutum*, and non-glandular type V trichomes on *L. esculentum* were also found by Simmons and Gurr (2005) and Luckwill (1943). The glandular trichome types IV and VI are the predominant trichomes associated with negative effects on pests in the genus *Lycopersicon* (Simmons and Gurr, 2005). The fact that there was no significant correlation of type VI trichome density with mite fecundity and longevity suggests that its association with resistance to arthropods is chemical rather than physical. Type VI glandular trichomes of *L. hirsutum* var. *glabratum* contain much higher levels of 2-tridecanone and 2-undecanone than in *L. esculentum* (Chatzivasileiadis et al., 1999). These methyl ketones are toxic to *T. urticae* (Chatzivasileiadis and Sabelis, 1997).

Snyder and Carter (1984; 1985) reported that mite survival was lower in *L. hirsutum* var. *hirsutum* that possessed fewer type VI trichomes than in *L. esculentum*, and concluded that trichome type IV could be responsible for the high levels of resistance in this species. Snyder and Carter (1985) and Maluf et al. (2001) reported a positive relationship between the density of type IV trichomes in *L. hirsutum* var. *hirsutum* and its hybrids with *L. esculentum* mortality as well as repellence of spider mites. Type IV trichomes of *L. hirsutum* var. *hirsutum* contain the sesquiterpene zingiberene that confers high levels of resistance to *T. evansi* (Weston et al., 1989; Maluf et al., 2001). The present study agreed with Gurr and McGrath (2002), who reported a positive effect of type V trichomes on an arthropod pest. These authors demonstrated that these trichomes were positively correlated with larval survival of the potato tuber moth *Phthorimaea operculella* (Zeller) in *L. hirsutum*. However, according to Simmons and Gurr (2005), this might be an artefact since plants with high densities of type V trichomes have low densities of type IV and VI trichomes and vice versa.

Much less is known about resistance and resistance mechanisms in *L. peruvianum*. Picanço et al. (1997) reported differences in resistance of three accession of *L. peruvianum* to *Tetranychus ludeni* Zacher and the eriophyid mite *Aculops lycopersici* (Massee), but did not compare these accessions to susceptible cultivated tomatoes. Silva et al. (1992), observed similar oviposition of *T. evansi* on the *L. peruvianum* accession LA 444-1 and the cultivated tomato variety Angela Gigante. By contrast, the *L. peruvianum* accession LA 2185 tested in the present study was resistant to *T. evansi*. This suggests that there is a large difference in *L. peruvianum* accessions with regard to mite resistance.

The spider mites clearly preferred the susceptible variety Money Maker to the resistant wild accessions PI 134417 and LA 2185 in both bioassays. However, preference was not clearly linked to performance in comparisons between different *L. esculentum* accessions. Yano et al. (1998), reported a significant correlation between host-plant acceptance and fecundity of *T. urticae*. The relative indifference towards PI 134417 and in one case LA 2185 implies lack of attractive volatiles and their precursors from these accessions. Gonçalves et al. (1998), had earlier demonstrated that volatiles of type VI trichomes in accession PI 134417 contain high concentrations of the methyl ketones that cause repellency to spider mite species like *T. urticae* and *Tetranychus ludeni* Zacher. Dicke (1986) has shown that spider mites react to host-plant emitted volatiles in an olfactometer. Contrary to our results, van den Boom et al. (2003), and Yano et al. (1998), did not find attraction of *T. urticae* to lima bean leaf sections compared to a plastic disc

in experiments comparable to the glass slide bioassay. Van den Boom et al. (2003), hypothesized that the amount of volatiles emitted by the small leaf sections was not enough to influence the choice of mites. As the major dispersal mechanism of spider mites is by wind volatiles might in practice influence the decision of the mite to stay on the plant it landed on or leave.

CONCLUSION AND RECOMMENDATION

The trichome density on the abaxial surface of leaves affects fecundity and lifespan of tomato spider mite. Plant emitted volatiles play an important role in host-plant selection of spider mites. If this is mainly caused by attraction to odours from suitable host-plants or repellence by odours from unsuitable plants remains to be investigated. The fact that spider mites are not attracted by small leaf section of a suitable host-plant compared to a plastic disc (van den Boom at al. ,2003, Yano et al., 1998), but clearly preferred small leaf discs of suitable plants to unsuitable plants in our experiments leads to the hypothesis that repellence is more important than attraction. As spider mites in nature often reach host-plants through passive dispersal in wind (Yano et al., 1998) this repellence effect might trigger their movement away from unsuitable hosts.

ACKNOWLEDGEMENTS

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