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Influence of canopy height and fertilization levels on the resistance of *Lycopersicon hirsutum* to *Aculops lycopersici* (Acari: Eriophyidae)

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Abstract. The objective of this work was to study the effect of NK fertilization levels and canopy height on the resistance of *Lycopersicon hirsutum* and *Lycopersicon esculentum* to *Aculops lycopersici* (Acari: Eriophydae). The effects of NK fertilization levels and canopy height in the leaf size and density of trichomes and their effects on tridecan-2-one (2-TD) and undecan-2-one (2-UD) limiting the attack of *A. lycopersici* on tomato plants were assessed. Different NK fertilization levels had no effect on the resistance of *L. hirsutum* to *A. lycopersici*. No significant differences were found in attack rates of this mite on leaves of the top and median parts of *L. hirsutum* canopy. The type and density of trichomes were the main determining factor of *A. lycopersici* attack on tomato plants. High trichome densities and type VI glandular trichomes which produce tridecan-2-one are important resistance factors on tomato plants. *L. hirsutum* showed a high resistance level to *A. lycopersici* due to high densities of type VI glandular trichomes and consequently higher levels of tridecan-2-one in its leaves.

Key words: Tomato plants, susceptibility, mite, tridecan-2-one, nitrogen, potassium

Introduction

Aculops lycopersici (Massee, 1937) (Acari: Eriophyidae) is one of the tomato pests in Brazil, both young and adults feeding on the plants. Large colonies feed on tomato leaves and cause rupture of the parenchyma, thus reducing leaf size and resulting in abnormal plant growth. Older infested leaves fall to the ground and infested fruits acquire a white and pale colour (Vanetti, 1983).

As soon as the first symptoms are seen, insecticides are used to control this mite species due to the lack of other control alternatives (Picanço *et al*., 1997). For this reason it is necessary to develop tomato varieties resistant to this arthropod. Some wild tomatoes are resistant to mites due to the presence of tridecan-2-one (2-TD) and

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undecan-2-one (2-UD) on exudates of leaf glandular trichomes which are toxic to *A. lycopersici* (Carter and Snyder, 1986; Weston *et al*., 1989; Snyder *et al*., 1993).

One of the limitations of using *L. hirsutum* f. *glabratum* (PI 134417) is that its resistance depends on fertilization levels and canopy height of tomato plants. When NPK fertilization is increased, trichome density decreases as does plant resistance to insects (Barbour *et al*., 1991). This was also demonstrated by Leite *et al*. (1995) who observed larger oviposition of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), another tomato pest in South America, on leaves of the median height than on those of the apical and basal parts of tomato canopies.

The objective of this work was to quantify level of 2-TD, 2-UD, N and K, with densities and type of trichomes and leaf area in the top and median parts of the canopy of three-month-old plants of *L. hirsutum* f. *glabratum* (PI 134417), and relate these factors to the attack rate of *A. lycopersici*.

Materials and methods

This study was carried out at the Federal University of Viçosa, in Viçosa, State of Minas Gerais, Brazil, from June to October 1996. When the tomato plants were one month old, a total of 15 pots (three pots per block) were put inside the greenhouse with two-month-old plants of *L. esculentum* with attack symptoms of *A. lycopersici* for use as the infestation source. When plants of *Lycopersicon* were three months old and showing attack symptoms, the population density of *A. lycopersici* was visually assessed using a 12.5 × magnifying lens on both faces of three leaves randomly chosen from each canopy height.

The experiment used a split-plot design with the tomato species and fertilization levels as the main plots, and canopy height as the sub-plots with five replicates. Each replicate was constituted by one three-month-old tomato plant in a 5 liter polyethylene pot. The following sources of variation were studied: (1) two tomato species [*Lycopersicon esculentum* cv. Santa Clara (susceptible) and *Lycopersicon hirsutum* f. *glabratum* – PI 134417 (resistant)]; (2) canopy height [top and median height of canopy]; and (3) fertilization levels [$N_{100}K_0$ (100 mg of N/kg soil), $N_{100}K_{200}$ (100 mg of N and 200 mg of K/kg soil), $N_{300}K_0$ (300 mg of N/kg soil), and $N_{300}K_{200}$ (300 mg of N and 200 mg of K/kg soil]), where the $N_{300}K_{200}$ level corresponds to recommended fertilization level for tomato plants in Brazil (CFSEMG, 1989). Leaves on the lower part of the tomato plants were not evaluated because they were senescent at the time of the study. Chemical analysis of the soil was made prior to beginning the experiment. After this analysis, minimum levels (plants with mineral deficiency) and maximum levels (plants without mineral deficiency) of N and K were established and used for this experiment. K was added as KCl and mixed with the soil one month before starting the experiment. The N source was urea which was added to the pots as an aqueous solution one week before sowing. When the plants were two months old fertilization was maintained with a total of 0.30 g of urea being added to each pot.

Two sets of plants were selected for study following the experimental design reported above. Mite studies were carried out in the first set, while the second set was used for plant related determinations (i.e., levels of 2-TD, 2-UD, nitrogen and potassium and trichome density). In the second set of plants, three leaves were collected from each plant part during the morning and 5 g of them were extracted with 50 ml of distilled hexane for 24 h to extract 2-TD and 2-UD (Leite, 1997). The extracted solution was dried using Na_2SO_4 (anhydrous) and evaporated at 30 °C in rotatory evaporator. The oil obtained was redissolved in 1 ml of hexane and analyzed by gas chromatography using a glass column packed with methyl phenyl silicone (OV 17, 1%). The chromatograph (GC Instruments, State of São Paulo, Brazil) was programmed from 150 °C to 220 °C at 6 °C/min. The injector and detector were maintained at 260 °C and 280 °C respectively and 1 ml of sample was used for the injection. The flow rates of H_2/N_2/air were 30, 30 and 300 ml/min respectively.

Individually collected leaves from each replicate were stored in 70% ethanol, cleared with 10% NaOH for two hours and commercial sodium hypochlorite (20%) for 18 hours for trichome density evaluation. After washing and during dehydration, the leaves were coloured and mounted between slides using Canadian balsam. Samples were coloured for three minutes in fast green (Johansen, 1940). Trichome density (trichomes/mm^2) was assessed in cleared leaves from the median of three leaflets of the top part of each leaf (Channarayappa *et al*., 1992). Twenty-four fields (area of one field = 0.60 mm^2) were analyzed in the mid-part of each leaf area (equidistant between the principal vein and the margin of the leaf). Trichome count in the epidermis (adaxial and abaxial) was made by focalization of these fields in the collected leaves. The trichomes were classified as glandular or non-glandular in both genotypes, despite the existence of seven distinct trichomes types within the genus *Lycopersicon* (Channarayappa *et al*., 1992). This was done because the accessions of *L. hirsutum* show only glandular trichomes (types I, IV, VIc, and VII) and mainly of the type VI (producer of 2-TD and 2-UD), unlike *L. esculentum* which shows mainly non-glandular trichomes (types III, Va, and Vb) (Channarayappa *et al*., 1992). One leaf per replicate was collected to measure leaf area (cm^2) using the Delta T Leaf Area Measurement System (Bacarin, 1995).

Nutrient concentration was determined using three leaves from each canopy height. The leaves were placed in paper bags and dried at 67 °C in an air forced oven for three days, and then ground in a Wiley mill (20 mesh), and analyzed for their mineral content. K was determined with plutometer flame (Coleman, model 22) and N was analyzed by the Nessler method (Jackson, 1958).

The results of *A. lycopersici* attack, leaf area, trichome density, concentrations of 2-TD, 2-UD, N and K were submitted to analysis of variance and to Scott–Knott groupment analysis test at 5% significance (Scott and Knott, 1974), using the System of Statistical and Genetics Analysis of the Federal University of Viçosa (Euclides, 1983). Regression analysis was also carried out using the same software (Euclides, 1983).

Results

There were significantly higher levels of N and K on leaves when higher fertilization levels of these two nutrients were used (Table 1). Increased levels of N and K were observed in leaves from the apical part of the canopy compared with the median part for both tomato species (Table 1). There was no significant effect of NK fertilization on leaf area (168.12 cm^2) and trichome density (9.93/mm^2) in *L. hirsutum* ($df = 16$, $P > 0.05$, $n = 48$). There was also no significant effect of canopy height on 2-UD concentration in *L. hirsutum* ($df = 16$, $P > 0.05$, $n = 48$). However, the higher the canopy height, the greater the trichome density and 2-TD production in *L. hirsutum* (Fig. 1). Trichome density also increased with the canopy height in *L. esculentum*, but an increase in leaf area caused a degree of trichome density in this species (Fig. 1).

Bigger leaves were observed in *L. esculentum* fertilized with $N_{300}K_0$ (370.76 cm^2) and $N_{300}K_{200}$ (370.73 cm^2) than with $N_{100}K_0$ (281.75 cm^2) and $N_{100}K_{200}$ (260.33 cm^2) ($df = 16$, $P < 0.05$, $n = 48$). A higher trichome number was found on leaves of *L. esculentum* with $N_{100}K_0$ fertilization (13.57/mm^2, followed by $N_{100}K_{200}$ (11.30/mm^2), than with $N_{300}K_0$ (10.55/mm^2) and $N_{300}K_{200}$ (9.20/mm^2) ($df = 16$, $P < 0.05$, $n = 48$). *L. esculentum* and *L. hirsutum* showed 9.69% and 97.12% of glandular trichomes respectively. Increased trichome densities were observed in the abaxial faces rather than in the adaxial in *L. esculentum* (13.66 and 7.11/mm^2 respectively) and in *L. hirsutum* (11.76 and 6.35/mm^2 respectively) ($df = 16$, $P < 0.05$, $n = 48$).

Table 1. Effect of NK levels and canopy height on N and K concentration on leaves of *Lycopersicon esculentum* and *Lycopericon hirsutum* ($df = 16$, $P < 0.05$, $n = 48$)

Fertilization	Nutrient concentration on leaves (% on dry matter)			
	Nitrogen		Potassium	
	L. esculentum	*L. hirsutum*	*L. esculentum*	*L. hirsutum*
$N_{100}K_0$*	2.28 C**	2.97 A	1.83 B	2.79 C
$N_{100}K_{200}$	2.15 C	2.56 B	3.11 A	3.60 B
$N_{100}K_0$	3.03 A	3.08 A	1.69 B	2.70 C
$N_{300}K_{200}$	2.70 B	3.05 A	3.12 A	4.20 A
Canopy				
Apical	2.68 A	3.52 A	2.77 A	3.33 A
Median	2.01 B	2.46 B	1.84 B	2.65 B

* N_{100} and N_{300} = 100 and 300 mg of N/kg of soil, respectively. K_0 and K_{200} = 0 and 200 mg of K/kg of soil, respectively.
** Means followed by same letter within a column are not significantly different by the Scott-Knott groupment analysis test at $P < 0.05$.

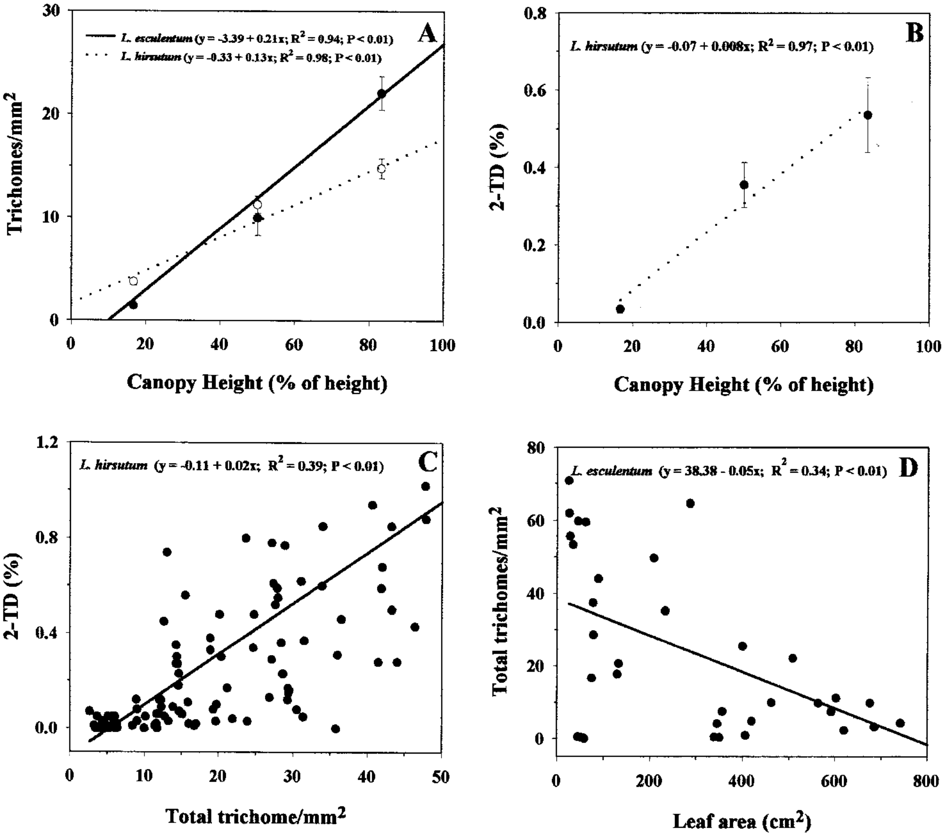

Figure 1. (A) Effect of canopy height on trichome density; (B) concentration of 2-TD; (C) effect of trichome density on 2-TD concentration; (D) effect of leaf area on trichome density in tomato species. The symbols represent the average of five replicates and the vertical bars indicate standard errors of the mean in Figs 1A and 1B.

Significantly larger numbers of *A. lycopersici* were observed in leaves of *L. esculentum* (6.60 mites/cm^2) than in *L. hirsutum* (0.11 mites/cm^2) in the different fertilization levels and canopy heights (df $= 32$, $P < 0.05$, $n = 80$). Numbers of *A. lycopersici* were similar at both canopy heights of *L. hirsutum*, whereas highest numbers of mites were observed in the median height (10.94/cm^2) than in the apical part (2.75/cm^2) of *L. esculentum* (df $= 32$, $P < 0.05$, $n = 80$). Number of mites on the adaxial and abaxial faces of *L. esculentum* (5.74 and 7.53/cm^2) and *L. hirsutum* (0.13 and 0.08/cm^2) were similar (df $= 32$, $P > 0.05$, $n = 80$). The optimum levels of N and K for development of *A. lycopersici* in *L. hirsutum* were 2.0% for both nutrients as shown in Fig. 2B and 2C; N in *L. esculentum* was 1.4% on dry matter (Fig. 2A). The K level on leaves of *L. esculentum* did not have a significant effect on the mite density for leaves of this tomato species.

The increase in trichome density in both tomato species was detrimental to *A. lycopersici* (Fig. 3A, B). The harmful effect of trichome density of *L. esculentum* in the mite species is mainly mechanical, unlike the effect of the trichomes of *L. hirsutum* which are glandular and produce the toxic compound 2-TD. The higher the level of 2-TD leaves of *L. hirsutum*, the lower the mite density observed as would be expected (Fig. 3C).

Discussion

L. hirsutum was more resistant to *A. lycopersici* than *L. esculentum* when compared to other tomato pests (Giustolin, 1991; Channarayappa *et al*., 1992; Silva *et al*., 1992; Leite, 1997). Higher NK fertilization levels led to the accumulation of increased levels of the two nutrients in leaves of *L. esculentum* and *L. hirsutum* as expected. However, the NK fertilization levels did not have any direct effect on the resistance factors of *L. hirsutum* to *A. lycopersici*, such as trichome density and levels of 2-TD and 2-UD, unlike that observed by Barbour *et al*. (1991), who noticed that with increased NPK fertilization (20–20–20) from 1.4 g to 2.8 g/kg of soil, there was a decrease in the trichome-based resistance of *L. hirsutum* f *glabratum* PI 134417. This difference may be due to the influence of phosphorus in the study by Barbour *et al*. (1991) and also because in our study all glandular trichomes were pooled together.

Despite *A. lycopersici* showing maximum densities at specific N and K leaf concentrations, these two nutrients seem to play a minor role in affecting the resistance of *L. hirsutum* to this mite species. On the other hand, higher N fertilization levels led to an increase in leaf area in *L. esculentum* and a decrease in trichome what might favour the attack of *A. lycopersici* on this species. Maia and Busoli (1992) also reported positive effects of high N levels on the density of *Tetranychus urticae* (Koch) (Acari: Tetranichidae) in cotton plants, while Tanzini *et al*. (1993) observed negative effects of high K levels in *Caliothrips brasiliensis* Morgan (Thysanoptera: Thripidae). Unfortunately the cause of these responses on the arthropod species were not determined.

Trichome type and density seem to be the most important factors determining the resistance of tomato plants to *A. lycopersici*. The susceptible tomato species *L. esculentum* presents mainly non-glandular trichomes while the resistant species *L. hirsutum* presents mainly type VI glandular trichome which produces 2-TD and 2-UD, compounds which have insecticide and miticide effects (Carter and Snyder, 1986; Weston *et al*., 1989; Giustolin, 1991; Snyder *et al*., 1993; Leite, 1997). Therefore it seems that glandular trichomes are a more efficient defence against tomato pests than non-glandular trichomes. However, non-glandular trichomes still play an important role in protecting *L.esculentum* against *A. lycopersici*, as their density is negatively correlated with the mite density. This explains the higher mite

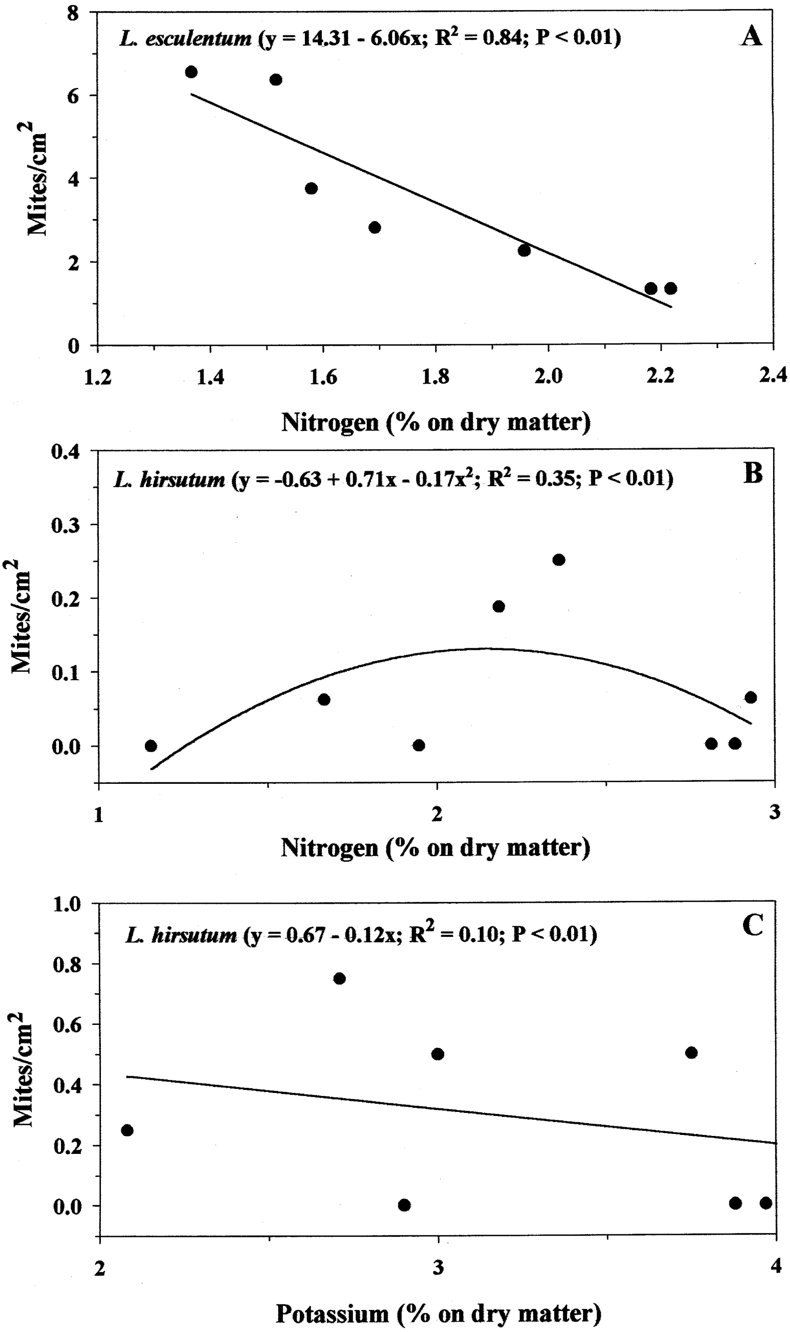

Figure 2. Effect of N and K on leaf levels in the leaf density of *Aculops lycopersici*. The symbols represent the average of five replicates.

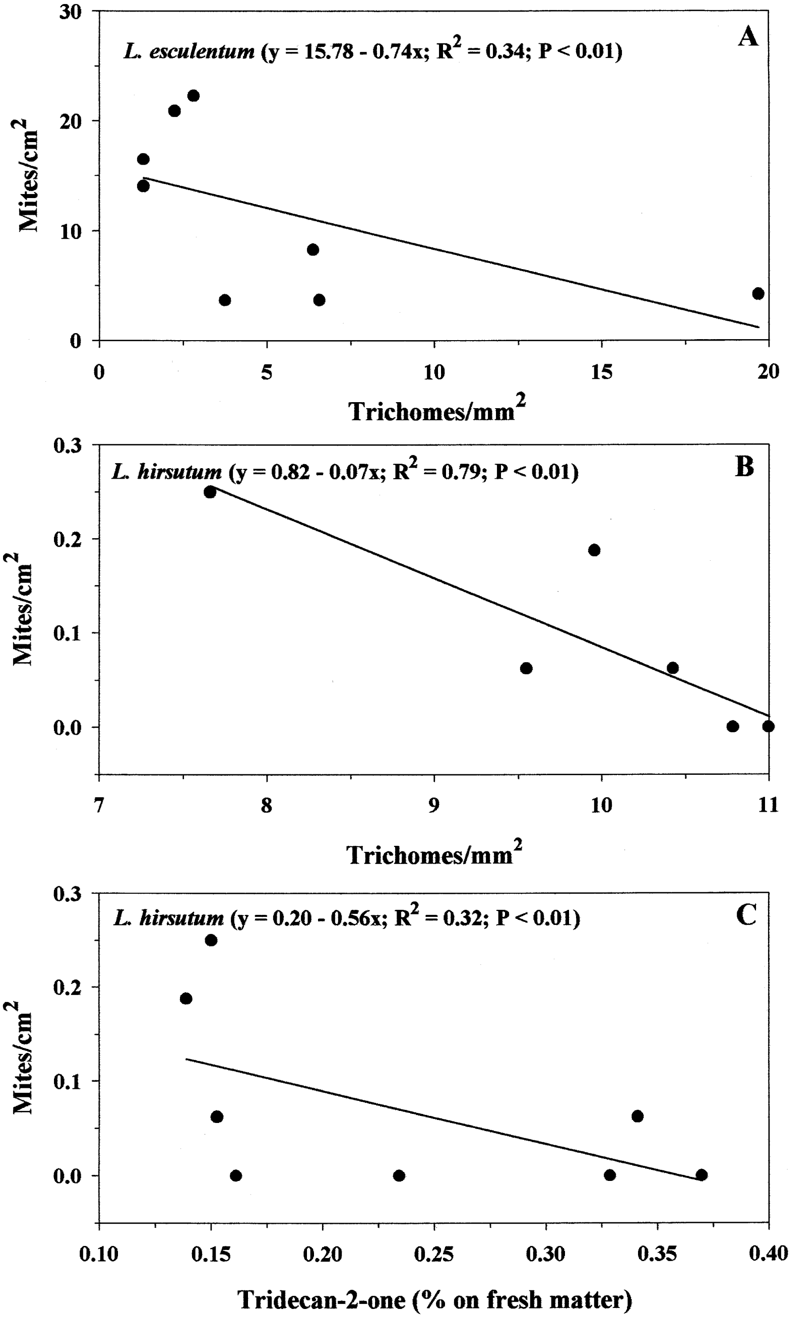

Figure 3. (A and B) Effects of trichome density and (C) concentration of 2-TD in the leaf density of *Aculops lycopersici*. The symbols represent the average of five replicates.

densities observed in the median part of the *L. esculentum* plants, which has lower trichome density than the apical part of the plant.

The partition of *A. lycopersici* infestation in *L. hirsutum* is distinct from *L. esculentum*. There is an increase in trichome density from the bottom to the median and top parts of the canopy of *L. hirsutum* plants. Because the main trichome of *L. hirsutum* is the glandular type VI trichome producer of 2-TD, there is also a higher concentration of 2-TD in leaves from the median and apical parts of the plant (Carter and Snyder, 1986; Weston *et al*., 1989; Snyder *et al*., 1993). The trichome density and 2-TD concentration were negatively correlated with the density of *A. lycopersici*, and seem to be the main resistance factors of *L. hirsutum* to this mite pest. The harmful effect of 2-TD has already been reported in the tomato pests *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and *Tetranychus urticae* where repellent and antibiotic effects were observed (Dimock *et al*., 1982; Carter and Snyder, 1986; Giustolin, 1991; Leite, 1997). Another compound produced by glandular type VI trichomes of tomato leaves is the 2-UD, but we were not able to observe any effect of this compound on *A. lycopersici* compared to those observed by Giustolin (1991) in *T. absoluta*.

In summary we reported the resistance of *L. hirsutum* to *A. lycopersici* and did not observe any effect of NK fertilization levels on this trait. Furthermore attacks by *A. lycopersici* did not differ in the median and apical parts of the *L. hirsutum* plants. However the median parts of the canopy of *L. esculentum* plants were subjected to more intense attacks by this mite pest. The type and density of trichomes seem to be the main factors determining the attach of *A. lycopersici* on tomato plants. In addition, the influence of NK fertilization and canopy height are not strong enough to suppress the effect of these resistance traits in *L. hirsutum*. Therefore the reported limitations to using *L. hirsutum* as a source of resistance are probably not the result of the effects of fertilization levels and canopy height and require further investigation.

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