INTENSITY OF *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) AND *Liriomyza* spp. (Diptera: Agromyzidae) ATTACKS ON *Lycopersicum esculentum* Mill. LEAVES

Intensidade de ataque de *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) e de *Liriomyza* spp. (Diptera: Agromyzidae) em folhas de *Lycopersicum esculentum* Mill.

Germano Leão Demolin Leite[1], Marcelo Picanço[2], Gulab Newandran Jham[3], Flávio Marquini[4]

ABSTRACT

The objective of this study was to determine the effect of height of leaves in the canopy of plants, leaf organic compounds, concentrations of leaf nitrogen and potassium, leaf trichomes and cristalliferous idioblasts densities on the attack intensity of three tomato (*Lycopersicon esculentum* Mill. cv. Santa Clara) crops by *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and *Liriomyza* spp. (Diptera: Agromyzidae), under field conditions. The experimental design was arranged in randomized blocks, with three replications, each being one tomato crop. Analysis of variance and the Tukey's multiple range test (5% significance) were used to test the effect of canopy height, trichome and cristalliferous idioblasts densities on the number of mines produced by *T. absoluta* and *Liriomyza* spp. and on the number of *T. absoluta* eggs. Pearson's correlation (5% significance) was used to evaluate the relationships between leaf organic compounds, leaf N and K concentrations, leaf trichome and cristalliferous idioblasts densities and the number of mines produced by *T. absoluta* and *Liriomyza* spp. and the number of *T. absoluta* eggs. Highest insect attack occurred in the final stage of the culture. A higher number of *Liriomyza* spp. mines/leaf was recorded in the lower (1.50) than in the upper (0.02) level of the tomato plants, the opposite was observed for the number of *T. absoluta* eggs/leaf (0.13 and 0.57, respectively). The number of *T. absoluta* mines/leaf concentrated more on the median (10.23) and apical regions (8.63) than on the basal (4.93). No significant effect of the trichomes and cristalliferous idioblasts densities of leaves was noted on *T. absoluta* and *Liriomyza* ssp. populations. Apparently, the terpenes affected oviposition of *T. absoluta* while leaf potassium affected *Liriomyza* spp. attack.

Index terms: Tomato, population dynamics, tomato leafminer, terpenes.

RESUMO

Objetivou-se com este estudo determinar as influências da posição das folhas no dossel das plantas, dos compostos orgânicos foliares, das concentrações de nitrogênio e potássio foliar, das densidades de tricomas e dos idioblastos cristalíferos foliares na intensidade de ataque de *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) e de *Liriomyza* spp. (Diptera: Agromyzidae) em três cultivos de tomate (*Lycopersicum esculentum* Mill. cv. Santa Clara) em condições de campo. O desenho experimental foi em blocos casualizados com três repetições, sendo cada um dos cultivos de tomate. Foram realizados análise de variância e teste de média de Tukey (5% de significância) para testar o efeito de dossel nas densidades de tricomas e de idioblastos cristalíferos, no número de minas de *T. absoluta* e de *Liriomyza* spp. e o número de ovos de *T. absoluta*. Foi usada correlação de Pearson (5% de significância) para avaliar as relações entre compostos orgânicos foliares, concentrações de N e de K foliares, densidade de tricomas e de idioblastos cristalíferos e o número de minas de *T. absoluta* e de *Liriomyza* spp. e o número de ovos de *T. absoluta*. O maior ataque desses insetos ocorreu no estágio final da cultura. Maior número de minas de *Liriomyza* spp. foi observado nas folhas basais (1,50), se comparando com as apicais (0,02), sendo o contrário observado para o número de ovos de *T. absoluta* (0,13 e 0,57, respectivamente). Minas de *T. absoluta*/folha concentraram-se mais nos terços medio (10,23) e apical (8,63) do que no basal (4,93). Não foi detectada influência das densidades de tricomas e de idioblastos cristalíferos foliares nas populações de *T. absoluta* e de *Liriomyza* ssp. Possivelmente, os terpenos afetaram a oviposição de *T. absoluta*, enquanto o potássio foliar afetou o ataque de *Liriomyza* spp.

Termos para indexação: Tomate, dinâmica populacional, traça-do-tomateiro, terpenos.

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1. Agronomist; D.Sc.; Professor; NCA/UFMG; Montes Claros, MG, 39404-006, CP: 135; gldleite@ufmg.br. Corresponding author.
2. Agronomist; D.Sc.; Professor; Universidade Federal de Viçosa (UFV); Viçosa, MG, 36571-000; picanco@mail.ufv.br.
3. Chemist; PhD; Professor; UFV. E-mail: gulab@mail.ufv.br.
4. Agronomist; M.Sc.; Graduate student; UFV.

INTRODUCTION

The tomato leafminers, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and *Liriomyza* spp. (Diptera: Agromyzidae) are important tomato pests in South America, with several morphological and chemical factors of plants influencing their populations (LETOURNEAU et al., 1996; PICANÇO et al., 1998; LEITE et al., 1999a). The NK fertilization can affect the degree of attack from insects (MARSCHNER, 1995): an excess of N and deficiency in K have been reported to lead to higher accumulation of amino acids in plants, leading to a higher degree of insect attack (MARSCHNER, 1995). Also, the leaf position on the plant canopy has been reported to influence *T. absoluta* and *Liriomyza* spp. attacks, with preferential oviposition on the apical leaves probably due to the fact that these leaves are more tender and of higher nutritional value (SILVA et al., 1998; LEITE et al., 1999a, b; OLIVEIRA, 1999).

The possible effect of trichomes on insects can result from chemical and/or mechanical factors. The chemical factor could partly be due to exudates produced by glandular trichomes which can contain compounds that are toxic to insects (FERY e KENNEDY, 1987; LEITE et al., 2001). Other possible resistance factors include mechanical barriers, density, length and form of trichomes (LEITE et al., 1999a, b). The presence of crystals in the leaves is another factor that can difficult feeding by insects (FRANCESCHI e HORNER JÚNIOR, 1980; LEITE et al., 1999c), with high occurrence of calcium oxalate crystals in Lycopersicon (LEITE et al., 1997; LEITE et al., 1999c).

The objective of this study was to determine the effects of height of leaves in the canopy of plants, leaf organic compounds, concentrations of leaf nitrogen and potassium, leaf trichomes and cristalliferous idioblasts densities on the intensity of attack of three “Santa Clara” tomato crops, by *T. absoluta* and *Liriomyza* spp., under field conditions.

MATERIAL AND METHODS

The experiment was conducted using three “Santa Clara” tomato crops (crops n°. 1, 2 and 3) from April to November of 1999 in Guidoval, Minas Gerais State, Brazil. Cultural practices utilized have been described by Filgueira (2000). The experimental design was arranged in random blocks, with three replications, each being one tomato crop. Each tomato crop had 1000 plants spaced 0.5 m apart within rows and 1.0 m between rows, trellised and pruned above the 7th branch. The four outside rows and the first ten plants on each side of the row formed the outer border and the remaining crop was considered to be the experimental plot.

The number of leaf mines produced by *T. absoluta* and *Liriomyza* spp. and the eggs of *T. absoluta* at the lower, middle and upper portions of 30 plants/crop (one leaf/plant) was determined weekly (LEITE et al., 1999a, b; OLIVEIRA, 1999, LEITE et al., 2001).

In order to estimate trichome and cristalliferous idioblast densities, one apical leaflet from each portion of the canopy of nine plants/crop (one leaflet/plant) was collected monthly. These leaflets were placed in white transparent plastic bags, immediately sealed, and stored in 70% ethanol for further evaluation. Leaflets were cleared in NaOH (10%) for 2h and in sodium hypochlorite (20%) for 18h. After washing and during dehydration, the materials were stained for three min. by immersing in fast green (JOHANSEN, 1940) and mounted between slides using Canadian balsam. The trichomes (adaxial and abaxial) and cristalliferous idioblast densities were calculated using a light microscope and by counting the number of trichomes or cristalliferous idioblasts in the leaflet. For each sample, twenty-four sections of 0.60 mm^2 each between the main vein and the margin were analyzed. The trichomes were classified as glandular and non-glandular (LEITE et al., 1999c). Three countings for each part of the canopy (one leaflet/counting) were made for each sampling of the three crops.

For determination of concentrations of leaf N and K, a fully expanded leaf from the upper part of the canopy of each of 12 plants/crop was collected monthly. The leaves were placed in Kraft paper bags, dried in a forced air circulation oven at 67°C for 3 days and ground in a Wiley mill (20 mesh). Concentration of leaf K was determined with Flame Photometer and concentration of leaf N was analyzed by the Nessler method (JACKSON, 1958). Three replications (four leaves/replication) were made for each sampling of the three crops.

For gas chromatography/mass spectrometry (GC/MS) analysis, fully expanded leaves from the upper part of the canopy of fifty tomato plants/crop were obtained monthly. The leaves were collected and placed in plastic bags and sealed. Fresh leaves (10g) were cut with scissors and immersed in 100ml of distilled hexane for 24 h. The hexane extract was dehydrated with anhydrous Na_2SO_4, evaporated at 30°C in a rotatory evaporator, sealed in nitrogen and stored in a freezer. One reading was made for each sampling of the three crops.

The hexane extracts were analyzed by GC/MS equipped with an auto sampler and a computer based system to accumulate data, and a mass spectra database with 160,000 compounds using the following conditions: initial oven temperature of 33^0C was programmed to 80^0C at 20^0C min.$^{-1}$ and finally raised to 250^0C at 5^0C min.$^{-1}$. The injector and transfer line temperatures were 180 and 280^0C, respectively. The split ratio was five, with He as the carrier gas. All analyses were carried on the DB 1 fused capillary column (30mx 0.25mm and film thickness of 0.25 µm). The mass spectrometer was scanned between 40 – 550 amu and the minimum area used for peak integration was 200.000 ions sec.$^{-1}$. The retention times for the peaks with total ion current (TIC) higher than 2 x 10^6ions sec.$^{-1}$ were recorded and the compounds identified using the mass spectra database. Only compounds with a similarity index (SI) greater than 80% were considered.

Analysis of variance and the Tukey's multiple range test (5% significance) were used to test the effect of canopy height on trichome and cristalliferous idioblasts densities, and the number of mines produced by *T. absoluta* and *Liriomyza* spp. and the number of *T. absoluta* eggs. Pearson's correlation (5% significance) were used to evaluate the relationships between leaf organic compounds, leaf N and K concentrations, leaf trichome and cristalliferous idioblasts densities and the number of mines produced by *T. absoluta* and *Liriomyza* spp. and the number of *T. absoluta* eggs.

RESULTS AND DISCUSSION

The 1 and 2 tomato crops were less attacked by *T. absoluta* as compared to the 3, while the opposite was observed for *Liriomyza* spp. (Figure 1A, B and C). In crops that suffered higher attack by these insects, the attack was more intense at the end of the growing season (Figure 1A, B and C), probably due to the fact that these crops were far from other tomato crops. Thus, they were apparently not colonized by *T. absoluta* nor *Liriomyza* spp.

No effect of leaf N and K was observed on the *T. absoluta* attack and positive effect of K (r = 0.56) was observed on the *Liriomyza* spp. attack. Letourneau et al. (1996) observed that damage to tomato foliage from leafminers did not increase with increased N concentration of the 17 commercial farms. Kaneshiro e Johnson (1996) noted that, although pupal weights of *Liriomyza trifolii* (Burgess) larvae increased significantly with the leaf nitrogen increase, *L. trifolii* fecundity was not significantly affected by nitrogen in the leaves upon which the females developed. However, Leite (1997) reported that an increase in the leaf N content of *L. esculentum*, grown in a greenhouse, reduced the larval mortality, higher pupal weight and *T. absoluta* egg eclosion. Also, this author observed that an increased K fertilization reduced the number of large mines/leaf in *L. esculentum* and the rate of egg eclosion of *T. absoluta* in wild tomate *Lycopersicum hirsutum* f. *glabratum* C.M. Mull (PI 134417).

No significant effect of the trichomes and cristalliferous idioblasts densities of leaves was noted on *T. absoluta* and *Liriomyza* ssp. populations under field conditions, such as observed by Leite et. al. (1998, 1999a, b, c) with *T. absoluta* on *L. esculentum* in greenhouse. A higher leaf trichome density/mm^2 was recorded on the abaxial (21.29 ± 1.52) than on the adaxial surface (8.19 ± 0.57) with the trichomes being mostly non-glandular (99.65%), such as observed by Leite et al. (1999c). The glandular trichomes and cristalliferous idioblast densities were reported to show negative effects on populations of *M. persicae* in *L. hirsutum* f. *glabratum* (PI 134417) (LEITE et al., 1999c). However, *L. esculentum* possesses mainly non-glandular trichomes (types III, Va, and Vb) (CHANNARAYAPPA et al., 1992), which are known to be less efficient than the glandular trichomes of *L. hirsutum* in the defense against insect attack (LEITE et al., 1999a, b, c; LEITE et al., 2001).

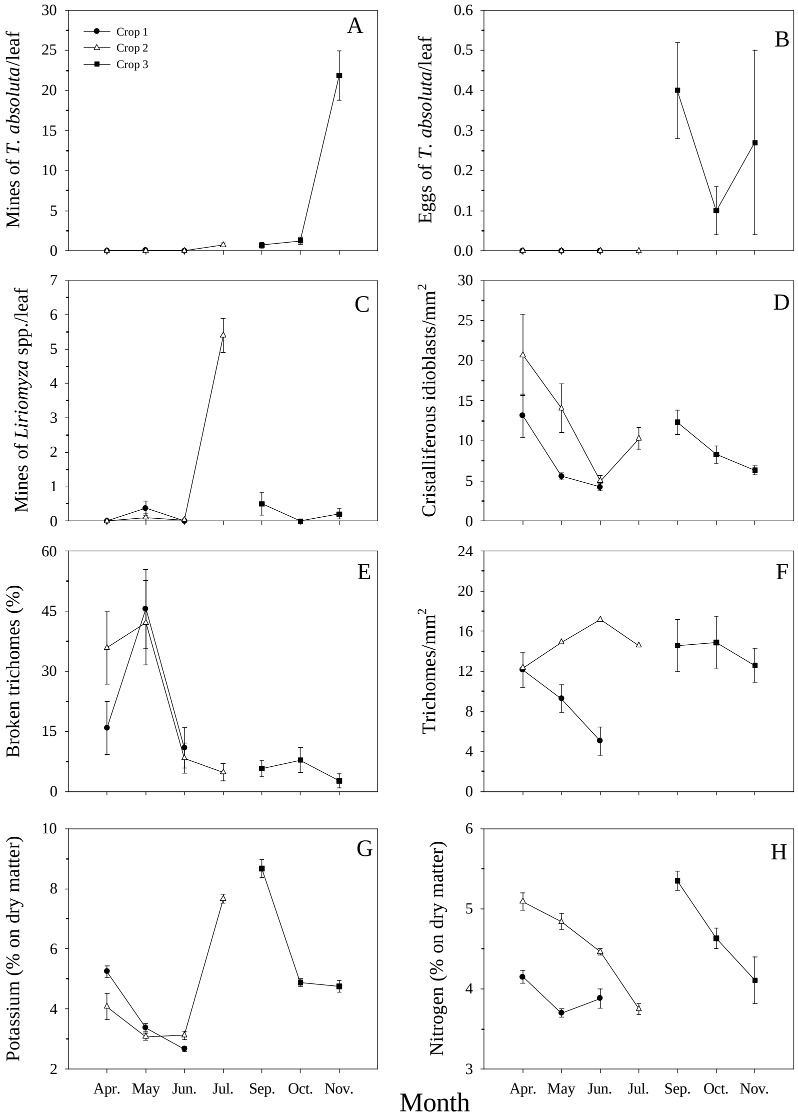

FIGURE 1 – Population dynamics of mines (A) and eggs (B) of *Tuta absoluta*/leaf, and mines of *Liriomyza* spp./leaf (C), densities of crystalliferous/mm^2 (D) and percentagem of broken trichomes (E), trichomes (adaxial + abaxial/2)/mm^2 (F), and potassium (G) and nitrogen (H) concentrations (% on dry matter) in the tomato *Lycopersicon esculentum*. Symbols represent averages of 30 leaves for figures A, B and C, nine evaluations for D, E and F and three evaluations for G and H and vertical bars standard erros of the mean.

A higher number of *Liriomyza* spp. mines was recorded in the lower than in the upper level of the tomato plants (Figure 2A). The opposite was observed for the number of *T. absoluta* eggs (Figure 2B). *T. absoluta* mines tended to concentrate on leaves on the middle and upper levels (Figure 2C). Leaf trichome and cristalliferous idioblast densities were higher on the upper level than on the middle and lower levels (Figure 2D and E) and the percentage of broken trichomes was higher in the middle and lower levels than at the upper levels (Figure 2F). This results are similar to the ones observed by Leite et al. (1999c) in greenhouse for *L. esculentum* and *L. hirsutum*.

The results on the preferential deposition of *T. absoluta* and *Liriomyza* spp. eggs on the apical leaves were in agreement with those reported by Coelho et al. (1984), Silva et al. (1998) and Oliveira (1999). As leaves get older, *T. absoluta* mines tended to concentrate in the middle parts of the canopy while those of *Liriomyza* spp. on the lower part of the plant canopy (LEITE et al., 1999a; OLIVEIRA, 1999). The preferential oviposition in the upper leaves was probably due to the fact that these leaves are more tender, as they have lower calcium content (part of the cellular wall) and percentage of leaf fibre insoluble in acid detergent as compared to the middle and lower leaves (MARSCHNER, 1995; SILVA et al., 1998; LEITE et al., 1999a; WEI et al., 2000) and a higher nitrogen content (LEITE et al., 1998).

Four peaks of organic compounds with retention time (r_t) of 25.488, 28.238, 42.802 and 48.356 min were recorded in the total ion chromatogram (TIC) of the hexane extracts of the *L. esculentum* leaves on GC/MS analysis (Figure 3). The peaks with r_t of 25.488, 42.802 and 48.356 min were identified as palmitic acid, hexacosane and triacontane with similarity index (SI) of 87, 95 and 90%, respectively. The peak with (r_t) of 28.238 min was identified as one of the following terpenes: mircene/farnesol/α-humulene/trans caryophyllene with a SI of 83%. These compounds have not been reported in the literature for *L. esculentum*. Although these compounds were identified with a very high SI index, their identity should be confirmed by other methods. These compounds can be isolated in large quantities and their

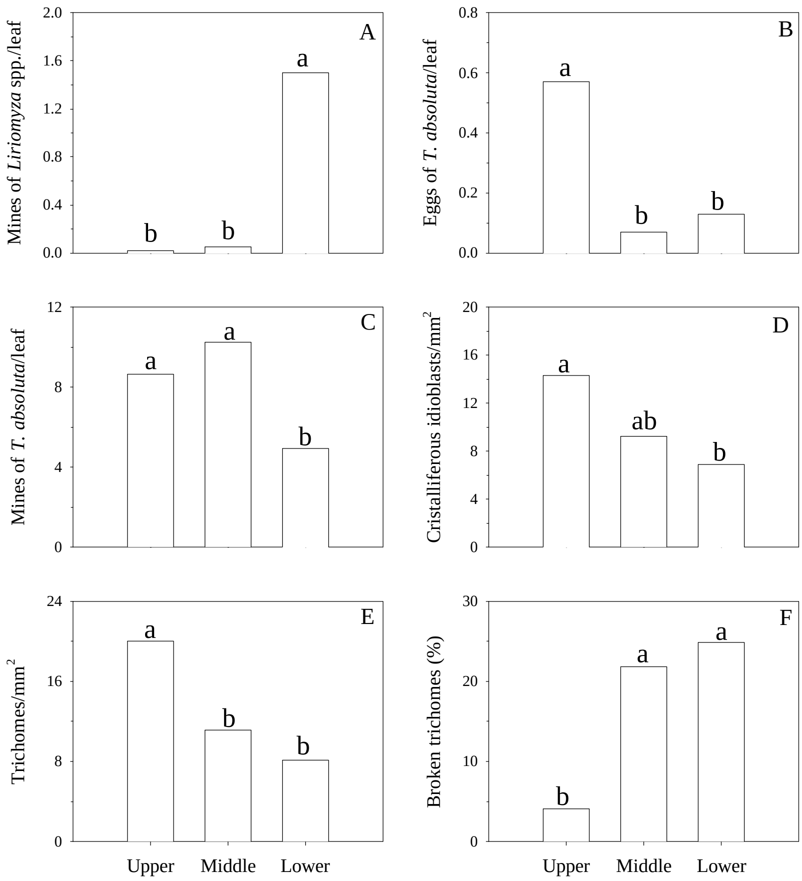

FIGURE 2 – Number of mines of *Liriozyza* spp./leaf (A), numbers of eggs(B) and mines/leaf (C) of *Tuta absoluta*, densities of crystalliferous idioblasts/mm^2 (D) and trichome density (adaxial + abaxial/2)/mm^2 (E), and percentage of broken trichomes (F) in the tomato *Lycopersicon esculentum* in different levels of the canopy plants. Means followed by the same letter do not differ by Tukey's multiple range test ($P < 0.05$).

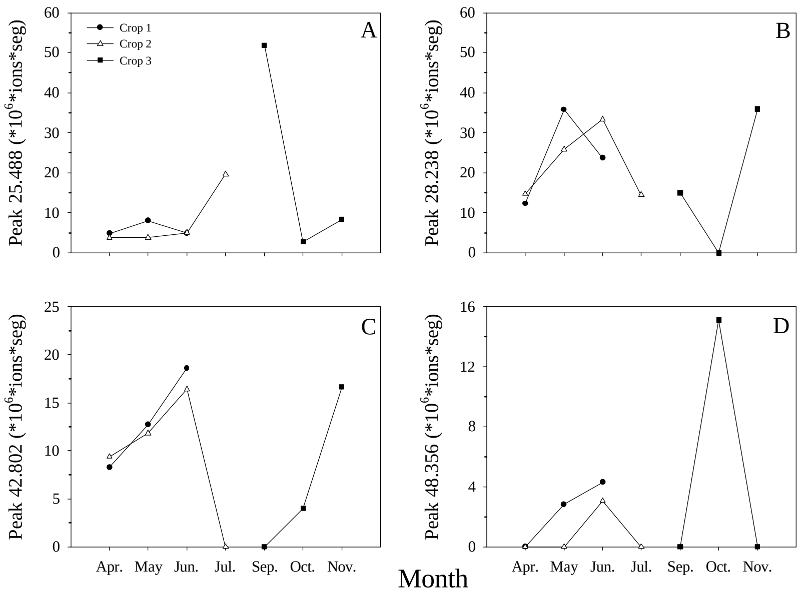

FIGURE 3 – Peaks of organic compounds leaves with retention time of 25.488 (A), 28.238 (B), 42.802 (C) and 48.356 (D) min. in the tomate *Lycopersicon esculentum*. Symbols represent one evaluation.

identification with more precise spectroscopic methods and the conduction of bioassays with the compound with r_t of 25.488 min. are of great interest, since it positively correlated with *T. absoluta* oviposition (r = 0.75).

CONCLUSIONS

The tomato leafminers, *T. absoluta* and *Liriomyza* spp., initiate their attack in the upper leaves of the canopy (site of preferential oviposition) with their mines concentrating in the middle and lower part of the plant canopy. When the initial insect population is low, a higher attack occurred at the end of the growing season, showing the importance of eliminating crop residue and rotating crops using plants that are not hosts of *T. absoluta* and *Liriomyza* spp.

The non glandular trichomes are not important to regulate insect density in these species. Apparently, terpenes are positively associated with the oviposition of *T. absoluta* while leaf K appears to positively favor *Liriomyza* spp. attack.

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