

EFFECT OF LEAF CHARACTERISTICS, NATURAL ENEMIES AND
CLIMATIC CONDITIONS ON THE INTENSITIES OF *MYZUS*
PERSICAE AND *FRANKLINIELLA SCHULZEI* ATTACKS
ON *LYCOPERSICON ESCULENTUM*

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ABSTRACT

The objective of this study was to determine the effects of height within the canopy, leaf chemical composition, levels of leaf nitrogen and potassium, densities of leaf trichomes and crystalliferous idioblasts, natural enemies, total rainfall and median temperature on the intensities of *Myzus persicae* (Sulzer) (Homoptera: Aphididae) and *Frankliniella schulzei* (Trybon) (Thysanoptera: Thripidae) attacks in six tomato plantations *Lycopersicon esculentum* var. "Santa Clara" in two counties in Brazil. Our results indicate that *M. persicae* can be an important tomato pest in regions of mild temperature while, *F. schulzei*, can be in higher temperature regions. *F. schulzei* tended to attack in the initial phase of the culture while *M. persicae*, from the middle to the final phases. Both *M. persicae* and *F. schulzei* concentrated their attack on the leaves in the apical part of the plants rather than in the middle and basal parts. Natural enemies, especially the parasite *Adialytus* spp. (Hymenoptera: Braconidae) and spiders, can be important agents for the control of the aphid and thrips population in tomato, respectively. No correlation was detected between levels of leaf N and K, leaf chemical composition, leaf trichome (99.65% non-glandular), and crystalliferous idioblasts with these pests.

KEY WORDS: Tomato, allelochemical, fertilization levels, trichomes, crystalliferous idioblasts, natural enemies.

RESUMO

EFEITO FOLIAR CARACTERÍSTICO, INIMIGOS NATURAIS E CONDIÇÕES CLIMÁTICAS NAS INTENSIDADES DE ATAQUE DE *MYZUS PERSICAE* E *FRANKLINIELLA SCHULZEI* EM *LYCOPERSICON ESCULENTUM*. O objetivo deste trabalho foi determinar os efeitos de dossel das plantas, composição química foliar, níveis foliares de nitrogênio e potássio, densidades de tricomas e de idioblastos cristalíferos foliares, inimigos naturais, pluviosidade total e temperatura média nas intensidades de ataque de *Myzus persicae* (Sulzer) (Homoptera: Aphididae) e de *Frankliniella schulzei* (Trybon) (Thysanoptera: Thripidae) em seis plantações de tomate *Lycopersicon esculentum* var. "Santa Clara" em dois municípios do Brasil. Os resultados indicam que *M. persicae* pode ser uma praga importante de tomate em regiões de clima mais ameno enquanto *F. schulzei* em locais de maior temperatura. *F. schulzei* apresentou tendência de atacar mais na fase inicial da lavoura enquanto *M. persicae* durante o meio para fase final do cultivo. *M. persicae* e *F. schulzei* concentrou os seus ataques mais as folhas do terço apical do dossel das plantas do que às folhas dos terços mediano e basal. Inimigos naturais, especialmente o parasitóide *Adialytus* spp. (Hymenoptera: Braconidae) e as aranhas podem ser importantes agentes controladores de pulgões e de tripses em tomate, respectivamente. Não foi detectado efeito entre níveis foliares de N e de K, compostos químicos foliares, tricomas foliares (99,65% non-glandular) e idioblastos cristalíferos com essas pragas.

PALAVRAS-CHAVE: Tomate, aleloquímicos, níveis de fertilização, tricomas, idioblastos cristalíferos, inimigos naturais.

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INTRODUCTION

The "green peach aphid" *Myzus persicae* (Sulzer) (Homoptera: Aphididae) and the thrips *Frankliniella schulzei* (Trybon) (Thysanoptera: Thripidae) are tomato (*Lycopersicon esculentum*) pests in Brazil (LEITE *et al.*, 1997; MIRANDA *et al.*, 1998a; PAULA *et al.*, 1998). Several factors can influence the number of the *M. persicae* and *F. schulzei* in tomato. Temperature and rainfall besides natural enemies have been cited as the most important factors that influence the development of aphids and thrips (WALKER *et al.*, 1984; NAKATA, 1995; GONÇALVES, 1997; HOOKS *et al.*, 1998; MIRANDA *et al.*, 1998a; VENZON *et al.*, 1999; FUNDERBURK *et al.*, 2000; TAGASHIRA & HIROSE, 2001). Crop fertility status can affect the degree of attack of aphids and thrips as well as the position of the leaves in the plant canopy (MARSCHNER, 1995; WILLIAMS, 1995; DEKOGEL *et al.*, 1997; LEITE *et al.*, 1997; MIRANDA *et al.*, 1998a; LEITE *et al.*, 1999a; BRODBECK *et al.*, 2001; CISNEROS & GODFREY, 2001; NEVO & COLL, 2001).

Trichomes are another factor that can influence the *M. persicae* and *F. schulzei* attacks on the tomato plant. The effect of trichomes on insects can be due to chemical and/or mechanical factors. The chemical effect in part could be due to exudates produced by glandular trichomes which contain ketones that are toxic to insects (FERY *et al.*, 1984; KENNEDY & SORENSON, 1985; FERY & KENNEDY, 1987; LEITE *et al.*, 1999a,b,c; LEITE *et al.*, 2001). Other possible resistance factors include mechanical barriers, density, length and form of trichomes (NORRIS & KOGAN, 1980; LEITE *et al.*, 1997; LEITE *et al.*, 1999a,b).

FRANCESI & HORNER (1980) have associated the presence of crystals in the leaves of several vegetables and LEITE *et al.* (1999a), in *L. hirsutum f. glabratum* (PI 134417), with the resistance to attack of herbivores. The occurrence of calcium oxalate crystals in the plants of Solanaceae (METCALFE & CHALK, 1957) and in *Lycopersicon* (LEITE *et al.*, 1997; LEITE *et al.*, 1999a) is frequent. Hence, the objective of this study was to evaluate the effects leaf chemical composition, levels of N and K, leaf trichome and crystalliferous idioblasts densities, height within the canopy, natural enemies, total rainfall and median temperature on the intensity of the "green peach aphid" *M. persicae* and the thrips *F. schulzei* in six tomato plantations in two counties in Brazil, under field conditions.

MATERIAL AND METHODS

This experiment was conducted using three tomato plantations (about 200 m apart) in Viçosa County (20°44'38.7" S, 42°49'18" W and 649 m), from April to July and from February to December 1999, and

three tomato plantations (about 1 km apart) in Guidoal County (21°08'36" S, 42°47'54" W and 239 m), from April to July and from September to December 1999. All tomato plantations were *Lycopersicon esculentum* var. "Santa Clara" and both counties are located in Minas Gerais State, Brazil. Cultural practices utilized in this study were described by FILGUEIRA (2000). The experiment design was arranged in random blocks, with three repetitions, each one being a tomato plantation. The tomato plantations had 1,000 plants spaced 0.5 m apart within rows and 1.0 m between rows, trellised and pruned above the 7th branch, maintaining the main branch. The four periphery rows and the first ten plants on each side of the row formed the outer border and the remaining plantation was considered to be the useful area.

The beating tray method (BUTLER *et al.*, 1993; Stansly, 1995; Miranda *et al.*, 1998b) was used to estimate the number of *M. persicae* and *F. schulzei* as well as the predators and parasitoids on the bottom, middle and apical parts of 30 plants/plantation (one leaf/plant), monthly in Guidoal County and weekly in Viçosa County. This method consisted in beating an expanded leaf of each part of the plant canopy inside a 34 x 26 x 5 cm white tray and counting the insects inside it. Arthropods were collected with an aspirator or tweezers and held individually in 8 x 2 cm glass flasks containing 70% ethanol for identification.

In order to estimate trichome and crystalliferous idioblast densities, one apical leaflet from each part of the canopy (bottom, middle and apical) of nine plants/plantation (one leaflet/plant) was collected monthly in both counties, but, in 1998, these morphological characteristics were not evaluated. These leaflets were placed in white transparent plastic bags, immediately sealed, and stored in 70% ethanol for posterior evaluations. Prior to that, leaflets were cleared for 2h in NaOH (10%) and 18h in sodium hypochlorite (20%). After washing and during dehydration, the materials were stained for three minutes by immersing in fast green (JOHANSEN, 1940) and mounted between slides using Canadian balsam. The trichomes (adaxial and abaxial) and crystalliferous idioblasts densities were calculated using a light microscope and by counting the number of trichomes or crystalliferous idioblast at the median part of the apical leaflet (LEITE *et al.*, 1999a). For each sample, twenty-four fields (area of one field = 0.60 mm²) in the median part (field equidistant between the median vein and the margin) were analyzed. The trichomes were classified as glandular or non-glandular (LEITE *et al.*, 1999a). Three evaluations for each part of the canopy (one leaflet/evaluation) were done for each monthly collection of the five plantations.

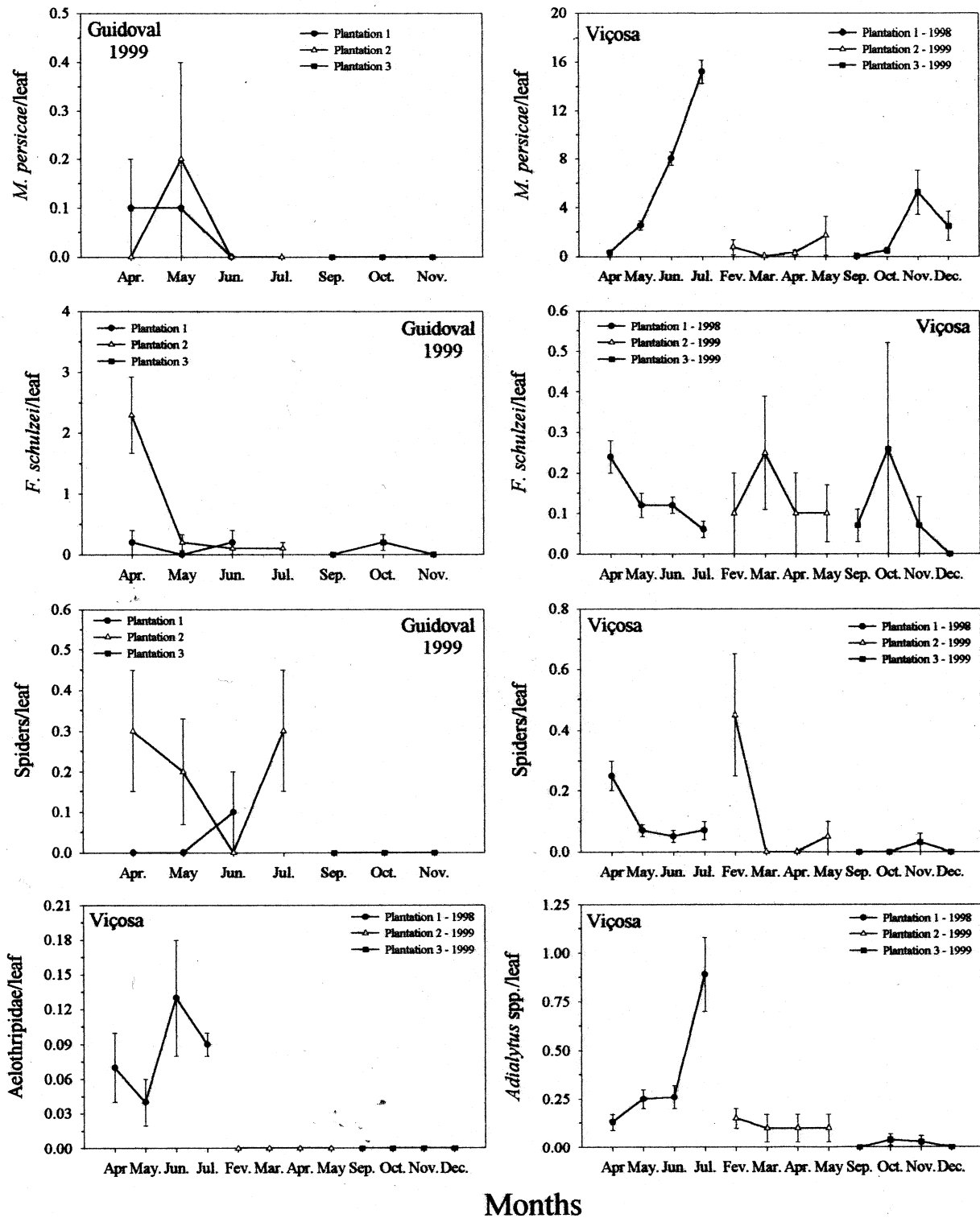


Fig. 1 - Population fluctuation of *Myzus persicae*/leaf, *Frankliniella schulzei*/leaf and spiders/leaf in plantation of tomato *Lycopersicon esculentum* in Viçosa and Guidoival counties, and population fluctuation of *Adiatylus* spp./leaf and *Aelothripidae*/leaf in plantation of *L. esculentum*, in Viçosa County. Symbols represent averages of 30 leaves and vertical bars indicate standard errors of the mean.

For determination of leaf N and K, an expanded leaf from the apex of each of 12 plants/plantation was collected monthly in both counties, but, in 1998, these nutritional characteristics were not determined. 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). K was determined with a Flame Photometer (Coleman, Model 22) and N

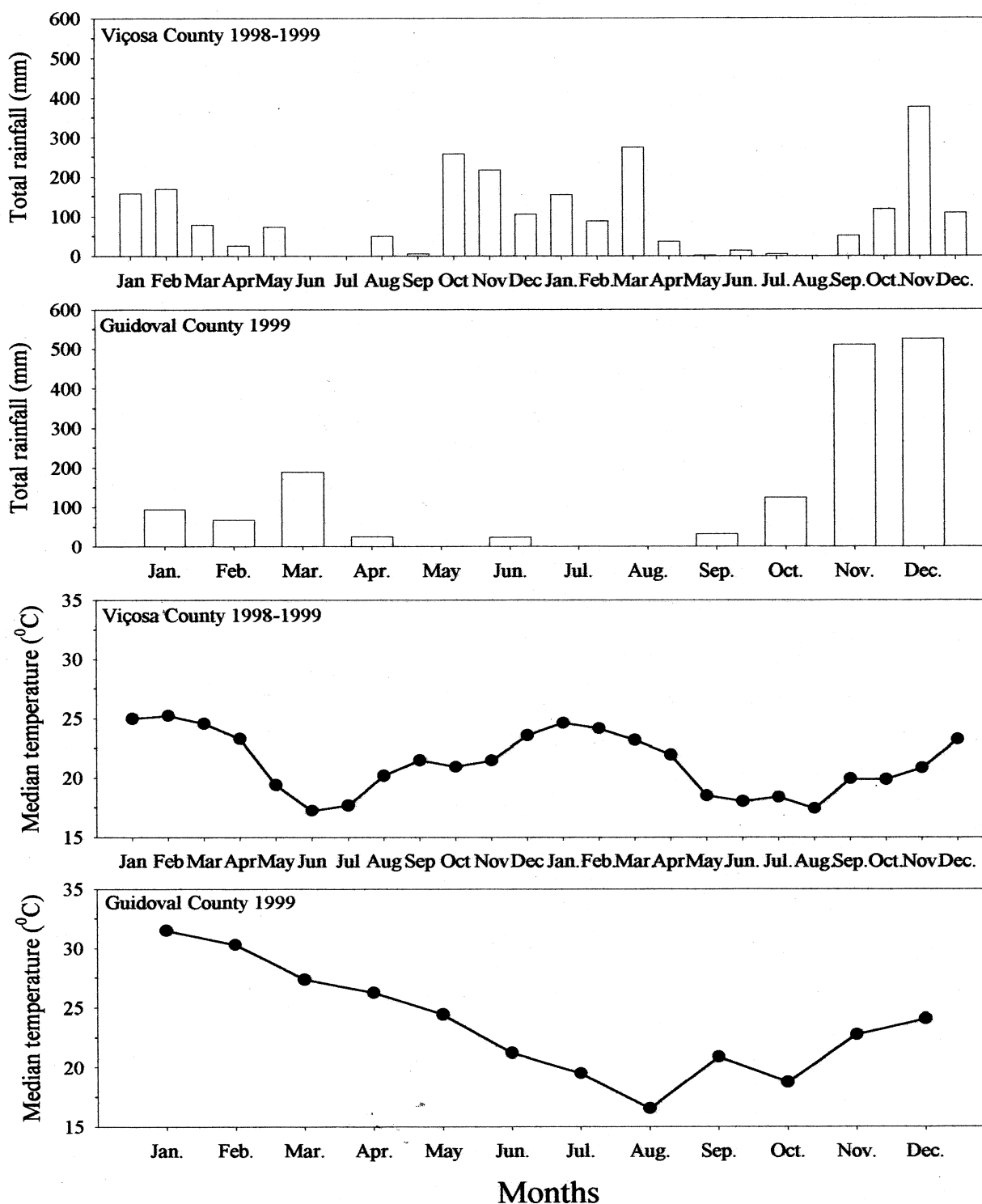
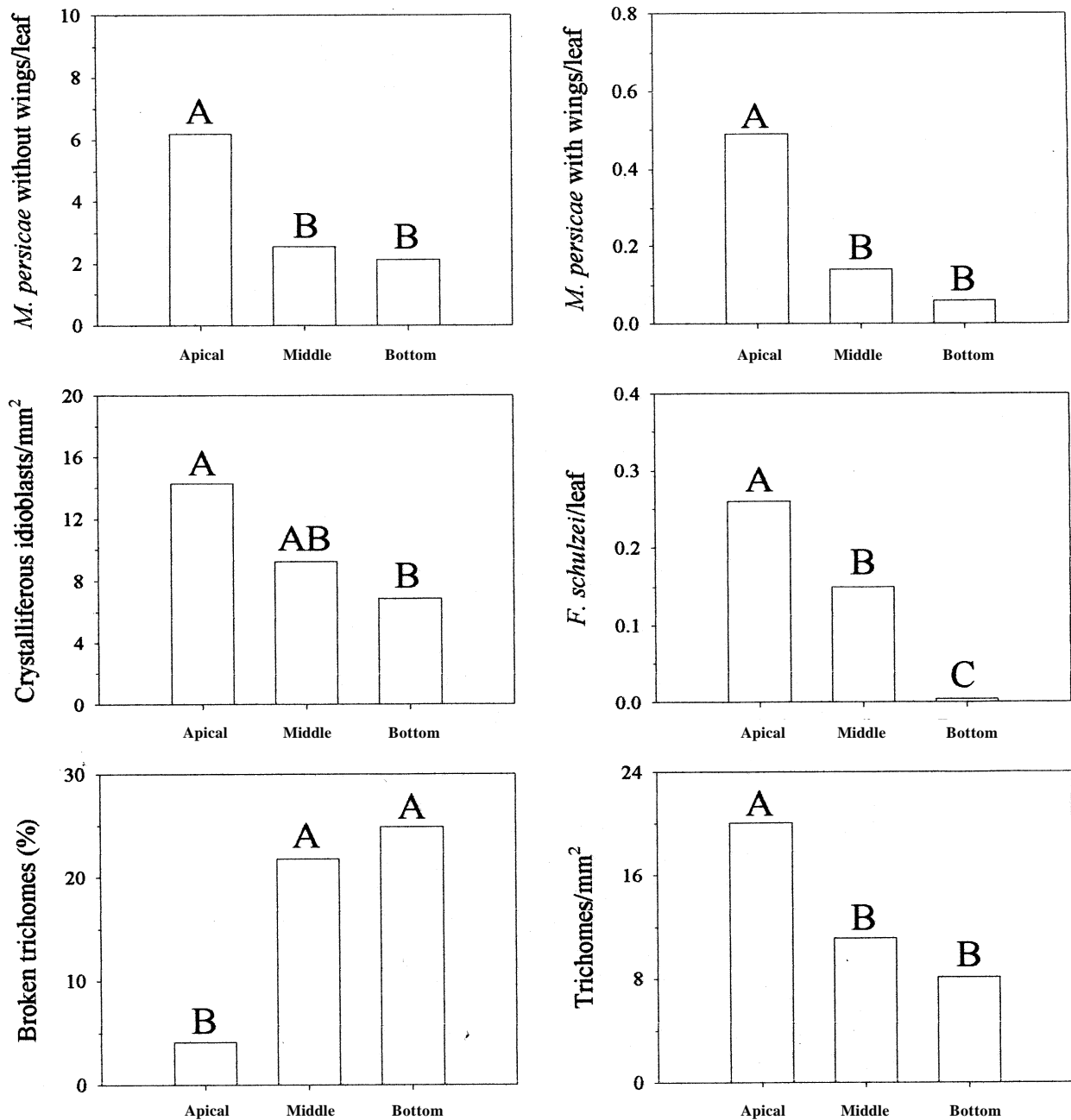


Fig. 2 - Total rainfall distribution (mm) and median temperature (minimum + maximum/2) (°C) in Viçosa County (1998-1999) and Guidoival County (1999). The symbols represent, in general, the rainfall accumulated and the average of the temperature during 30 days.

was analyzed by the Nessler method (JACKSON, 1958). Three evaluations (four leaves/evaluation) were done for each monthly collection of the five plantations.

For GC/MS analysis, fully expanded leaves from the apex of fifty tomato plants/plantation were

obtained monthly in both counties, but, in 1998, these analyses were not conducted. The leaves were collected and placed inside plastic bags, sealed and transported to the laboratory. Fresh leaves (10 g) were cut with scissors and immersed in 100 mL of bidistilled hexane



Canopy part

Fig. 3 - Effect of plant canopy on numbers of *Myzus persicae* (with and without wings)/leaf, *Frankliniella schulzei*/leaf, densities of crystalliferous idioblasts/mm² and trichome density (adaxial + abaxial/2)/mm², and on percentage of broken trichomes in plantations of tomato *Lycopersicon esculentum*. Means followed by the same letter do not differ by Tukey's multiple range test ($P < 0.05$).

for 24 h. The hexane extract was dehydrated with anhydrous Na_2SO_4 , evaporated for dryness at 30°C in a rotatory evaporator, sealed in nitrogen and stored in a freezer until analysis. One evaluation was done for each monthly collection of the five plantations.

The hexane extracts were analyzed by gas chromatography/mass spectrometry (GC/MS) using a Shimadzu system (Model QP 5000) equipped with

an auto sampler, a computer-based system to accumulate data and a mass spectra database (John Wiley) with 160,000 compounds. The analyses were conducted using the following conditions: initial oven temperature of 33°C was programmed to 80°C at 20°C/min and finally to 250°C at 5°C/min. The injector and transfer line temperatures were 180° and 280° C, respectively. The split ratio was 5 with He as the

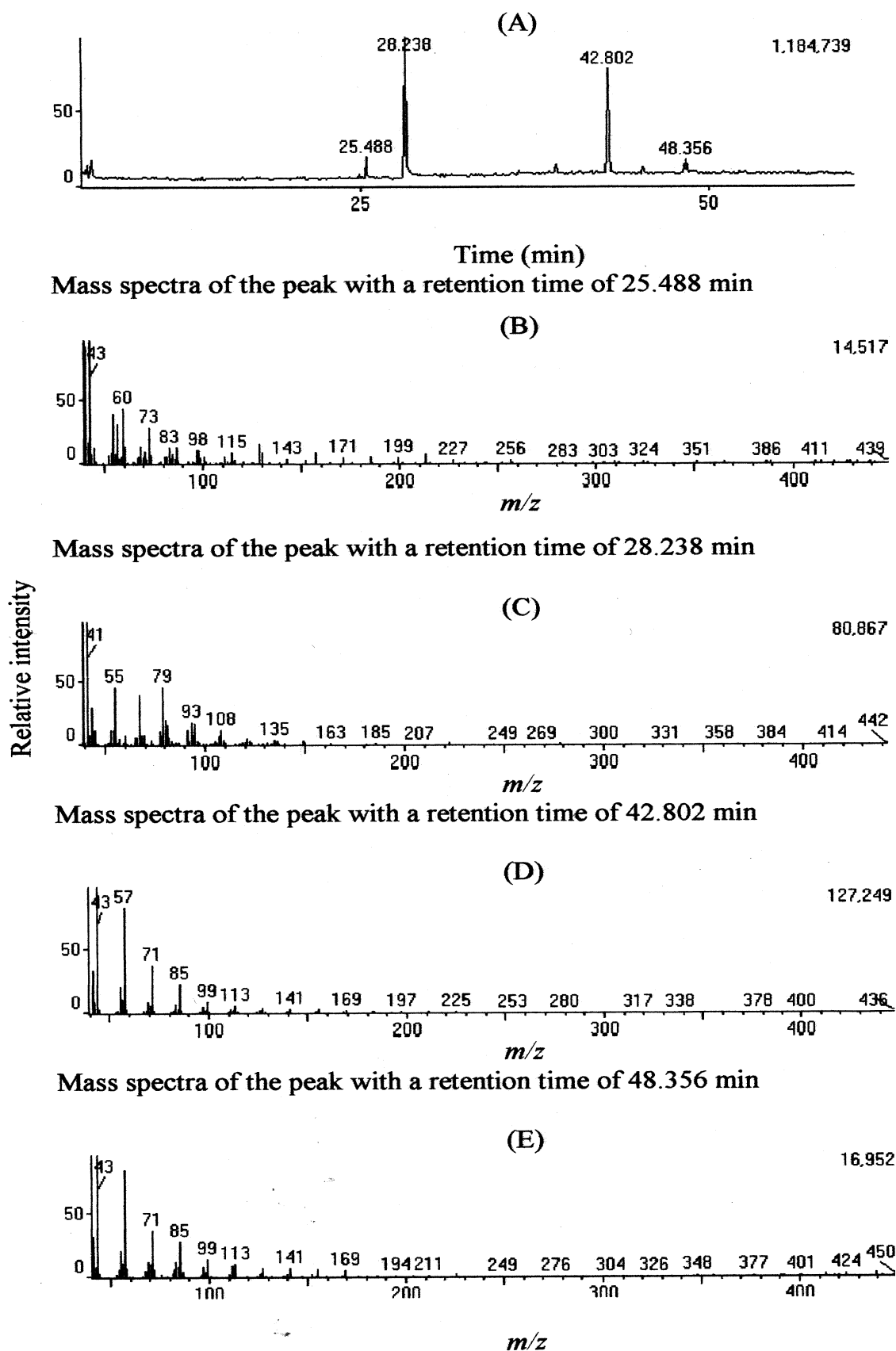


Fig. 4 - Total ion current of hexane extract of the tomato *Lycopersicon esculentum* (A) and mass spectra of peaks eluting at 25.488 (B), 28.238 (C), 42.802 (D) and 48.356 (E) min. Numbers in the upper right side of each figure represent the number of ions recorded.

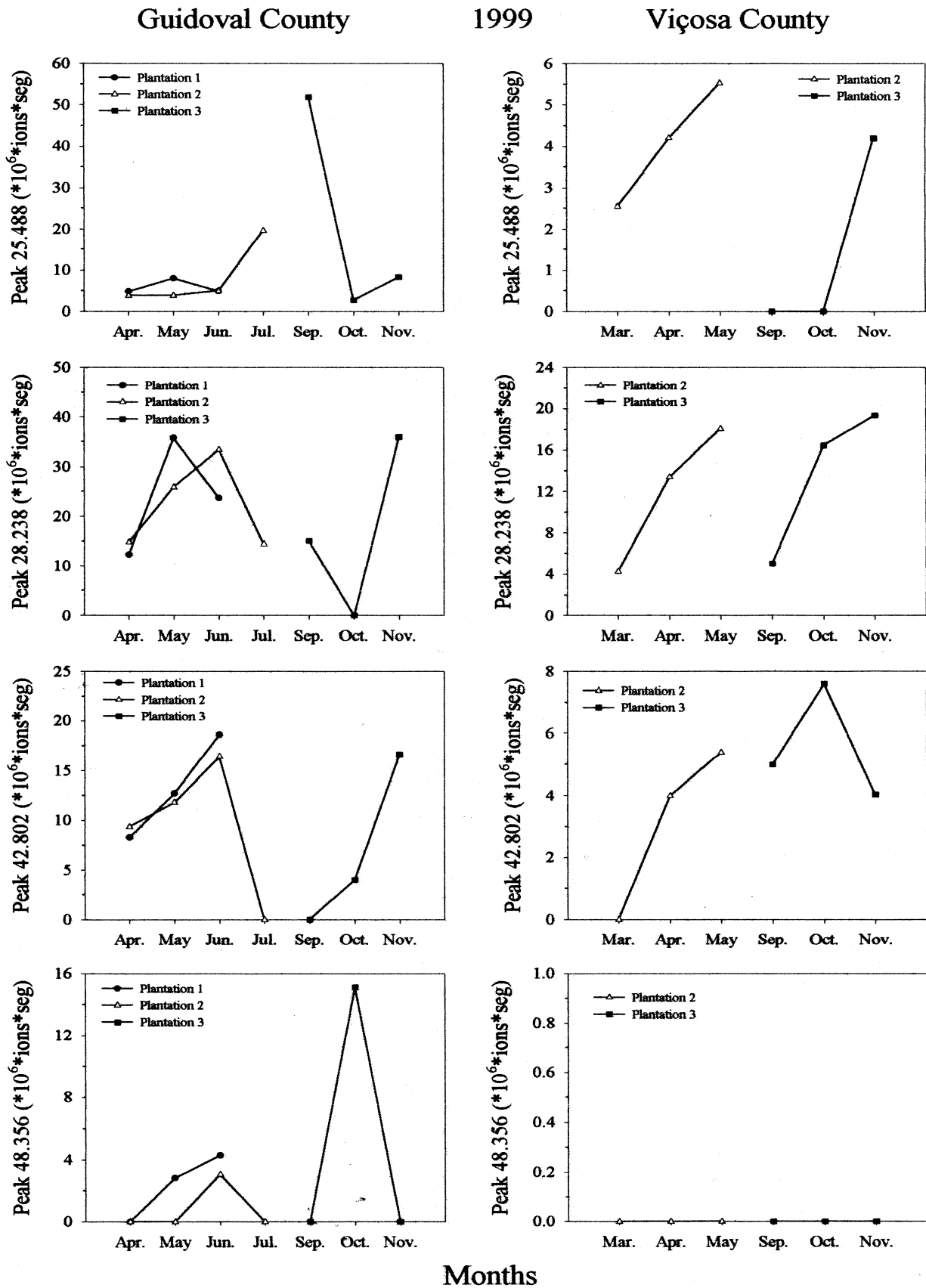


Fig. 5 - Peaks with retention times of 25.488, 28.238, 42.802 and 48.356 min. in leaves of tomato plants (*Lycopersicon esculentum*) from plantations located in Viçosa and Guidoival counties. Symbols represent one evaluation.

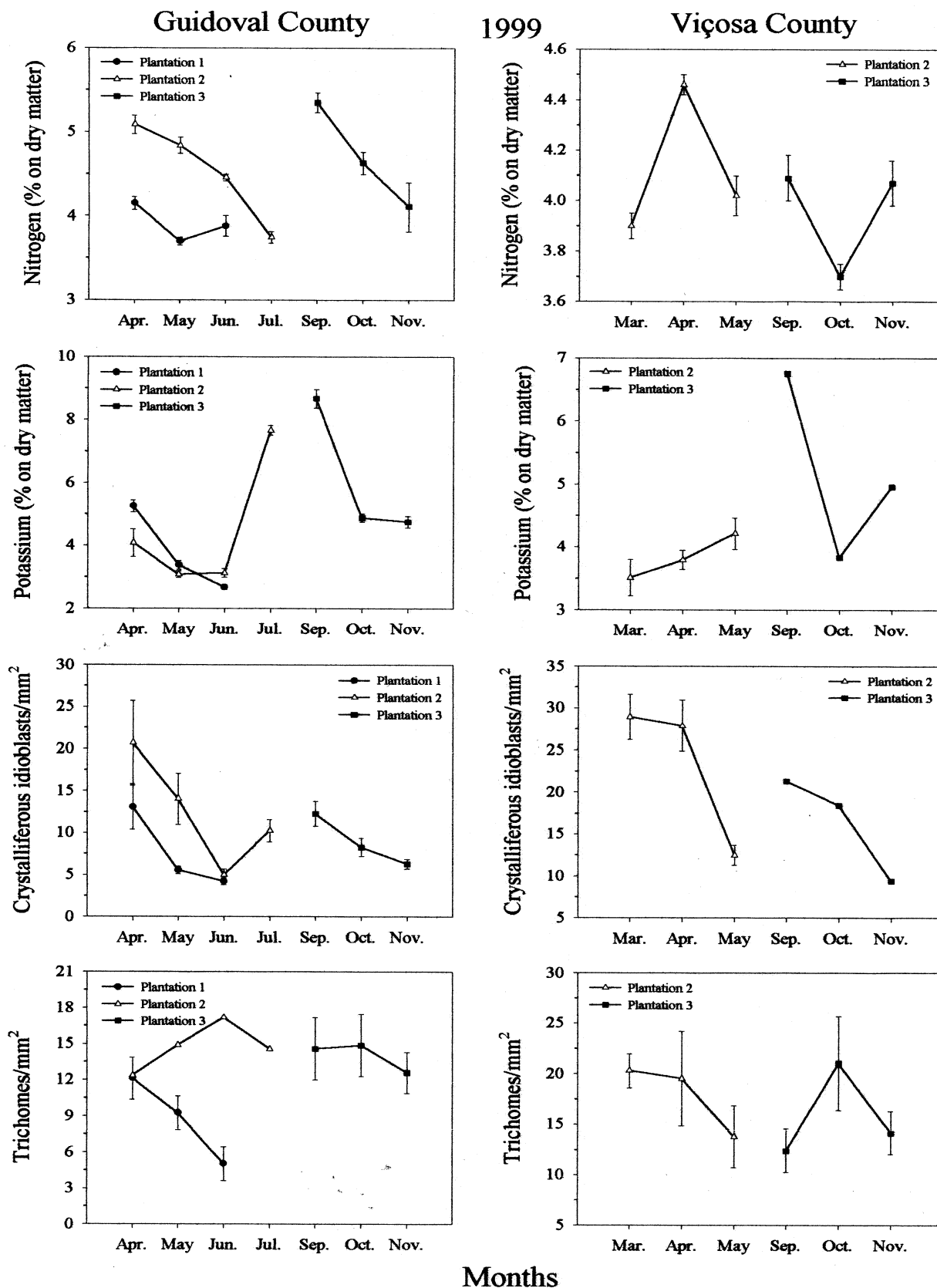


Fig. 6 - Nitrogen and potassium contents (% on dry material) and densities of crystalliferous idioblasts/mm² and trichomes (adaxial + abaxial/2)/mm² related to leaves of tomato plantations (*Lycopersicon esculentum*) in Viçosa and Guidoal counties. Symbols represent averages of three evaluations and vertical bars indicate standard errors of the mean.

carrier gas. All analyses were carried out on a DB 1 fused capillary column (J & W Scientific, USA, 30m x 0.25mm and film thickness of 0.25 μ m). The mass spectrometer was scanned between 40–550 amu and the minimum area utilized for peak integration was 200,000 ions/second. The retention times for the peaks with total ion current (TIC) higher than 2×10^6 ions/second were recorded and the compounds identified using the mass spectral database. Only compounds with a similarity index (SI) greater than 80% were considered as positive identifications. Further identification through standards was not attempted.

Total rainfall and median temperature (minimum + maximum)/2 data were obtained daily with a pluviometer and thermometer (minimum and maximum), respectively, in Guidoal County (1999) and in the "Estação Climatológica Principal" of the Federal University of Viçosa (UFV) (INMET/5°DISME/UFV) located in Viçosa County (1998-1999). Variance analysis and the Tukey's multiple range test ($P \leq 0.05$) were used in a System of Statistical and Genetics Analysis (SAEG) of the UFV (Euclides, 1983) in order to verify the effect of canopy height on trichome and crystalliferous idioblasts densities, on the number of natural enemies, and number of *M. persicae* and *F. schulzei*. Pearson's correlations ($P \leq 0.05$) were used in the SAEG (Euclides, 1983) to evaluate the relationships of leaf chemical composition, leaf N and K levels, leaf trichome and crystalliferous idioblasts densities, natural enemies, total rainfall and median temperature with the number of *M. persicae* and *F. schulzei*.

RESULTS

M. persicae population was higher in Viçosa compared to Guidoal, while *F. schulzei* population was slightly higher in Guidoal than in Viçosa (Fig. 1). *F. schulzei* attack tended to occur in the initial phase of the culture, while *M. persicae* attack occurred during the middle and final phases in both counties. Guidoal's average temperature was higher, with the county receiving more rainfall in 1999 ($23.62 \pm 1.34^\circ$ C and 1588.90 mm, respectively) compared to Viçosa in 1998 ($21.67 \pm 0.79^\circ$ C and 1139.00 mm, respectively) and in 1999 ($20.85 \pm 0.73^\circ$ C and 1224.80 mm, respectively) (Fig. 2). These data could partly explain the higher and the lower populations of *M. persicae* and *F. schulzei*, respectively in Viçosa than in Guidoal, since the median temperature correlated with these insects ($r = -0.54$, $P = 0.0082$ and $r = 0.40$, $P = 0.0449$, respectively), while the total rainfall did not significantly affect ($P > 0.05$) these populations (Figs. 1 and 2).

The parasitoids observed in the tomato plantations of Viçosa and Guidoal counties were *Adialytus* spp. (Hymenoptera: Braconidae) (0.17 ± 0.07 and $0.02 \pm$

0.02 , respectively) and Eulophidae (Hymenoptera) (0.03 ± 0.01 and 0.02 ± 0.01 , respectively) and the predators were spiders [*Misumenops* spp. (Thomisidae) and Anyphaenidae] (0.08 ± 0.04 and 0.09 ± 0.04 , respectively), Aelothripidae (Thysanoptera) (0.03 ± 0.01 and 0.02 ± 0.02 , respectively), Syrphidae (Diptera) (0.01 ± 0.01 and 0.00 ± 0.00 , respectively) and Chrysopidae (Neuroptera) (0.01 ± 0.01 and 0.00 ± 0.00 , respectively). A positive correlation between *M. persicae* and *Adialytus* spp. ($r = 0.84$, $P = 0.0004$) in Viçosa and *F. schulzei* and spiders ($r = 0.60$, $P = 0.0340$) in Guidoal (Fig. 1) was detected. No significant correlations ($P > 0.05$) between the pests and the rest of natural enemies was detected in either county (data not shown).

Both *M. persicae* and *F. schulzei* concentrated their attack on the leaves in the apical part of the plants rather than in the middle and basal parts (Fig. 3). Leaf trichomes and crystalliferous idioblast densities were higher in the apical part than in the middle and lower parts and the percentage of broken trichomes was higher in the middle and bottom parts than at the apical part (Fig. 3). However, no relationships ($P > 0.05$) was detected between trichome densities, mostly non-glandular (99.65%), and crystalliferous idioblasts with these pests in both counties (Figs. 1 and 3).

Four peaks with retention times of 25.488, 28.238, 42.802 and 48.356 min were recorded in the TIC (total ion chromatogram) of the hexane extracts of the *L. esculentum* leaves on GC/MS analysis (Fig. 4). The peaks with retention times of 25.488, 42.802 and 48.356 min were identified as palmitic acid, hexacosane and triacontane with SI of 87, 95 and 90%. The peak eluting at 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, their identity should be confirmed by other more precise spectroscopic methods such (NMR, IR, etc). However, no significant correlation ($P > 0.05$) was detected between the concentration of these peaks with *M. persicae* and *F. schulzei* populations (Figs. 1 and 5). No relationship ($P > 0.05$) was detected between leaf N and K with these pests in either county (Figs. 1 and 6).

DISCUSSION

Our results indicate that *M. persicae* can be an important pest in tomato-producing regions with mild temperatures, while *F. schulzei*, can be in higher temperatures. Similar results have been reported in onion, potato and tomato plantations and although no significant effect ($P > 0.05$) was observed in this research, rainfall was another important mortality

factor reported in the literature (WALKER *et al.*, 1984; NAKATA, 1995; GONÇALVES, 1997). The lack of influence of rainfall observed in this study was probably related to the tomato plant's architecture and its defense mode against insect attack and rainfall (LEITE, 2000).

F. schulzei attack tended to occur in the initial phase of the plant cycle while the attack of *M. persicae*, tended to occur towards the middle and the final phases for both the regions. In general, both aphids and thrips attack plants in their initial stages (WILLIAMS, 1995; PAULA *et al.*, 1998; LEITE *et al.*, 1999a; PICAÑO *et al.*, 1999), with the aphids population decreasing due to physiological changes during plant development (KRING, 1972; NOORTJE & LINDHOUT, 1992). High N levels have been associated with aphids and thrips infestation on lettuce, tomato and cotton (KENNEDY, 1958; BRODBECK *et al.*, 2001; CISNEROS & GODFREY, 2001; NEVO & COLL, 2001). However, LEITE *et al.* (1999a) did not detect the effect of N and K, at doses of 100 and 300 and 0 and 200 mg/kg of soil, respectively, on the intensity of *M. persicae* populations on the *L. esculentum* and *L. hirsutum* f. *glabratum* (PI 134417), in the greenhouse. We were unable to detect the effect of leaf N and K levels in tomato with aphids and thrips, which may be due to small variation of these nutrients in the leaves during the experimental period.

M. persicae and *F. schulzei* preferentially attack the apical leaves of tomato plant (LEITE *et al.*, 1997; MIRANDA *et al.* 1998a; LEITE *et al.*, 1999a). This was probably due to the fact that these leaves are more tender due to lower calcium content (part of the cellular wall) and lower percentage of leaf fiber insoluble in acid detergent as compared to the median and basal parts of the plants (MARSCHNER, 1995; SILVA *et al.*, 1998; LEITE *et al.*, 1999a), which favor feeding of these insects besides having better nutritional quality (higher N content) (CHATTERS & SCHLEHUBER, 1951; VAN LENTEREN & NOLDUS, 1990; LEITE *et al.*, 1998).

Compounds identified in this work have not been reported in the literature for tomato and probably are not present in the trichomes because they are almost all non-glandular types (99.65%). On the other hand, these compounds and the trichomes and the crystalliferous idioblasts densities were not correlated with aphids and thrips populations. This was expected because this tomato variety has been extensively bred to achieve higher productivity of fruits which could reduce the frequency of glandular trichomes, crystalliferous idioblasts and chemical compounds involved in the resistance to arthropods. However, the glandular trichomes and crystalliferous idioblast densities show negative effects on populations of *M. persicae* on wild tomato *L. hirsutum* f. *glabratum* (PI 134417) (LEITE *et al.*, 1999a). The specie *L. esculentum* possesses mainly non-glandular trichomes (types III, Va, and Vb) (CHANNARAYAPPA *et al.*, 1992; LEITE *et al.*,

1999a), which are known to be less efficient than the glandular trichomes of *L. hirsutum* in the defence against aphid attack (LEITE *et al.*, 1999a), besides a lower number of crystalliferous idioblasts than *L. hirsutum* (LEITE *et al.*, 1999a). The apical leaves had more trichomes and crystalliferous idioblasts than middle and bottom leaves, such as observed by LEITE *et al.* (1999a) for *L. esculentum* and *L. hirsutum* in greenhouse conditions.

The parasite *Adialytus* spp. can be the limiting factor for an *M. persicae* population in tomato such as observed by HOOKS *et al.* (1998) for *Aphis gossypii* (Glover) (Homoptera: Aphididae) in zucchini. Also, the presence of entomopathogenic fungi, coccinellid and syrphid larvae have been observed. However, MIRANDA *et al.* (1998a) showed the presence of the predatory ladybugs *Cycloneda sanguinea* (L.) and *Sciminius* sp. (Coleoptera: Coccinellidae) besides Syrphidae larva (Diptera), adults of *Condylostylus erectus* (Diptera: Dolichopodidae), spiders and parasitoids of the family Braconidae on tomato plants attacked by aphids. Only the ladybugs were correlated with the number of individuals of aphids. Within the natural enemies of thrips, the parasites of the Eulophidae family and the predator of Anthocoridae family appear to be the most important (VENZON *et al.*, 1999; FUNDERBURK *et al.*, 2000; TAGASHIRA & HIROSE, 2001). Low densities of these parasitoids and predator were observed in this work, and, probably due to this, did not correlate with *F. schulzei* populations in tomato.

In summary, *M. persicae* can be an important pest in tomato-producing regions with mild temperature, while *F. schulzei*, can be in higher temperature regions. Natural enemies, especially the parasite *Adialytus* spp. and spiders, can be important controlling agents of the aphid and thrips population in tomato. Hence, it appears necessary to study measures aiming to maintain and to increase populations of these natural enemies during and between cultivation periods. These measures can include plantation of surrounding strips with host plants of other aphid species that do not attack tomato plants (because they will serve as shelter and food for natural enemies in periods when this plant is not cultivated) and use of insecticides, always selective, only when these pests reached nominal thresholds. Such measures could allow the farmers to maintain populations of aphids and thrips low, not causing economic losses. Spraying with insecticides should be directed towards the apical leaves, the region where *M. persicae* and *F. schulzei* concentrate their attack.

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Received on 17/6/02

Accepted on 28/11/02