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INFLUENCE OF FEEDING OF *FRANKLINIELLA OCCIDENTALIS* PERGANDE (THYSANOPTERA:THRIPIDAE) ON THE POLYPHENOLIC COMPLEX IN THE LEAVES

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Abstract

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Production of polyphenolic complex compounds in plants contributes to protecting them against herbivores and often determines their forage nutritional value and the insect preference for them. The thrips *Frankliniella occidentalis* is one of the most important insect pests on vegetable crops in greenhouses on the island of Crete (Greece). We recently showed that its preference for several vegetable cultures is variable, ranging from highest levels for eggplant to lowest ones for tomato plants. To test whether this differential preference was associated with the production of polyphenolic compounds in the leaves, we compared the profile of polyphenols in control (undamaged) and damaged leaves (evoked by *F. occidentalis* attack) of eggplant and tomato, by using reverse phase HPLC. We showed that feeding of *F. occidentalis* causes alterations in the quantity of several phenolic acids (chlorogenic acids) and flavonic glucosides (rutins), which are believed to play an important role in plant defence against insect pests. These alterations are different in tomato leaves compared to those of eggplant, indicating that changes in the quantities of compounds from the polyphenolic complex might be associated with the different preference of *F. occidentalis* for feeding on these vegetable hosts.

Key words: plant defence mechanisms, antinutritive components, trips, *Frankliniella occidentalis*, eggplant, tomato

Introduction

Plants are constantly exposed to threats from the outside environment. Because they are confined to the place where they grow, they have developed ingenious molecular strategies to defend themselves against the biotic and abiotic stresses they may be

confronted with. Many plants respond to attack by herbivores by changing in various ways, and some of these changes make them more resistant to subsequent herbivory. They produce chemicals, such as alkaloids, cyanogenic glycosides, terpenoids, flavonoids, and tannins, which are not directly involved in the process of growth but act as deterrents to insect and mi-

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crobial attack (Karban and Baldwin, 1997). These secondary compound metabolites often concentrate in the leaves to affect pests by inducing toxicity, reducing the nutritive value of the food by depressing intake or utilization of nutrients.

Many studies have demonstrated the importance of phenolic compounds in plant defense and their impact on forage nutritional value. Bi et al. (1997) showed that induced resistance in cotton plants involves both a shift in the oxidative status of the host plant, and a decline in host nutritional quality. Feeding of insect pests resulted in the activation of oxidative enzymes and changes of the redox status of the tissue (production of hydrogen peroxide and reactive oxygen radicals). In addition, damaged tissues had altered phenolic metabolism after herbivory, as the production of the phenolic prooxidants syringic, ferulic, gallic, and the most toxic, chlorogenic acids, increased. Subsequent ingestion of those tissues by the insect pest caused lipid peroxidation, oxidation of proteins, and release of free iron in the larval midgut, resulting in oxidative damage to gut epithelium.

Plants vary in their primary and secondary metabolites, which may affect the suitability of the host for either feeding or oviposition. Plant defensive compounds such as phenolics, tannins and alkaloids have been shown to increase resistance. They can be either constitutive components or induced by environmental factors such as UV light, herbivory, or pathogen infection, and may act by deterring insect infestation. One plant hormone, ethylene, induced by at least three different thrips on different hosts, causes abscission and senescence of particular plant tissues (Wien and Rosingh, 1980; Kendall and Bjostadt, 1990; Rieske and Raffa, 1995). Flavonoids and carotenoids impart particular floral hues to plants (Ananthakrisman and Gopichandran, 1993) and these may also affect the attractiveness as well as the acceptability and suitability of the host.

Material and Methods

The analysis of the polyphenolic complex of damaged and undamaged leaves was performed at the

Institute of Tobacco and Tobacco Products –Plovdiv by the method of Liquid Chromatography. Two different vegetable cultures were used: eggplant (*Solanum melongena* L.) - the most heavily attacked by *F. occidentalis*, and tomato (*Solanum lycopersicum* L.), the least preferred amongst six vegetable cultures tested in a previous study (Papadaki, 2003). The plants for this experiment were grown at the laboratory of the Dept. of Entomology at the Agricultural University-Plovdiv. The plants were placed into 8 plastic chambers 60X20X40 cm, covered with fine netting to prevent invasion of other insects. In half of the chambers a sufficient number of thrips was released to feed on the plants. The control plants were kept free from thrips and other pests. After 15 days of feeding leaf samples were collected from the damaged and undamaged plants.

HPLC of polyphenols

The leaf material was extracted with $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (60:40, v/v). Fraction from the extract was processed with cartridge C18 according to Court et al. (1991). Polyphenols were determined by using the reversed phase HPLC method (Snook and Chortyk, 1982). Chromatography was carried out at the following conditions allowing maximum separation of chlorogenic acid from its isomer 4-O-caffeoyl quinic acid, namely: liquid chromatograph Perkin Elmer (Perkin Elmer Ltd., Beaconsfield, Buckinghamshire, England) equipped with LC 290 binary pump, LC290 UV/VIS detector and LCI-100 integrator; analytical column Kromasil LC18, 150 mm, 5 μm , 4q6 mm i.d. (Supelco Park, Bellafonte, PA, USA); single wave-length at 340 nm; eluent flow rate 1.0 mL/min-1, sample volume 20 μL ; solvent composition A= $\text{CH}_3\text{OH}:\text{H}_2\text{O}:\text{CH}_3\text{COOH}$ (5:93:2); B= $\text{CH}_3\text{OH}:\text{H}_2\text{O}:\text{CH}_3\text{COOH}$ (86:12:2). Gradient elution profile was 100% A, 0 min; 20 min to 85% A; 5 min to 80% A; 17 min to 45% A.; 5 min to 100% A or 5 min to 0% A for cleaning.

The reproducibility of the method is good with a typical standard deviation of about 2% for chlorogenic acid and rutin. Peaks of chlorogenic acid and rutin were identified by using reference compounds. Peak assignments for those components where no

references were available (neochlorogenic and 4-0-caffeoyl quinic acid) were based on data for retention time according to Snook and Chortyk (1982).

Pattern recognition methods PRM was performed according to the Package software program "Patrec" (Pharmaceutical Institute, Sofia, Bulgaria) (Dimov et al., 1987). Processing of HPLC profiles was done using all peaks present in each sample. HPLC profiles were processed on the basis of 13 peaks both for tomatoes and for eggplant, having statistically significant height, selected from the samples studied. The matrix for PRM was designed using the retention time of the peaks and the percent ratio of the height of each peak to the sum of the heights of all selected peaks. PRM data are presented as indexes of similarity (I_s , %). I_s of 98% are considered as a limit value. Above this value differences are not significant.

Results and Discussion

Several peaks were identified in the chromatographic profiles of tomato and eggplant leaves: neochlorogenic acid $t_R=9.60$; chlorogenic acid $t_R=16.57$; 4-0-caffeoylquinic acid $t_R=19.13$; rutin $t_R=35.13$; K-3-rutinoside $t_R=38.37$. They were subsequently compared according the method of Pattern Recognition in order to evaluate their quantitative and qualitative changes. The index of similarity (I_s , %) between the two chromatographic profiles for damaged and undamaged leaves of tomato was 96% which indicates that there is difference in quantitative and qualitative composition of polyphenolic acids (the limit value of I_s , %, above which the samples are undistinguishable is 98%).

Feeding of *F. occidentalis* caused alterations in the relevant quantity of several polyphenolic complex compounds (Table 1), such as phenolic acids (chlorogenic acid) and flavonic glucosides (rutin). For the phenolic acids the sum of the areas (A) in the damaged leaves increased by 12.5% and for the flavonic glucosides – by 9%. The increase was greater in the content of phenolic acids. In the damaged leaves the quantity of 4-0-caffeoylquinic acid ($t_R=19.40$) and of the peak $t_R=23.3$ (Figure 1a and 1b).

Table 1
Quantitative changes of Polyphenolic acids and flavonic glucosides as a result of feeding of *F. occidentalis*

Polyphenols	Plant sample			
	Tomato		Eggplant	
	Undamaged	Damaged	Undamaged	Damaged
Neochlorogenic acid (5-0-caffeoylquinic acid)	0.052%	0.059%	0.059%	0.094%
Chlorogenic acid (3-0-caffeoylquinic acid)	0.11%	0.12%	0.58%	0.72%
4-0-caffeoylquinic acid	0.013%	0.01%	0.2%	0.14%
Rutin	0.13%	0.12%	0.011%	0.017%

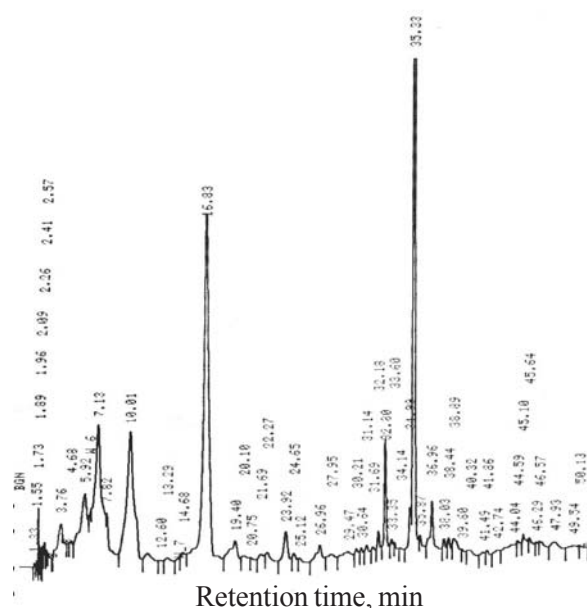


Fig. 1a. HPLC profile of polyphenols in undamaged leaves of tomato: $t_R=10.01$ was identified as neochlorogenic acid; $t_R=16.83$ as chlorogenic acid; $t_R=19.40$ as 4-0-caffeoylquinic acid; $t_R=35.33$ as rutin and $t_R=38.89$ as K-3-rutinoside

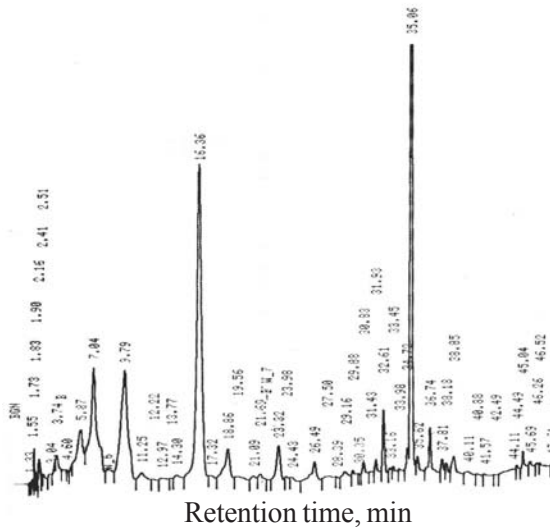


Fig. 1b. HPLC profile of polyphenols in damaged leaves of tomato: tr=9.60 was identified as neochlorogenic acid; tr=16.57 as chlorogenic acid; tr=19.13 as 4-0-caffeoylquinic acid; tr=35.13 as rutin and tr=38.37 as K-3 rutinoside

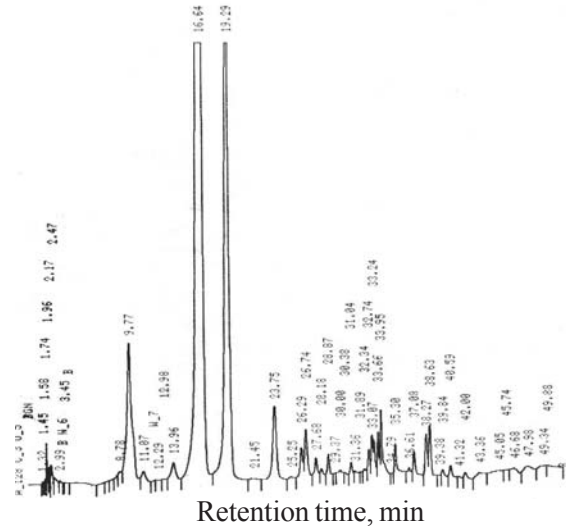


Fig. 2a. HPLC profile of polyphenols in undamaged leaves of eggplant: tr=9.60 was identified as neochlorogenic acid; tr=16.57 as chlorogenic acid; tr=19.13 as 4-0-caffeoylquinic acid; tr=35.13 as rutin and tr=38.37 as K-3-rutinoside

Three more peaks were observed in the profile, which had statistically significant quantities and changed significantly:

- tR = 23.32 A = 2350
- tR = 32.61 A = 2640
- tR = 36.74 A = 2931

- tR = 21.56 A = 1707
- tR = 31.99 A = 3970
- tR = 36.32 A = 4536

For the leaves of eggplant, which were much heavily damaged the index (Is, %) between the two chromatographic profiles was 94%, which indicates a significant difference in the contents and composition of polyphenolic components.

In the polyphenolic complex of eggplant leaves, we identified 5 more peaks (Figures 2a and 2b) with significantly altered quantities:

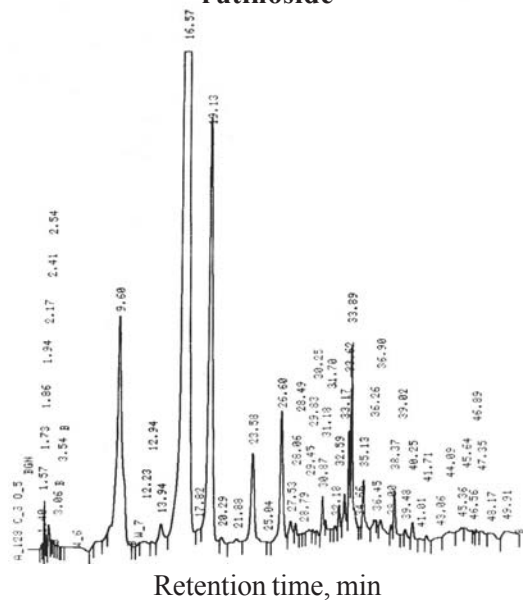


Fig. 2b. HPLC profile of polyphenols in damaged leaves of eggplant: tr=9.60 was identified as neochlorogenic acid; tr=16.57 as chlorogenic acid; tr=19.13 as 4-0-caffeoylquinic acid; tr=35.13 as rutin and tr=38.37 as K-3-rutinoside

tR = 23.75 A = 5425
 tR = 26.74 A = 2828
 tR = 30.38 A = 487
 tR = 32.74 A = 1271 (izocvercitrine)
 tR = 38.27 A = 2035 (cvercitrin)

tR = 23.25 A = 5030
 tR = 26.44 A = 7611
 tR = 30.76 A = 2074
 tR = 32.52 A = 3362
 tR = 38.01 A = 1045

For the phenolic acids in the damaged leaves the sum of the areas (A) increased by 18%, and for the flavonic glucosides – by 46%. There is again a tendency of increase of the polyphenolic complex compounds, including both phenolic acids and flavonic glucosides. The increase is greater in flavonic glucosides. In the damaged leaves the quantity of 4-0-caffeoylquinic acid (tR=19.13) and the peaks with tR=23.25 and tR=38.01 decrease. As a whole, the changes in the polyphenolic complex are greater in the leaves of eggplant, which were more heavily damaged.

We showed that feeding of *F. occidentalis* causes significant changes in polyphenolic complex compounds. Additional experiments have been planned to test how these changes influence further infestation by the pest and if different host plants react in a different way.

Conclusions

Feeding of *F. occidentalis* causes alterations in the relevant quantity of several phenolic acids (chlorogenic acids) and flavonic glucosides (rutins), which are believed to play an important role in plant defence against insect pests. These alterations were different in tomato compared to eggplant leaves, indicating that changes in the production of polyphenolic complex compounds might be associated with the preference of *F. occidentalis* for these vegetable hosts.

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