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Seasonal variations in the leaf surface composition of field grown grapevine plants

DANIELA I. BATOVSKA*, IVA T. TODOROVA and SIMEON S. POPOV

Institute of Organic Chemistry, Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str. Bl. 9, Sofia, 1113, Bulgaria

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Abstract: The leaf surface is the first barrier of grapevine plants towards various environmental stressors causing damage in vineyards. For this reason, identification of leaf surface metabolites in grapevine and their putative role in plant–environment interactions is important for viticulture. In this study, the leaf surface components of 16 grapevine plants (*Vitis vinifera*) growing in an experimental vineyard were analyzed in two consecutive seasons – the summer and the autumn of 2007. Forty-eight individual metabolites typical of the cuticular plant wax were identified by gas chromatography–mass spectrometry (GC––MS). They belonged to the following groups of compounds: hydrocarbons, sterols, terpenes, free and esterified fatty acids, alcohols, aldehydes and ketones. The metabolic profiles of the summer and the autumn samples were statistically different ($P < 0.05$), which was mainly attributed to the specific insects present in the two seasons and to the adaptation of the grapevine to lower temperatures.

Keywords: GC–MS; leaf surface metabolites; seasonal variations; *Vitis vinifera*.

INTRODUCTION

The leaf surface of grapevine (*Vitis vinifera* L.) is rich in metabolites and constitutes the first line of defense towards various biotic and abiotic stressors. The individual components identified to date belong to the classes of terpenoids, steroids, free and esterified fatty acids and heterocyclic compounds.[1] Their qualitative and quantitative composition varies from one grapevine to another depending on age, breeding conditions, season, *etc*.[2,3] The study of these variations is an important task because it can have implications for a better understanding and manipulation of the biochemical processes related to the adaptability of grapevines. Furthermore, there is an increasing demand for the development of grapevine varieties with increased disease resistance and stress tolerance.[4]

* Corresponding author. E-mail: danibat@orgchm.bas.bg
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Recently, the first, to the best of our knowledge, investigation of the seasonal variations of the leaf surface composition of grapevine seedlings was conducted.[3] The obtained metabolic profiles of summer samples included terpenes, sterols, fatty acids and heterocyclic compounds. Most of the components underwent alteration in autumn. For example, the sterol and fatty acid contents decreased, mono- and diterpenoids and the heterocyclic compounds were missing, while hydrocarbons and alcohols appeared. The observed variations proceeded under conditions of reduced environmental impact because the plants were grown in a greenhouse. Extending such an investigation to grapevines growing in open fields could provide interesting data for their adaptability to various environmental factors.

In this study, attention was focused on 16 grapevine plants growing in an experimental field. Their acetone extracted leaf surface components were readily obtained and analyzed by GC–MS in the summer and in the autumn of 2007. The GC–MS analysis did not give exact quantitative data because the ion current generated depended on the characteristics of the investigated compounds and hence was not a true quantification. However, this method provides valuable data that can be used for comparison between the same compounds as well as for determination of structural diversity. Using this approach statistically significant seasonal differences were observed for the leaf surface compositions of the investigated grapevine plants. The results are discussed in the light of the possible biological functions of the respective components on the grapevine leaf surface.

EXPERIMENTAL

Plant material

Fresh and apparently healthy leaves were collected once from 16 grapevine plants, in a totally randomized design, in the summer (June) and the autumn (September) of 2007. The samples were stored at –20 °C until extraction. The plants were two-year-old seedlings originnating from the self-pollination of the Bulgarian wine-making variety Storgozia. They were grown in an experimental vineyard of the Institute for Agriculture and Seed Science, located near the town of Rousse, Bulgaria. The studied grapevine seedlings were spaced 1.20 m between plants and 3.5 m between rows with dripper irrigation. The original plant material is deposited in the grapevine collection of the institute.

Sample preparation

On the day of their collection, the leaves were transported in a cooler bag to the Institute of Organic Chemistry in Sofia and immediately elaborated as previously described.[1] Briefly, the fresh leaves of each seedling (around 1.0 g) were dipped one by one into acetone (40 mL) for not more than one minute. The resulting extracts were filtrated, evaporated to dryness and analyzed by GC–MS. The yields in % of fresh weight were as follows: 0.1–3.4 %, mean value 0.6±0.8 %, for the summer samples and 0.2–0.8 %, mean value 0.4±0.2 %, for the autumn samples.

GC–MS analysis of the leaf surface components

The analysis was performed using a Hewlett Packard 6890 GC System Plus MS 5973 (Hewlett Packard, Palo Alto, CA, USA) equipped with a capillary column HP5-MS (30 cm, 0.25 mm, 0.25 μm film thickness, Agilent Technology, USA). The carrier gas was helium at a flow rate of 0.8 mL/min. The temperature program was 100–300 °C (10 min isotherm) at 5°/min. The method of electron-impact ionization was applied. The ion source was set at 230 °C and the ionization voltage was 70 eV.

Identification of compounds

The GC–MS identification was based on the interpretation of the mass spectral fragmentation, followed by comparisons of the spectra obtained with those of authentic samples. Computer searches in a HP Mass Spectral Library NIST98 (Hewlett Packard, Palo Alto, CA, USA) were also applied. When the spectra of some isomers were similar and they could not be identified unambiguously, comparisons of the GC retention times obtained under the same conditions were used. When there were no suitable authentic samples and/or spectra for comparison, no identification was proposed.

Statistical analysis

The statistical differences between the chemical compositions of the summer and the autumn samples were calculated using the nonparametric, Wilcoxon matched pairs test. Statistically significant values of $P < 0.05$ were accepted.

RESULTS AND DISCUSSION

Surface layers of fresh leaves collected from 16 grapevine plants were obtained in the summer and in the autumn of 2007 and analyzed by GC–MS (see Experimental).

In total, 48 individual metabolites were identified (Tables I and II). Together, they outlined a typical profile of leaf cuticular wax of higher plants, which is a mixture of mainly long-chain aliphatic hydrocarbons, fatty acids, alcohols, ketones and aldehydes.[5] These are hydrophobic compounds that form a protective layer on the interface between the leaves and environment. The grapevine plants were grown in an open field and were exposed to the effects of various environmental factors. For this reason, their acetone extracted leaf surface metabolites were totally different from those obtained from a previous investigation of greenhouse grown grapevines.[3]

The summer and the autumn samples showed statistically significant differences in the total hydrocarbons, sterols, terpenoids, free fatty acids, alcohols, aldehydes and ketones present (Table III). This was also true for 36 of the identified individual compounds, which is evidence for presence of substantial seasonal variations. The seasonal ratios and possible functions of the compounds showing statistically significant variation are also presented in Table III and are discussed below.

Hydrocarbons were present predominantly in the summer samples. They were entirely *n*-alkanes with chains ranging in length from 18 to 31 carbons. Long chain hydrocarbons normally occur over areas of plants exposed to the air.

TABLE I. GC–MS data for the surface components of grapevine leaves collected in the summer of 2007 (% of the total ion current)

Surface metabolic components	Plant															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Hydrocarbons	19.8	29.0	48.1	13.4	28.5	10.8	20.9	16.1	16.1	19.0	10.4	19.7	34.4	15.5	17.8	12.8
Octadecane	–	–	–	–	–	–	<0.1	<0.1	<0.1	<0.1	<0.1	0.2	–	0.1	<0.1	–
Nonadecane	–	–	–	–	–	–	<0.1	0.3	<0.1	<0.1	<0.1	0.2	<0.1	0.2	<0.1	–
Eicosane	1.1	0.5	0.4	0.8	0.8	0.7	1.0	1.3	0.9	0.6	1.3	0.8	0.7	0.6	1.3	0.6
Heneicosane	0.5	<0.1	<0.1	0.4	<0.1	0.3	0.4	0.6	0.4	0.3	<0.1	0.4	<0.1	0.4	0.5	<0.1
Docosane	<0.1	<0.1	0.2	0.3	<0.1	0.3	<0.1	0.5	0.3	0.4	<0.1	0.4	0.3	0.3	0.5	0.3
Tricosane	0.6	<0.1	0.9	0.5	0.7	0.4	0.6	0.9	0.5	0.5	1.1	0.5	0.7	0.7	0.6	0.4
Tetracosane	0.8	<0.1	0.5	0.3	0.8	0.3	0.7	1.0	0.3	0.4	0.7	0.6	0.8	0.6	0.8	0.4
Pentacosane	1.1	1.1	2.0	1.6	1.7	1.3	1.9	0.9	1.7	1.2	2.1	1.1	1.8	3.5	1.8	1.9
Heptacosane	6.2	10.5	9.3	3.4	6.2	2.1	4.8	3.2	4.5	5.3	3.2	4.1	7.2	2.9	3.3	9.2
Nonacosane	8.2	16.9	30.8	6.1	16.0	4.7	10.1	6.6	6.6	9.1	2.0	9.6	19.9	5.8	7.8	–
Hentriacontane	1.3	<0.1	4.0	<0.1	2.3	0.7	1.4	0.8	0.9	1.2	–	1.8	3.0	0.4	1.2	–
Terpenes	8.1	16.3	11.8	15.2	10.5	22.8	17.3	7.3	8.0	6.8	9.3	7.9	5.5	116.6	8 8.4	–
δ-Tocopherol	–	1.2	–	–	2.7	1.7	<0.1	–	–	–	–	–	–	–	–	–
β-Amyrine	1.9	1.2	5.2	<0.1	<0.1	1.6	11.7	1.1	4.4	2.0	<0.1	2.4	1.9	2.5	<0.1	–
Lupeol	5.0	13.9	6.3	15.2	5.9	19.3	5.6	5.5	2.5	4.8	9.3	5.3	2.6	13.7	7.4	–
6,10,14-Trimethyl-pentadecan-2-one	0.5	–	0.3	–	–	–	–	0.7	0.6	–	<0.1	<0.1	0.5	0.2	0.7	–
4,8,12,16-Tetramethylheptadecan-4-olide	0.7	–	<0.1	–	1.9	0.2	–	<0.1	0.5	–	<0.1	0.2	0.5	0.2	0.3	–

TABLE I. Continued

Surface metabolic components	Plant															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Fatty acids	1.1	0.8	1.5	1.1	0.8	2.2	0.7	3.5	0.9	3.1	2.6	2.1	1.8	1.5	1.4	0.6
Tetradecanoic acid	–	–	–	–	–	0.5	<0.1	0.6	<0.1	0.5	<0.1	0.6	0.5	0.4	<0.1	–
Hexadecanoic acid	1.1	0.8	1.5	1.1	0.8	1.7	0.7	1.5	0.9	2.2	1.6	1.5	1.3	1.1	1.4	0.6
Octadecanoic acid	–	–	–	–	–	<0.1	–	1.4	–	0.4	1.0	<0.1	–	–	–	–
Esters	0.8	2.3	0.5	0.6	1.7	1.4	2.1	0.9	1.7	1.8	<0.1	1.0	0.6	1.1	2.2	13.8
Methyl tetradecanoate	–	2.3	–	–	1.7	0.8	1.5	<0.1	1.1	1.1	–	0.5	–	0.7	1.2	13.8
Decyl isobutyrate	0.8	<0.1	0.5	0.6	<0.1	0.6	0.6	0.9	0.6	0.7	<0.1	0.5	0.6	0.4	1.0	<0.1
Alcohols	–	<0.1	–	<0.1	<0.1	0.3	<0.1	0.5	0.3	0.3	<0.1	0.8	<0.1	0.5	0.4	<0.1
Tetradecanol	–	<0.1	–	<0.1	<0.1	0.3	<0.1	0.5	0.3	0.3	<0.1	0.5	<0.1	0.3	0.4	<0.1
Hexadecanol	–	–	–	–	–	–	<0.1	<0.1	<0.1	<0.1	<0.1	0.3	–	0.2	<0.1	–
Ketones	1.7	1.4	<0.1	<0.1	3.2	1.3	2.4	2.3	1.2	1.8	<0.1	0.5	0.5	0.6	0.5	0.8
4-Methyl-3-penten-2-one	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.6	<0.1	0.5	0.5	0.6	0.5	0.8
14,16-Hentriacontanedione	1.7	1.4	<0.1	<0.1	3.2	1.3	2.4	2.3	1.2	1.2	<0.1	<0.1	<0.1	<0.1	<0.1	–

TABLE II. GC–MS data for the surface components of grapevine leaves collected in the autumn of 2007 (% of the total ion current)

Surface metabolic components	Plant															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Hydrocarbons	8.7	9.4	15.0	4.9	13.0	3.3	10.0	7.9	25.9	16.9	15.0	3.3	16.4	13.7	5.7	12.5
Nonadecane	–	0.1	–	–	–	–	0.5	–	–	–	–	–	–	–	–	–
Eicosane	–	0.2	0.3	–	–	–	–	<0.1	0.3	0.2	–	–	<0.1	–	–	–
Heneicosane	–	0.2	<0.1	0.1	–	–	–	0.2	<0.1	0.4	–	–	<0.1	0.1	<0.1	–
Docosane	0.3	0.2	0.2	0.1	–	0.1	0.2	0.2	0.3	0.5	–	0.1	0.5	0.1	<0.1	–
Tricosane	1.1	1.0	0.6	0.3	0.4	0.3	0.7	0.6	1.8	1.8	1.2	0.5	0.8	0.3	0.3	0.7
Tetracosane	1.2	0.9	1.4	0.5	0.4	0.4	1.0	0.6	2.4	2.0	1.7	0.1	1.4	0.4	0.5	0.9
Pentacosane	0.8	1.8	1.5	1.3	0.8	0.7	1.9	1.6	7.7	4.7	4.2	1.0	1.6	2.5	1.1	2.9
Heptacosane	1.2	1.6	2.3	0.9	1.4	0.6	2.1	1.3	9.1	3.1	4.7	0.8	2.0	2.3	1.8	3.2
Octacosane	1.9	0.3	1.3	0.3	<0.1	–	0.4	0.4	1.9	1.1	1.3	–	1.0	6.8	0.5	–
Nonacosane	0.8	1.4	1.4	0.7	1.3	0.6	0.8	0.9	2.4	2.1	1.9	0.5	1.2	0.8	1.0	1.1
Triacontane	0.6	0.6	0.9	–	1.2	–	–	0.4	–	–	–	0.3	–	0.4	–	–
Hentriacontane	0.8	1.1	5.1	0.7	7.5	0.6	2.4	1.7	–	1.0	–	–	7.9	–	0.5	3.7
Sterols	0.6	0.8	1.4	0.3	<0.1	1.0	2.0	1.3	2.1	–	–	1.7	1.7	0.3	0.2	0.7
Sitosterol	0.6	0.8	1.4	0.3	<0.1	1.0	2.0	1.3	2.1	–	–	1.7	1.7	0.3	0.2	0.7
Terpenes	5.4	50.9	70.3	87.9	76.4	89.8	60.1	60.3	54.5	62.3	62.4	84.1	50.4	76.2	87.8	61.5
γ-Terpinene	–	–	–	0.1	0.3	0.3	0.5	0.4	–	0.2	–	–	–	–	–	1.1
Neophytadiene	–	0.3	0.2	0.2	1.3	0.6	0.5	0.4	0.4	0.6	–	0.3	0.2	–	0.1	1.1
Neophytadiene (isomer)	–	0.5	0.7	0.2	0.6	0.5	0.3	0.4	0.6	1.2	–	0.1	–	–	<0.1	0.6
α-Tocopherol	0.5	1.1	0.8	0.4	1.0	0.7	–	1.0	–	–	–	0.3	2.0	0.1	0.2	2.2
β-Amyrine	0.9	2.6	43.3	2.0	2.0	2.9	33.7	4.3	35.4	12.9	10.5	14.5	14.0	7.6	1.8	30.5
Lupeol	2.9	45.0	25.3	84.4	71.2	84.5	24.5	53.2	16.7	46.5	51.9	68.5	34.2	68.5	85.7	25.3
4,8,12,16-Tetramethylheptadecan-4-olide	1.1	1.4	–	0.6	–	0.3	0.6	0.6	1.4	0.9	–	0.4	–	–	–	0.7

TABLE II. Continued

Surface metabolic components	Plant															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Esters	–	6.8	1.3	0.8	0.4	–	0.6	0.3	2.6	0.7	–	0.1	0.7	0.3	<0.1	<0.1
Methyl hexadecanoate	–	3.7	–	0.5	0.4	–	0.2	<0.1	0.5	–	–	0.1	–	–	<0.1	<0.1
Ethyl linoleate	–	0.8	0.4	–	–	–	–	–	–	–	–	–	–	–	–	–
Methyl eicosanoate	–	1.7	–	0.2	–	–	–	–	–	–	–	–	–	–	–	–
Methyl tetracosanoate	–	0.6	0.9	0.1	–	–	0.4	0.3	2.1	0.7	–	–	0.7	0.3	–	<0.1
Aldehydes	0.2	0.6	0.3	0.6	0.8	0.4	3.0	0.4	1.2	1.9	0.9	1.7	0.5	0.1	0.6	4.5
2-Pentenal	–	–	–	–	–	–	0.4	–	–	<0.1	–	0.2	–	–	0.2	–
Nonanal	<0.1	0.1	<0.1	<0.1	<0.1	<0.1	0.4	<0.1	<0.1	<0.1	–	0.1	<0.1	–	<0.1	1.2
Decanal	0.1	0.1	–	0.4	0.8	0.3	1.3	0.4	0.6	0.7	0.9	0.5	–	–	0.2	1.2
2-Decenal	–	0.2	–	0.1	–	–	0.4	–	<0.1	<0.1	–	0.3	–	–	–	0.5
2-Undecenal	0.1	0.2	0.3	0.1	–	0.1	0.5	–	0.6	1.2	–	0.6	0.5	0.1	0.2	1.6
Ketones	0.4	9.6	1.0	3.0	4.2	1.7	7.1	5.9	5.2	4.5	<0.1	2.4	2.4	0.9	2.2	8.0
4-Methylacetophenone	<0.1	0.2	–	0.6	0.8	0.3	1.5	<0.1	1.0	0.7	–	0.5	–	–	0.4	1.4
4-(3-Cyclohexen--1-yl)-3-buten-2--one	–	–	–	0.1	0.8	0.5	1.0	2.2	–	0.9	–	0.2	–	–	–	4.5
4-(3-Cyclohexen--1-yl) 3-buten-2--one (isomer)	–	–	–	–	–	0.2	0.4	1.2	–	0.3	–	–	–	–	–	1.5
Pentadecanone	–	8.3	0.3	0.3	0.6	0.2	1.4	0.6	0.7	0.4	–	0.3	0.2	–	0.1	0.6
Nonadecanone	–	0.4	0.7	–	–	–	0.7	0.4	1.7	0.2	<0.1	–	0.4	0.2	0.2	<0.1
Pentacosanone	–	0.4	–	0.2	–	–	0.6	0.3	1.8	0.7	–	0.2	–	0.1	0.3	<0.1
14,16-Hentriacontandione	0.4	0.3	<0.1	1.8	2.0	0.5	1.5	1.5	<0.1	1.3	<0.1	1.2	1.8	0.6	1.2	<0.1

TABLE III. Statistically significant seasonal variations in the leaf surface composition of 16 field grown grapevine plants, observed in the summer and the autumn of 2007

Main groups of leaf surface components	Individual components showing statistically significant seasonal variations	Statistical significance, P	Seasonal appearance of the leaf surface components		Possible biological function on the grapevine leaf surface
			Summer	Autumn	
Hydrocarbons		0.0040	++[a]	+	Prevention of desiccation, displaying of specific pheromone functions
	Octadecane	0.0100	+	–	
	Eicosane	0.0004	++++++++++++++	+	
	Heneicosane	0.0060	++++	+	
	Heptacosane	0.0050	++	+	
	Octacosane	0.0010	–	+	
	Nonacosane	0.0006	+	+++++++++	
	Triacontane	0.0200	–	+	
Sterols	Sitosterol	0.0010	–	+	Adaption of cell membranes to lower temperatures in autumn
Terpenes		0.0005	+	++++++	Protection against oxidative stress, displaying of specific pheromone functions
	γ-Terpinene	0.0200	–	+	
	Neophytadiene	0.0010	–	+	
	Neophytadiene (isomer)	0.0020	–	+	
	α-Tocopherol	0.0020	–	+	
	β-Amyrine	0.0300	+	+++++++	
	Lupeol	0.0005	+	+++++++	
	6,10,14-Trimethylpentadecane-2-one	0.0080	+	–	
Fatty acids		0.0004	+	–	Strengthening of cell membranes, prevention of desiccation, defense against plant pathogens
	Tetradecanoic acid	0.0050	+	–	
	Hexadecanoic acid	0.0004	+	–	
	Octadecanoic acid	0.0400	+	–	

TABLE III. Continued

Main groups of leaf surface components	Individual components showing statistically significant seasonal variations	Statistical significance, P	Seasonal appearance of the leaf surface components		Possible biological function on the grapevine leaf surface
			Summer	Autumn	
Esters		>0.05[b]	++	+	Interaction with specific insects
	Decyl isobutyrate	0.0004	+	–	
	Methyl tetradecanoate	0.0030	+	–	
	Methyl hexadecanoate	0.0080	–	+	
	Methyl tetracosanoate	0.0050	–	+	
Alcohols		0.0010	+	–	Displaying of specific pheromone functions
	Tetradecanol	0.0010	+	–	
	Hexadecanol	0.0100	+	–	
Aldehydes		0.0004	–	+	Defense against plant pathogens, signal function, displaying of specific pheromone functions
	Nonanal	0.0010	–	+	
	Decanal	0.0010	–	+	
	2-Decenal	0.0050	–	+	
	Undecenal	0.0010	–	+	
Ketones		0.0020	+	+++	Displaying of specific pheromone functions
	4-Methyl-3-penten-2-one	0.0004	+	–	
	Methylacetophenone	0.0020	–	+	
	4-(3-Cyclohexen-1-yl)-3-buten-2-one	0.0120	–	+	
	4-(3-Cyclohexen-1-yl)-3-buten-2-one (isomer)	0.0400	–	+	
	Pentadecanone	0.0010	–	+	
	Nonadecanone	0.0030	–	+	
	Pentacosanone	0.0050	–	+	

[a]The symbols "+" and "–" show the presence and absence of the metabolite in the samples, respectively; one symbol "+" refers to one unit of available metabolite; [b]the only main group of surface compounds without statistically significant seasonal variation

They are efficient in maintaining the internal water balance in leaves by preventing desiccation and also affect the absorption of chemicals and microbes. All of the identified hydrocarbons are known semiochemicals and most probably are involved in plant–insects relationships.[6] However, they affect the behavior of different insects and most probably, the qualitative difference in the hydrocarbon composition between the summer and the autumn samples was due to the presence of different pests in the experimental field during these two seasons.

The terpenoid level in the grapevine leaf surface increased significantly in the autumn, which was mainly due to the accumulation of compounds with triterpenoid biosynthesis, such as β-amyrine, lupeol, and sitosterol. The pentacyclic triterpenes, β-amyrine and lupeol, are supposed to be toxic to insects, due to their ability to inhibit acyl chain packing in the lipid bilayers of the insect membranes.[7] Hence, their function on the leaf surface in grapevines is most probably connected to the repulsion of some insects appearing in the field in the autumn. Sitosterol, which is the commonest plant sterol, is able to regulate membrane fluidity and plays a role in the adaptation of membranes to temperature.[8] Its level in plant leaves increased significantly due to acclimation to the lower temperatures in autumn.[9] Moreover, the terpenoid profiles of the summer and autumn samples were significantly different. Some of the individual components were present solely either in the summer or in the autumn samples, which is related to their possible specific functions in the grapevine leaf surface. Thus, 6,10,14-trimethylpentadecan-2-one (hexahydrofarnesyl acetone), which is connected to the biogenesis of chlorophyll, appeared only in the summer while the chlorophyll breakdown products neophytadienes (2 isomers) were present only in the autumn. The senescing leaves also contained the antioxidants α-tocopherol and γ-terpinene, which were absent in the summer. α-Tocopherol is the major vitamin E compound in leaf chloroplasts, where it deactivates photosynthesis-derived reactive oxygen species and scavenges lipid peroxyl radicals in the thylakoid membranes.[10] It is generally assumed that increases in α-tocopherol contribute to plant oxidative stress tolerance and most probably this compound aids the adaptation of the grapevine to the autumn conditions. γ-Terpinene exerts synergistic effects with other plant antioxidants and may also be involved in specific plant–pests interactions.[6,11]

Fatty acids were observed only in the summer samples. The three identified acids, tetradecanoic (myristic), hexadecanoic (palmitic) and octadecanoic (stearic) acids are known to strengthen cell membranes in higher plants.[12] In this way, they prevent plants from desiccation, leakage of important minerals and volatiles, and also hamper the infiltration of pathogens into the leaves. Their absence in the autumn undoubtedly increases the permeability of the cell membrane, which is one of the common features accompanying senescence.[13]

Esterified fatty acids did not show statistically significant seasonal alterations. However, this was not valid for four of their individual representatives. Decyl isobutyrate and methyl tetradecanoate were found only in the summer samples while methyl hexadecanoate and methyl tetracosanoate were present only in the autumn samples. These compounds probably interact with specific insects, present in the two seasons.[6] The same probably holds for the long chain alcohols tetradecanol and hexadecanol, which were identified only in the summer samples.

Most carbonyl compounds possess allelochemical functions in plants. Such compounds predominated in the autumn samples. Amongst them, fatty aldehydes appeared only in the autumn. These compounds are emitted by plants as a response to insect attacks.[14] With regards to their possible role on the leaf surface, it should be mentioned that the unsaturated aldehydes, 2-decenal and 2-undecenal, play role in the pathogen defense of some plants and also perform important signal functions in plants.[15,16] Amongst the ketones, 4-methyl-3-penten-2-one, also known as mesityl oxide, was identified only in the summer samples. In grapes, this compound is a precursor of 4-mercapto-4-methylpentan-2-one, which has an impact on the odor of wines and a lot of grape varieties.[17] The function of 4-methyl-3-penten-2-one in the grapevine leaf surface is not clear. The other identified ketones were found only in the autumn samples.

CONCLUSIONS

Collectively, the present data show that the leaf surface layers of 16 grapevine plants (*Vitis vinifera*) are the source of metabolites typical of cuticular plant wax, which indicate certain interactions between the plant and the environment. Differences in their composition during two consecutive seasons, the summer and the autumn of 2007, were statistically significant. It is suggested that these differences were mainly due to the specific insects available in the two seasons and to the adaptation of grapevine to lower temperatures.

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ИЗВОД

СЕЗОНСКЕ ПРОМЕНЕ У САСТАВУ ПОВРШИНЕ ЛИСТА ВИНОВЕ ЛОЗЕ КОЈА РАСТЕ У ПОЉУ

DANIELA I. BATOVSKA, IVA T. TODOROVA и SIMEON S. POPOV

Institute of Organic Chemistry, Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str. Bl. 9, Sofia, 1113, Bulgaria

Површина листа винове лозе је прва заштита од утицаја околине који изазивају оштећење винограда. За гајење винове лозе је, према томе, важно идентификовати метаболите повр-

шине листа и њихову улогу у интеракцији биљка–околина. У овој студији је анализиран састав површине листа 16 врста винове лозе (*Vitis vinifera*), током две узастопне сезоне – лета и јесени 2007. Идентификовано је 48 метаболита типичних за восак кутикуле методом гасно–масене спектрометрије (GC–MS). Ова једињења припадају следећим групама: угљоводоници, стероли, терпени, слободне и естерификоване масне киселине, алкохоли, алдехиди и кетони. Метаболички профили летњих и јесењих узорака су статистички значајно различити ($P < 0,05$), што се може објаснити присуством специфичних инсеката у ове две сезоне и прилагођавањем винове лозе на ниже температуре.

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