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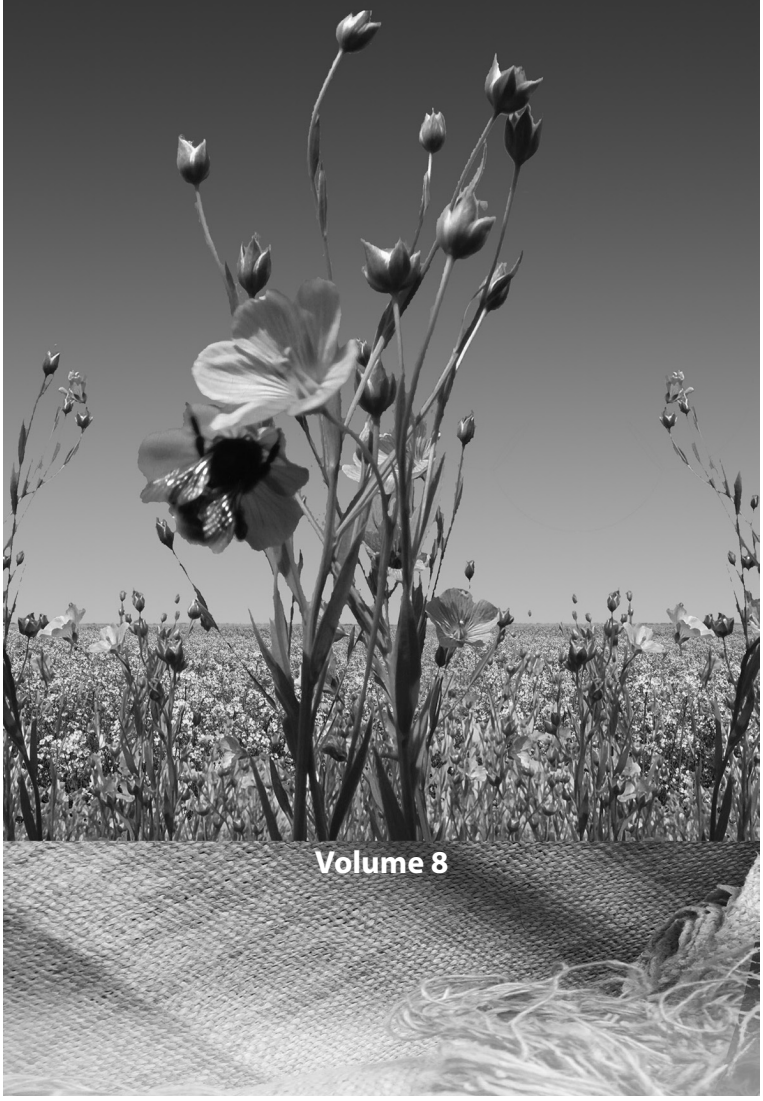

Volume 8

EDITURA UNIVERSITĂȚII AUREL VLAICU
ARAD

ISSN-L 2066-5687
ISSN 2069-8070

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Contents

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The 8th volume of the journal is like a new beginning for the Scientific Bulletin of ESCORENA. There are several changing in the life of the journal. The aims have been made broad and original papers from following directions will be published:

- *Production of bast, seed, leaf, and cellulosic fibers, hard fibers, protein fibers.*
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Degumming process of flax at industrial pilot scale

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Abstract

The enzymatic/ultrasounds degumming of flax fibers becomes an important alternative to conventional procedures. The present study shows the benefits of the ultrasounds in laccase degumming process at pilot scale. A pilot experiment using 1 % laccase and less than 30 minutes ultrasounds was performed, and high-quality degummed flax fibers were produced from flax tows using processes which have not been exploited commercially. We tested the influence of the ultrasounds/laccase system on the physical-mechanical and chemical properties of the flax fibers. The properties of the fibers are compared with those of the cotton fibers. Those properties allow us to obtain fine blend yarns (Tex 25) when the flax percentage is 50 %.

Keywords: flax, cotton, laccase enzyme, degumming, ultrasounds

Introduction

For flax degumming the conventional method is done using alkali solutions and high temperature with or without applying pressure (Hu et al., 2010; Aydin et al., 2012; Kozlowski et al., 2012). The conventional treatment reduces the quality of the fiber and produces waste waters highly polluting for the environment (Ranganathan et al., 2007). Consumption of large amount of chemicals and energy, it becomes economically not feasible. In the last years, the interest for using other methods instead of the conventional non-biological methods for flax degumming has increased.(Ko et al., 2011; Fu et al., 2012; Karaca et al., 2012).

Pectinase, cellulase and laccases had mostly used for degumming of flax tow in the industrial processes (Alix et al., 2012; Karaca, et al., 2012). There are many studies about Laccases (benzenediol:oxygen oxidoreductases; EC 1.10.3.2) intermediated degumming processes on different bast fibers (Kozlowski, et al., 2012). Laccases show low substrate specificity and high level stability in the extracellular environment in addition it is not necessary the usage of co-subtrate (Couto and Herrera, 2006). Low molecular mass mediators

such as *N*-heterocylic bearing N–OH groups (e.g. 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid, violuric acid, hydroxyl benzotryazole) should be used in the degumming process of the flax with laccase, due to the non-specificity of the enzymes (Bao et al., 1993; Kozlowski et al., 2006; Batog et al., 2008; Tauber et al., 2008).

Due to the fact that enzymes have low diffusion rates and the effect is concentrated on the outer fibers there are some strategies to improve these drawbacks. For example it can be used the ultrasound energy (Basto et al., 2007), microwaves (Sgriccia and Hawley, 2007), steam explosion (Kessler et al., 1998) could be a way to improve the diffusion of the enzymes to the interior of the technical fibers in order to release elementary fibers. Among them the ultrasonic energy has been used in different processes such as: mercerization, desizing, bleaching, scouring, and dyeing of natural fibers (Yachmenev et al., 2002; Yachmenev et al., 2004; Stanescu et al., 2010). The use of ultrasounds in textile wet processes reduce energy and chemicals consumption (Moholkar and Warmoeskerken, 2004; Moholkar et al., 2004; Sirghie et al., 2008). The combined ultrasound/hydrolytic enzymes processes conduct to less fibers damage and better results (Yachmenev, et al., 2002; Yachmenev et al., 2007) which consist in cottonised flax fibers with physical-mechanical properties similar to cotton.

Akin et al (2001) has reported one of the first results on pilot scale studies of enzyme/ultrasounds applied on retted flax fibers obtaining fine materials.

In the present study we show the benefits of the ultrasounds in laccase degumming process at pilot scale. We tested the influence of the ultrasounds/laccase system on the physical-mechanical and chemical properties of the flax fibers.

Material and methods

All chemicals were supplied by Sigma-Aldrich unless otherwise stated.

Enzyme concentration used in the present work was 1 % regarding to fiber mass. In every experiment 10 kg flax tow fibers were treated in two steps in an ultrasound thermostatic bath (Bandelin RM 210 UH, Badelin electronic Gmbh., Berlin, Germany), with a capacity of 300 liters. In the first step were used 1 % o.w.f laccase and 0.01 % HOBT (respect to enzyme concentration) as mediator, at 55 ^{0}C and ultrasound frequency of 35 kHz for 25 min in a liquid to fiber ratio 1:15. In the second step the fibers were rinse for 10 min in the same liquid ratio conditions (1:15).

After drying the cottonized flax fibers were evaluated from physical-mechanical point of view using High volume instrument (HVI) System for cotton in order to appreciate the degumming degree.

200 kg of cottonised flax fibers obtained from 20 independent experiments of 10 kg each were mixed with cotton fibers (140 kg) in two ways (50 % + 50 %; 70 % + 30 %) in order to obtain the blended yarns. The blends were spun on cotton type equipments as follows: 3C carding machine (Unirea SA, Cluj, Romania), LB drawing frame equipments (Unirea SA, Cluj, Romania), BD-200 S spinning equipments (Elitex OE, Avanco, Praha, Czech Republic).

Results and discussion

In a previous study (Sirghie et al.), we have been sown that the optimal concentration of enzyme for degumming of flax fibers had been 1 %. Industrial pilot scale experiments were conducted in order to prove the technical efficiency for this concentration of enzyme (o.w.f) in presence of ultrasounds. This value (in correlation with ultrasounds) is a compromise by economical point of view which can be used at industrial scale due to the high cost of pure enzymes.

A higher concentration of enzyme without ultrasounds gives the same results while an increasing amount of enzyme in ultrasound treatment conditions would offer better results but is more expensive. Since “degumming” effect was obtained at 1 % enzyme concentration a higher percent could determine better fibers quality but with higher costs.

The composition of flax fibers includes besides cellulose, hemicellulose, lignin and pectin, etc. (Kozlowski, et al., 2012). Pectin and lignin act mainly as bonding agents. The chemical compositions of cottonised flax fibers compared with flax tow are shown in Table 1.

Table 1. Chemical composition of row material (flax tow) and degummed flax fibers

Chemical composition	Flax tow ± SEM	Degummed flax fibers ± SEM
Hemicellulose (%)	16.0 ± 0.1	14.7 ± 0.7
Lignin (%)	4.5 ± 0.1	2.44 ± 0.06
Pectin (%)	1.5 ± 0.1	0.95 ± 0.12
Degree of polymerization	3510 ± 13	3229 ± 59

The hemicellulose, lignin and pectin percentages are decreasing significantly for degummed flax fiber compared with flax tow. Similar composition of flax fibers is described in other papers (see for review (Baley, 2002)). The same decreasing in pectin, lignin and hemicellulose amount in flax fibers was shown in many studies and should be desirable in order to avoid poor absorbency and wettability (Bismarck et al., 2002).

As it can be seen from Table 2 the physical-mechanical characteristics of degummed flax fibers are comparable with those of the cotton fibers, in terms of length, tenacity and elongation.

Table 2. The physical-mechanical characteristics of degummed flax fibers and cotton fibers

	Length (mm)	Tenacity (cN/Tex)	Micronaire	Elongation (%)	Impurities (%)
Cottonised flax fibers	32.6-35.4	26.7–29.4	6-7.6	4.1-5.06	0.7-3.9
Cotton fibers	28.96-31.01	27.4-31.6	3.7-4.6	5.8-6.1	0.05-0.36

Fineness is one of the most important factors affecting fiber quality for spinning into yarns.(Akin et al., 1997) Fineness of degummed flax (6-7.6 micronaire) obtained using our procedure is 150 % higher than one which used pure cotton (3.7- 4.6 micronaire). Fiber strength, which has been related to fineness in enzyme-degummed flax, and length are other important fibers parameters that, along with fineness, influence quality and production efficiency of blended yarns.

From the physical-mechanical properties of blended yarns presented in Table 3 it can be seen that these yarns fall in 100 % cotton yarn quality parameters especially for strength and elongation.

Table 3. The physical-mechanical characteristics of different blended yarns

Characteristics/ composition		Yarn 29 Tex 50 % Degumming flax + 50 % Cotton	Yarn 25 Tex 50 % Degumming flax + 50 % cotton	Yarn 31 Tex 70 % Degumming flax + 30 % cotton
Fineness	Tex	30.3	24.5	31.8
	CV%	9.6	8.4	3.4
Strength	CN	163	133	208
	CV%	17.4	21.0	17.1
Breaking length	Km	5.4	5.4	6.5
Elongation	%	5.1	5.7	6.7
	CV%	20.3	20.2	12.4
Torsion (OE)	Torsions/m	1264	1164	1062
	CV%	4.5	2.9	3.8
Uster irregularity	CV%	12.9	10.43	11.43
Uster/1000 M	Neps	1477	1595	978

The torsion, even if it is slightly higher than the yarns of 100 % cotton, is in the acceptable range. From the irregular values, it can be seen that the blended yarns are as

uniform as 100 % cotton yarns, a fact which indicates a very good degree of homogenization of fibrous mixture, thus confirming the results presented in Table 2 for degummed fibers.

The cost of our process of ultrasonic – enzymatic degumming of flax implies two different activities. The degumming process including water, energy, chemicals, drying of the fibers lead to a price of 0.88 EUR/kg. The price of cotton and flax is 2 EUR/kg. The second process included in the price is the spinning with the total costs of 1.33 EUR/kg. Taking in account all costs, the specific consumption for a blend yarn of 29 or 25 Tex is 1.4 EUR/kg while for a blend yarn of 31 Tex is 1.6 EUR/kg. The above mentioned costs were calculated for 300 kg mixtures in a pilot experiment. It is expected that the production of yarns in large amounts (over 1000 kg) may improve the specific consumption.

Conclusions

We showed that by using 1 % enzymes in ultrasound conditions for 25 min were obtained at industrial scale, degumming flax fibers of very good quality (similar to cotton fibers from physical-mechanical point of view). This procedure allowed us to obtain a fine blended yarn (Tex 25) when the flax percentage was 50 %. Such yarns has never been obtained at industrial scale except in one of our earlier report (Sirghie, et al., 2008).

Acknowledgement

This work was supported by project co-funded by European Union through European Regional Development Fund Structural Operational Program “Increasing of Economic Competitiveness” Priority axis 2, operation 2.1.2. ID project 679, cod SMIS CNSR 12638.

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Natural dyes in green walnut shells for textile materials dyeing

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Abstract

The purpose of this study was to obtain and characterize a natural dye from green walnut shells. By thin layer chromatography a single brown spot was obtained and attributed to naphthoquinones. Naphtoquinones presence was also proved by the UV/VIS and the IR spectra.

Keywords: natural dyes, green walnut shells, chemical composition, UV-VIS, IR, thin layer chromatography.

Introduction

During the last years an increased interest was noted for the use of natural dyes in textile dyeing process. Particularly are concerned natural vegetable dyes. The main advantage of using them is the fact that their source is renewable, biodegradable and reduces environmental impact on the environment. Still the use of natural dyes involves some disadvantages such as requirement of large amounts of plant material, limited success in dyeing synthetic materials, need for the use of mordents, poor light stability (Taylor, 1986). However, especially in case of natural fibers, replacing synthetic dyes with natural ones represent a not too distant perspective.

The worldwide use of walnut green leaves and shells for natural dyeing of textiles has ancient roots. Recent studies brought them in the attention of specialist since it was found to have, besides coloring properties, good insecticidal effect - thus helping to the fiber protection (Wang et al., 2009). Regarding their chemical composition we found few researches performed leading to the identification of several targeted compounds like juglanins, methyl palmitate, ellagic, gallic and caffeic acids and some volatiles (such as pinocarvone, pinocarveol, myrtenal, myrtenol, caryophyllene epoxide, verbenol, verbenone, terpinolene) (Wang et al., 2009; Chen et al., 2008; Buttery et al., 2000; Segundo et al., 1998).

The purpose of this study was to gather as many information on the chemical composition of the walnut green shells extract by using a series of qualitative analysis such as Borntrager reaction, thin layer chromatography, UV-VIS spectroscopy and infrared spectroscopy thus completing the information already available.

Material and methods

Benzene, ethanol, potassium hydroxide (p.a.), glacial acetic acid and ammonia solution (25%) were purchased from Reactivul Bucureşti.

For this study it has been used green nut shells harvested in June when it seems to be richest in active principles (Segundo et al., 1998). A quantity of 400 g of these shells was crushed in a mortar and then was subjected to an extraction with hot benzene in a Soxhlet apparatus until exhaustion. The benzene extract was filtered and then evaporated to dryness under a water bath. After cooling a crystalline precipitate was obtained. The precipitate was purified by recrystallization with ethanol. Further the obtained substance was dried and weighed. 18 g of dried extract were obtained.

Borntrager reaction

Borntrager reaction (Auterhoff et al., 1968) consist in extract treatment with a hot solution of 5 % KOH followed by acidification with acetic acid and dissolution in benzene, when a clearly phase is separated; the clear phase is afterwards treated with ammonia solution.

Thin layer chromatography (TLC)

For separation of the components from the obtained extract, 0.5 g was diluted with 1 ml benzene; methanol-chloroform 1:1 was used as mobile phase on 250 μm silica gel plate; eight spots were put at the starting line.

Ultraviolet and visible spectroscopy (UV-VIS)

UV-VIS absorption spectra were obtained using a SPECORD 200 spectrophotometer (Analytic Jena).

Infrared spectroscopy (IR)

The IR spectra were recorded in 4000-400 cm^{-1} by filling the extract under a form of thin film of KBr spectral pellets.

Results and discussion

The natural quinine pigments, supposed to be involved in the overall brown color of the walnut extract, range in color from pale yellow to almost black and there are over 1200

known structures. They contain the same basic chromophore, benzochinone. Those present as glycosides may be slightly water-soluble – but for the rest is more likely to be lipid-soluble and would be extracted from a crude plant extract together with carotenoids and chlophylls (Tamas et al., 1971).

Borntrager reaction

For confirming that a pigment is quinone type, simple color reactions are still very useful. The extracted sample prepared under the conditions showed above, lead to the apparition of characteristic red color (Segundo et al., 1998; Auterhoff et al., 1968).

Thin layer chromatography (TLC)

TLC on silica gel is a general procedure for separating quinones. Simple benzoquinones or naphtaquinones are very soluble and can be easily separated in pure benzene, pure chloroform or pure petroleum but complex solvent mixtures are required for those highly hydroxylated. Since they are colored, there is no difficulty to be detected in visible light (Harborne, 1998).

A picture of the obtained thin layer chromatogram is presented in Figure 1. The dye was quickly released from the starting line leading to a single strong brown spot. The compound type can be identified by mean of retardation factor value, R_f .

$$R_f = h_A / h_s \quad (1)$$

where h_A was the average distance from the start to the middle of the spots and h_s the distance from the start line to the solvent end line.

Thus calculated R_f was found to be 0.81 comparable with literature data for naphthoquinones (Kichner, 1977; Tamas et al., 1971).

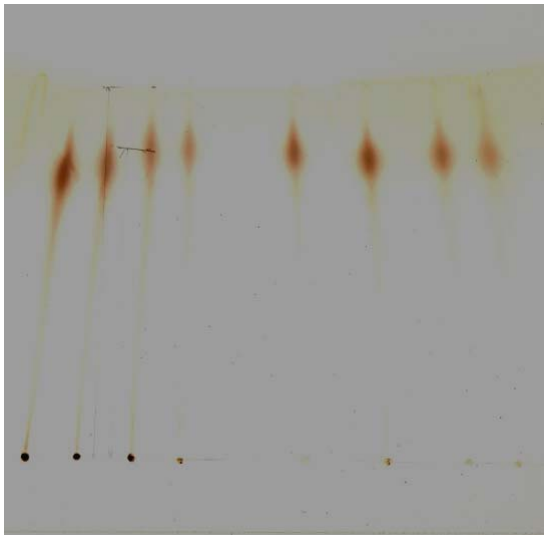

Figure 1. Thin-layer chromatogram

Ultraviolet and visible spectroscopy (UV-VIS)

The resulting spectra from UV-VIS analysis of the dye at different concentrations (30 µg/ml; 15 µg/ml; 7,5 µg/ml; 3,8 µg/ml) are shown in Figure 2. Three peaks appears around λ = 260 nm, λ = 340 nm and λ = 430 nm wavelengths, which complies with naphthoquinones spectra characterized with strong signals between 240-290 nm, respectively with an average signal around 335 nm and in region of 400-510 nm (Medeleanu et al., 1998).

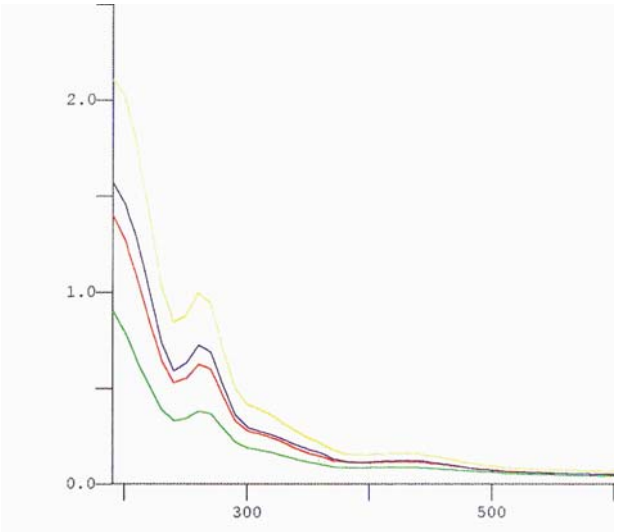

Figure 2. UV-VIS spectrum of the extract of green nut shells

Infrared spectroscopy (IR)

Unlike the UV-VIS spectroscopy which is more useful for quantitative analysis, the IR spectroscopy gives more information about the structural groups present in the green nut shells extract.

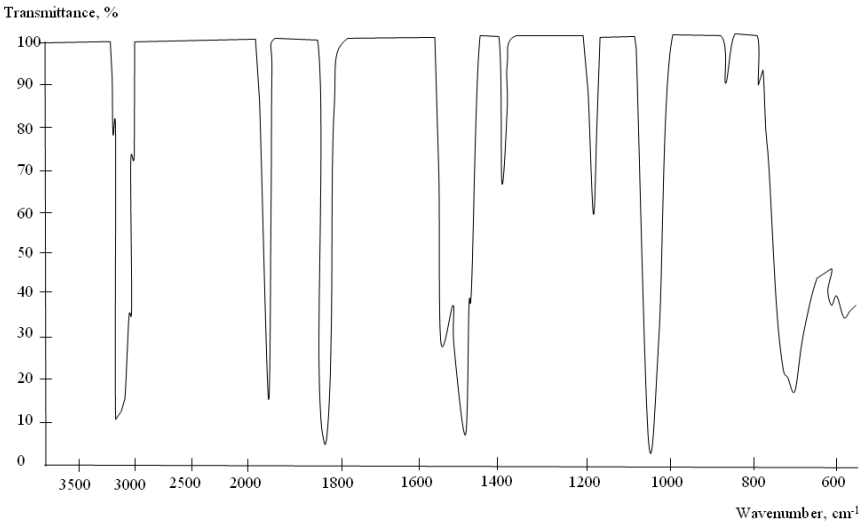

Figure 3. IR spectra of the green walnut shells extract

It is noted that the IR spectrum of the extract (Figure 3) contains several bands common to naphthoquinones namely (Balaban et al., 1983; Avram et al. 1966):

(i) the band from 3200 cm^{-1} indicates the presence of hydroxyl groups and C-H;

(ii) the band from 1860 cm^{-1} indicates the presence of C=O groups;

(iii) the band from 1550 cm^{-1} indicates the presence of aromatic (CHar) and saturated (C-C) structures existing in naphthoquinone as a derivative of naphthalene;

(iv) the band from 1090 cm^{-1} indicates the presence of C-O groups;

(v) the band from 700 cm^{-1} indicates the presence of C-H groups. These results are in accordance with other literature studies that found juglone (a naphtoquinone) to be one of the compounds responsible for the brown color (Segundo et al., 1998).

Conclusions

The purpose of this study was to find the compounds responsible for the brown color of the green walnut shells extract. All analytical techniques used (Borntrager reaction, thin layer chromatography, UV-VIS and IR spectroscopy) pointed to naphtoquinones derivatives. The results are completing and reinforcing the information already available in the literature data. Further studies regarding the dyeing of natural and synthetic textile materials using this dye will follow.

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Structural Investigation by Atomic Force Microscopy

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Abstract

Nanotechnology is an emerging field of research that has been widely applied in different fundamental science and engineering areas. An example of a nano-based device is the atomic force microscope, which is a widely used surface scanning apparatus capable of reconstructing at a nanometric scale resolution the 3D morphology of a wide variety of samples. Therefore, due to its versatility, sensitivity and unique capability to reveal the nanoscale structure of the samples, atomic force microscopy (AFM) produced, in the last years, a vast increase of reports of its use to determine the topography, electric properties, nanomechanics and even nanomanipulations of various samples in the fields of materials science, chemistry, physics, biology, microbiology, medicine, engineering, food products, forensic, etc.

Introduction

The development of new nanomaterials with a vast variety of applications in our day-life leaded to the need of use of new techniques for the structural and physico-chemical characterization. One of the advanced methods of investigation is the atomic force microscopy (AFM), which uses a microscope that was obtained after the extended of the research in the area of scanning tunneling microscopes for investigation of electrically non-conductive materials, such as proteins, DNA, etc. Binnig and Quate reported in 1986 the first invented AFM which used a very small probe-tip at the end of a cantilever (Binnig et al., 1986) and in 1989 was available the first commercial AFM. The general components of AFM are the following:

- laser: for an excellent spatial resolution and a high resolution over the photodiodes detector;

- photodiodes: for high sensitivity and detection in two dimensions (measures the deflection of the cantilever);
- feedback loop (controls *z*-sample position);
- cantilever (spring which deflects as probe tip scans the sample surface);
- probe tip (senses surface properties and causes the deflection of the cantilever);
- piezoelectric scanner (positions the sample (x, y, z) with high accuracy);
- computer (controls the system and performs the data acquisition, display and analysis).

AFM can image the surface topography with high magnifications, up to 1.000.000x, comparable with electronic microscopes, and in three dimensions, z-direction being usually higher than the horizontal x, y-plane.

Performing a data search in Web of Knowledge we found approximately 104.200 publications that reported research that used information obtained by using AFM, from which 85.600 were scientific articles from diverse areas of fundamental research and applied technologies such as: materials science 34.000; films (thin films, alloys) 25.600; chemistry 30.000; polymer science 7.700; composites 3.500; 2.600 cells and their functionality, dentins 157, etc. AFM imaging is a common technique (Barth et al., 2011; Ando, 2012) used for determination of carbon nanotubes (Baer et al., 2010; Rao et al., 2013; Tessmer et al., 2013), composites (Wang et al., 2013; Zhang et al., 2013), wood pulp and paper properties (Maximova et al., 2001; Koljonen et al., 2004; Maximova et al., 2004; Chhabra et al., 2005; Hou et al., 2006; Knutson et al., 2007; Ahola et al., 2008; Deng et al., 2008; Fatehi and Xiao, 2008; Wan et al., 2010; Wang et al., 2010; Gilli et al., 2012; Leitner et al., 2013; Miao and Hamad, 2013), biological samples and their mechanisms (Hoffmann and Dougan, 2012; Kalle and Strappe, 2012; Dufrene et al., 2013; Han et al., 2013; Miron-Mendoza et al., 2013; Singh, 2013) chemistry (Barth, et al., 2011; An et al., 2012), etc. For example AFM methods were use for imaging and measurements of DNA related research (Kalle and Strappe, 2012). A current challenge in the life was to reveal and to understand how biological systems change their structural, biophysical and chemical properties to adjust functionality. Addressing this issue has been severely hampered by the lack of methods capable of imaging biosystems at high resolution while simultaneously mapping their multiple properties. The recent developments in force-distance (FD) curve–based atomic force microscopy (AFM) enabled researchers to combine (sub) molecular imaging with quantitative mapping of physical, chemical and biological interactions.

Material and methods

An AFM device, namely NTEGRA Probe NanoLaboratory (NT-MDT, Moscow, Russia), equipped with an M Plan Apo 100x magnification objective that has the numerical aperture of 0.70 (Mitutoyo, Kawasaki, Japan) and a RPC-TVPCI camera which helps to locate the sample position were used. Software Nova_1644 for manipulating and analyzing the recorded data was employed. For storing the optical information a CCD camera was utilized. The samples were added to two-sided tape on sapphire support and the measurements were carried out under ambient conditions (temperature: 22 ± 1 ºC, relative humidity: 40 ± 10). Noncontact 'Golden' silicon cantilevers (NSG30 from NT-MDT, Moscow, Russia) with a resonance frequency of 320 ± 80 kHz, were used. All samples were measured in semicontact mode ("tapping" mode) to determine the topography images. Different surface areas of the samples have been investigated, as are mentioned in the figures.

Results and discussion

Atomic force microscope equipped with confocal Raman spectroscope is currently used in Institute of Technical and Natural Sciences Research-Development-Innovation of "Aurel Vlaicu" University from Arad, Romania (Figure 1). We performed imaging and measurements for a wide variety of samples such as composites (Popa et al., 2013), plant bast fibers (Figure 2 a, b), paper sheets (Figure 2 c, d), plants.

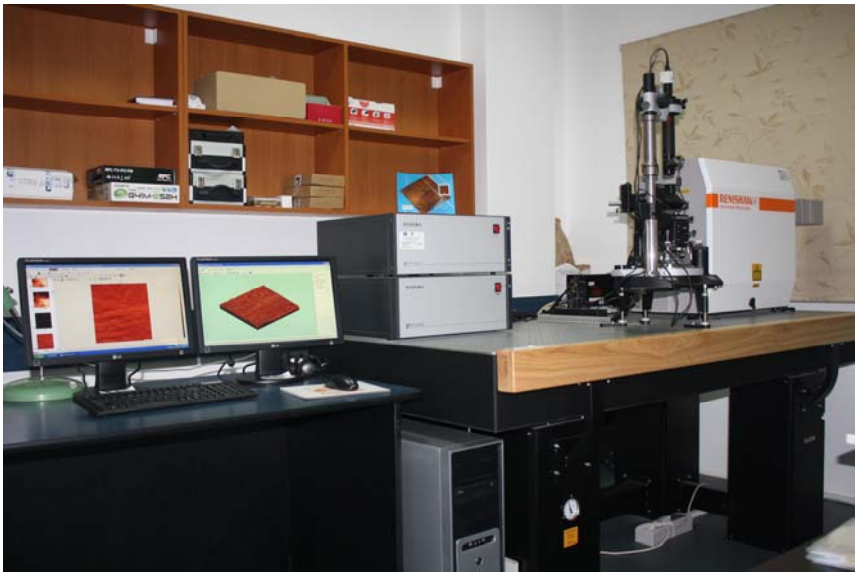

Figure 1. Atomic Force Microscope coupled with confocal Raman spectroscope used at ICDISTN of "Aurel Vlaicu" University.

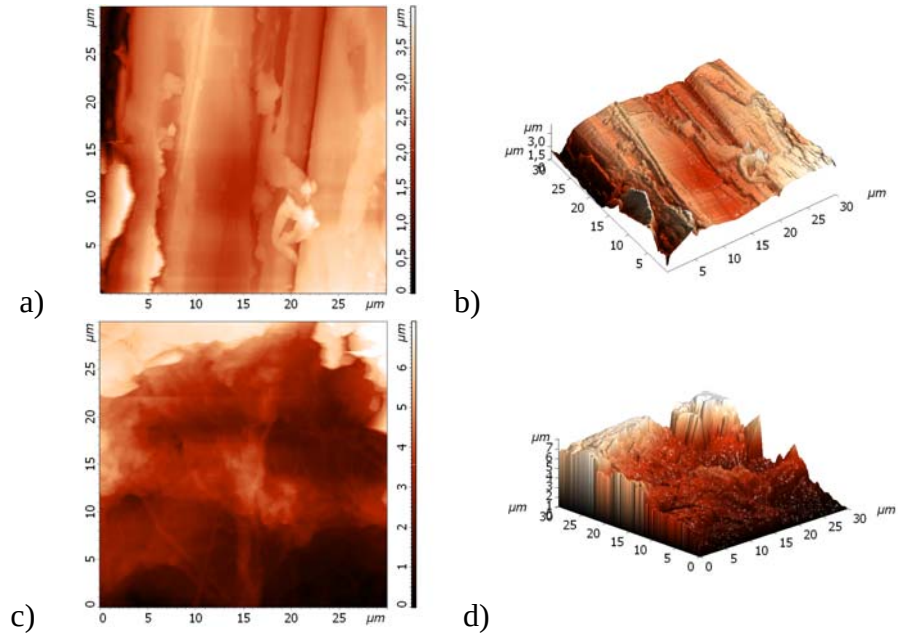

Figure 2. AFM images of the following samples:flax fiber a) 2D, b) 3D, paper sheet c) 2D, d) 3D.

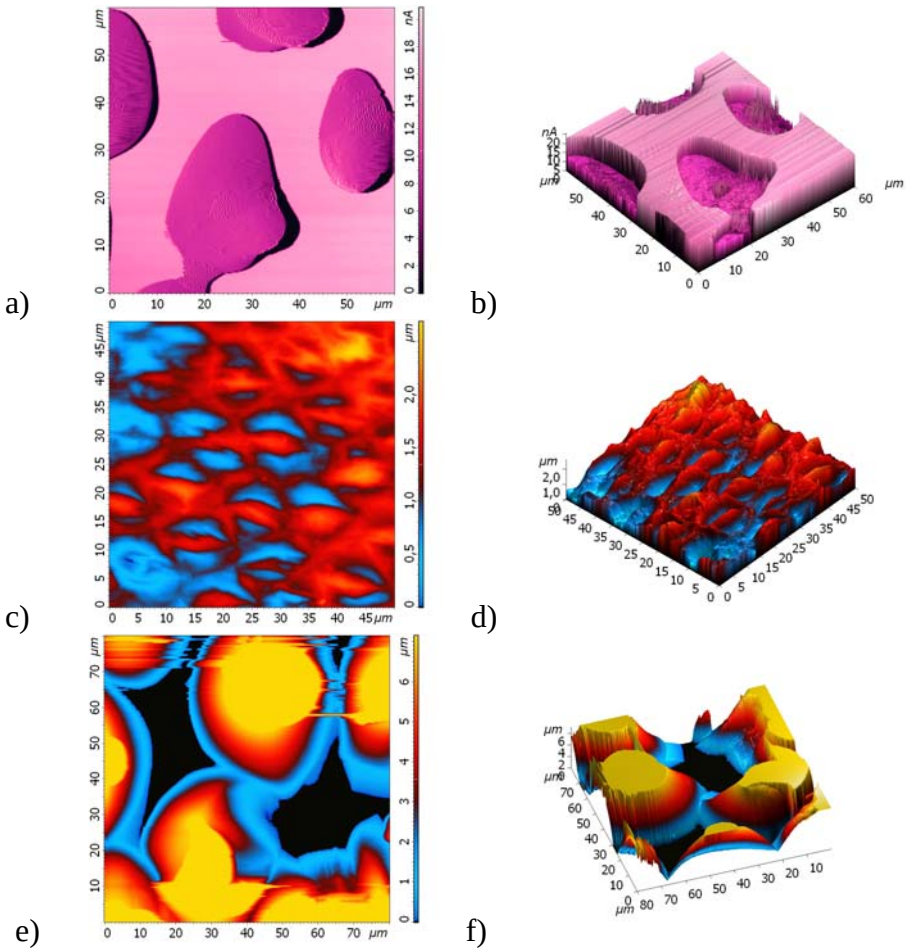

Figure 3. AFM images of petals of flowers: purple petal of *Hibiscus syriacus*: a) 2D, b) 3D; red petal of an *Hawaiian Hibiscus*: c) 2D, d) 3D; and protuberances from the edge of a petal of *Phalaenopsis amabilis* (Moth Orchid): e) 2D, f) 3D.

In Figure 3 are exhibited AFM images performed in semicontact topography (tapping) mode for Hibiscus petals and Moth Orchid petals. The AFM images unveiled the different morphologies of the petals measured.

Acknowledgements

This work was supported by project co-funded by European Union through European Regional Development Fund Structural Operational Program "Increasing of Economic Competitiveness" Priority axis 2, operation 2.1.2. ID project 679, cod SMIS CNSR 12638: "Bast plants – Renewable Strategic Resources for European Economy". - „BASTEURES". No. 210/2010 POS-CCE.

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Evaluation of Total Chlorophyll Content in Microwave-Irradiated *Ocimum basilicum* L.

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Abstract

Microwave stress is an important cause of stress today. This study was carried out to investigate the effect of microwave radiation derived from a wireless router on chlorophyll pigments from *Ocimum basilicum* L. Treatments included reference (no microwave), microwave-stressed plants and control plants (growth in normal condition). The extracts were analyzed using a Shimadzu UV-160A spectrophotometer for chlorophyll content determination. The content of chlorophylls a and b from plants subjected to microwaves was smaller than in the reference plants.

Introduction

Chlorophyll pigments are very important in the process of photosynthesis and chlorophyll concentration is closely related to the stress factors, this fact being shown by many researchers over time (Merzlyak and Gitelson, 1995; Penuelas and Filella, 1998).

Chlorophyll is one of the most important and most abundant photosynthetic pigments. The amount of chlorophyll is correlated with productivity and plant health (Dash and Curran, 2007). Chlorophyll content of leaves is also an indicator of stress, giving us vital information regarding plant responses to climate change events. When a plant is under stress, low chlorophyll content changes are evident in the initial stages. As stress increases, chlorophyll content decreases faster than other pigments (Bannari et al., 2007). These changes of chlorophyll content indicate the stress to which the plant was subjected (Radu et al., 1981). The chlorophyll content is, therefore, a plant stress response.

The adaptability of organisms to extreme conditions of life, environmental stress response mechanisms is an area of great current research. Nowadays, has become more

demanding the use of mobile telephony and wireless devices generating an exponentially increased level of electromagnetic radiations. Thus, there is a new stress factor - electromagnetic field, especially from the microwave area (Vashisth and Nagarajan, 2008).

The present study aimed to determine the effect of microwave field on chlorophyll pigments content from *Ocimum basilicum* L., a very important culinary spice and medicinal plant from *Lamiaceae* family.

Material and methods

In this paper the microwave influence on chlorophyll content from *Ocimum basilicum* L. was studied. The plants were grown in the laboratory, using seeds purchased from ARO Company (Romania). For this experiment were used plants grown in normal conditions and plants exposed to microwaves. For microwaves exposure, two identical anechoic chambers were used: a reference chamber and a microwave chamber.

At three weeks after seeding, vessels with sprung plants were placed in two identical anechoic chambers (E. Surducan et al., 2012), one being the reference chamber and the other with the active microwave field (Figure 1).

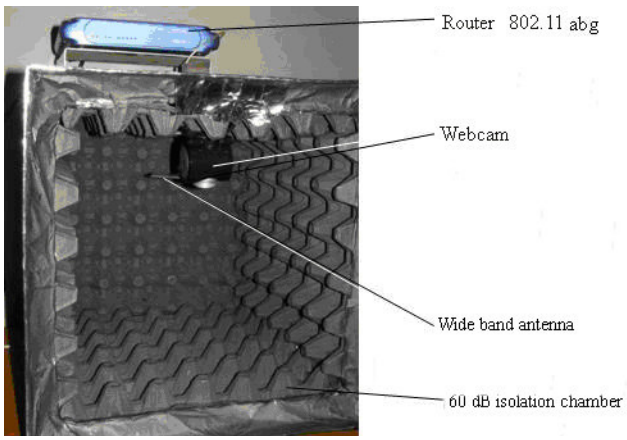

Figure 1. Installation to stimulate the plant development by applying a low power electromagnetic field.

The installation to stimulate the plant development by applying low power electromagnetic field consists in a closed chamber which contains the artificial light sources, humidity sensor, temperature and humidity sensors, video camera and a microwave generator that emits by means of an electromagnetic field antenna on the plants culture at a power level between $10W/m^2$ - $10mW/m^2$. The power is measured by a power meter with the probe located in the area where the plants are subjected to stimulation, and which is connected to a microcontroller and process computer which can interconnect a network of such premises (E.

Surducan et al., 2012). The reference chambers do not contain the microwave generator. The temperature and humidity in both chambers were continuously registered and a good correlation between the two was observed.

The irradiation of plants was performed with microwaves modulated by a WLAN communications protocol, in the 2.412 – 2.48 GHz frequency band. Irradiation was performed over a period of two weeks, within the plants were watered with 10 mL of ultrapure water at an interval of 3 days. After the irradiation period, the leaves were manually excised and subjected to chlorophyll extraction.

The weighed plant samples (1 g) were homogenized with an Ultraturrax in the presence of 20 mL 90 % acetone in water and then left for two hours with stirring. The extraction was repeated with 10 mL 90 % acetone in water until bleaching of the plant material, and then the samples were filtered. Each extraction was performed in three parallel samples.

The quantitative analysis of chlorophyll a and b pigments content from the samples extract was performed using a Shimadzu UV-160A spectrophotometer. The calculation of chlorophyll pigments concentration was carried out by reading the absorbances at 663 and 645 nm wavelengths, according to the following equations (1), (2), (3) (Arnon, 1949):

$$\text{Chl a} = \frac{[12.7(\text{A663}) - 2.69(\text{A645})]\text{xV}}{(1000\text{xW})} \quad (1)$$

$$\text{Chl b} = \frac{[22.9(\text{A645}) - 4.68(\text{A663})]\text{xV}}{(1000\text{xW})} \quad (2)$$

$$\text{Chl total} = \frac{[20.2(\text{A645}) + 8.02(\text{A663})]\text{xV}}{(1000\text{xW})} \quad (3)$$

where: Chl a – chlorophyll a, Chl b – chlorophyll b, Chl total – total chlorophylls content, A663 – sample absorbance at 663 nm, A646 – sample absorbance at 646 nm, V – volume of solvent, W – fresh weight of tissue extracted.

Results and discussion

In most of the cases any of stress factors by a specific intensity can lead to decrease of chlorophyll concentration in the plant. This was observed in the case of our plants (Figure 2).

a) b)

Figure 2. *Ocimum basilicum* L. plants: a) microwave-irradiated and b) reference.

It can be observed that reference plants have a darker green color than those microwave-irradiated.

The obtained results concerning the concentration of chlorophyll pigments in basil extracts are presented in Figure 3.

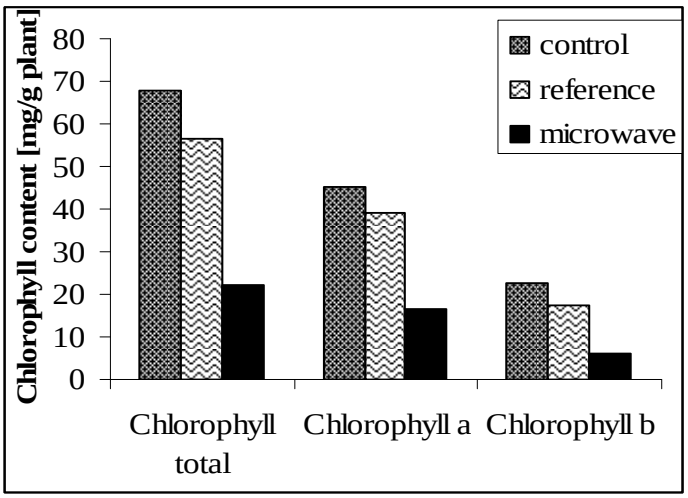

Figure 3. Content of chlorophylls in control, reference and microwave-irradiated plants of basil (*Ocimum basilicum* L.).

As can be seen in Figure 3, the chlorophyll content in control plants is higher than in the plants grown in the reference and microwave chambers. Also, chlorophylls a and b content from plants subjected to microwaves was lower than in non-irradiated plants.

The total chlorophyll content was 56.41 mg/g for the reference plants, and 22.26 mg/g for irradiated plants. Thus, the amount of total chlorophyll in irradiated plants is smaller by 60.54 % compared to reference plants.

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The influence of some calcium chloride products on post-harvest apple quality

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Abstract

The optimal time for harvesting is determined by the output destination: fresh consumption, industrialization, etc. For good fresh fruit consumption, harvest maturity is determined differently depending on species, variety, storage conditions, remoteness of consumer etc. In this experiment we studied the influence of some products based on calcium chloride upon 2 varieties of apple fruit from market in the west of Romania. For this purpose, immature fruits were harvested and stored in different temperature conditions. The following quality parameters were determined: fruit firmness, soluble carbohydrate content and starch test.

Introduction

The term "post-harvest treatment" is to some extent misleading because, as every manufacturer knows, apple treatments do not begin only after harvest but at the beginning of production, when selecting varieties of fruits and vegetables to be produced (Bucarciuc, 1991). Choosing apple varieties – with long-term preservation characteristics and resistance to transportation - is as important as any other work or process carried out in the orchard before harvesting or during handling and storage of fruits after harvest (Molina, 2006; Taya Pineiro, 2007). Across the globe, many countries set quality standards for fruits and vegetables that are imposed by law. To be marketed in those countries, fruits and vegetables must meet these standards (Unay, 2004).

Materials and methods

For testing Timac specific products for apples we decided to use the following varieties: **GENEROS** and **FLORINA** (Figure 1).

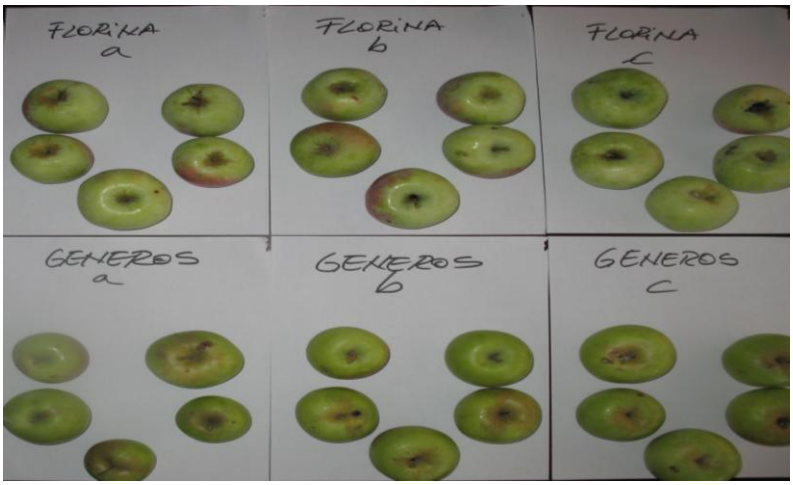

Figure 1. Studied apple varieties

The experiment was located in the Experimental Teaching Resort at USAMVB Timisoara on a total surface of 1440 m^2 using randomized blocks in plots of 80 m^2 and having 3 repetitions.

We used the following experimental variants: **a** - treatment with **calcium chloride** (control); **b -** treatment with **ELITE**; **c -** treatment with **RDM.** Treatments were performed at different stages of vegetation expressed with BBCH code (Table1) as follows:

Table1. Treatments applied

Treatment	Phenophase
Calcium chloride ,Elite, RDM	74 BBCH
Calcium chloride ,Elite, RDM	77 BBCH
Calcium chloride ,Elite, RDM	79 BBCH
Calcium chloride	81 BBCH
Calcium chloride	85 BBCH
Calcium chloride	89 BBCH

The main indices followed were: soluble carbohydrate content (digital refractometer), fruit firmness (penetrometer). Maturity starch-iodine test (AI) - This is a reliable method for determining the maturity of most varieties of apples and is the simplest indicator of apple ripeness. When the apples begin ripening, naturally accumulated starch turns into glucose. Fruits were stored / maintained in the following conditions: air-conditioned containers at a temperature of 4 º C; refrigerated containers at a temperature of 17 º C; room temperature: 27° C, during 12 days.

Results and discussions

Results regarding the determination of soluble carbohydrates content for the studied genotypes in BBCH 81 vegetation stage.

Table 2. Total content of soluble carbohydrates at Generos genotype

EXPERIMENTAL VARIANT	SOLUBLE CARBOHYDRATES CONTENT (%)									
	HARVEST MOM.	12 DAYS								
		27°C			17°C			4°C		
		VAL.	DIF.	%	VAL.	DIF.	%	VAL.	DIF.	%
CALCIUM CHLORIDE	10,65	14,6	3,95	37,09	13,41	2,76	25,92	13,1	2,45	23,00
ELITE	12,95	15,8	2,85	22,01	14,41	1,46	11,27	13,6	0,65	5,02
RDM	11,45	14,9	3,45	30,13	13,81	2,36	20,61	12,4	0,95	8,30

Regarding the soluble carbohydrates content (Table 2), an increase can be seen as followed: for **control** (calcium chloride) after 12 days of storage was found that for high temperatures (27 °C) the registered increase was of 37,09 % for Generos genotype, furthermore 25,92 % for fruits stored at 17 °C and 23 % for those stored at 4 °C. For Florina genotype the increase in soluble carbohydrates content has the following distribution: 21,91 % for fruits stored at 27°C, 10,84% for fruits stored at 17°C and 7,57% for those stored at 4°C. When applied the **Elite** fertilizer, the following differences were observed: for Generos genotype an increase of 22,01 % in soluble carbohydrates content for fruits held at 27°C, 11,27 % for fruit held at 17 °C and 5,02 % for those stored at 4 °C.

Table 3. Total content of soluble carbohydrates at Florina genotype

EXPERIMENTAL VARIANT	SOLUBLE CARBOHYDRATES CONTENT (%)									
	HARVEST MOM.	12 DAYS								
		27°C			17°C			4°C		
		VAL.	DIF.	%	VAL.	DIF.	%	VAL.	DIF.	%
CALCIUM CHLORIDE	12,55	15,3	2,75	21,91	13,91	1,36	10,84	13,5	0,95	7,57
ELITE	11,05	14,8	3,75	33,94	13,11	2,06	18,64	12,81	1,76	15,93
RDM	9,05	13,1	4,05	44,75	12,95	3,9	43,09	11,4	2,35	25,97

Florina genotype had the following distribution regarding the soluble carbohydrates content and storage conditions: the fruits held at a temperature of 27 °C registered with 33,94 % more carbohydrates than at harvest, those stored at 17 °C had with 18,64 % more and the fruits held at 4 °C registered an increase of 15,93 %. For the experimental variant were **RDM**

fertilizer was applied the results are: Generos genotype registered an 30,13 % increase of carbohydrates when fruits were stored at a temperature of 27°C, 20,61 % for fruits stored at 17 °C and only 8,30 % when the fruits were stored at a temperature of 4 °C.

In case of Florina genotype (Table 3) at 12 days after harvest the carbohydrate percentage was with 44,75 % higher for fruit stored at 27 °C. When kept at a temperature of 17°C the increased percentage was 43,09 % and fruits exposed to 4 °C showed a percentage increase of 25,97 % from time of harvest.

Experimental results regarding the determination of fruit firmness for studied genotypes in BBCH 81 vegetation stage

Table 4 Fruit firmness for Generos genotype

EXPERIMENTAL VARIANT	FRUIT FIRMNESS (lbs)									
	HARVEST MOM.	12 DAYS								
		27°C			17°C			4°C		
		VAL.	DIF.	%	VAL.	DIF.	%	VAL.	DIF.	%
CALCIUM CHLORIDE	12,48	7,81	-4,67	-37,42	10,18	-2,3	-18,43	11,13	-1,35	-10,82
ELITE	13,63	6,4	-7,23	-53,04	10,73	-2,9	-21,28	11,68	-1,95	-14,31
RDM	12,84	6,45	-6,39	-49,77	10,33	-2,51	-19,55	11,15	-1,69	-13,16

Regarding fruit firmness after 12 days of storage the following results were registered: for **control** (calcium chloride) when harvested the fruit from **Generos** variety had a 12,48 lbs firmness, for the experimental variant were **Elite** fertilizer was applied the fruit presented a 13,63 lbs firmness and those treated with **RDM** had 12,84 lbs (Table 4). After being stored for 12 days at different temperatures, their firmness modified as follows: fruit held at a temperature of 27 °C registered a 37,42 % percentage decrease in firmness, for **control** variant, fruit stored at 17 °C the decreased percentage was 18,43 % and those stored at 4 °C have a decrease in firmness of 10,82%. For the experimental variant treated with **Elite** fertilizer, fruit stored at a temperature of 27 °C registered a 53,04 % decrease in firmness, at 17 °C the decrease was of 21,28 % and those stored at 4 °C presented a decrease in firmness of 14,31 %. In the case of fruit were **RDM** fertilizer was applied their firmness decreased with 49,77 % for fruit stored at 27 °C, for those kept at 17 °C the decrease was of 19,55 % and for fruit kept at 4 °C the percentage was of 13,16 %.

Table 5 Fruit firmness for Florina genotype

EXPERIMENTAL VARIANT	FRUIT FIRMNESS									
	HARVEST MOM.	12 DAYS								
		27°C			17°C			4°C		
		VAL.	DIF.	%	VAL.	DIF.	%	VAL.	DIF.	%
CALCIUM CHLORIDE	16,7	6,22	-10,48	-62,75	11,86	-4,84	-28,98	12	-4,7	-28,14
ELITE	16,46	6,34	-10,12	-61,48	12,41	-4,05	-24,61	11,9	-4,56	-27,70
RDM	16,91	7,68	-9,23	-54,58	12,88	-4,03	-23,83	12,38	-4,53	-26,79

For **control** (calcium chloride) at harvest time the fruit from **Florina** genotype presented a 16,7 lbs firmness, for the variant were **Elite** fertilizer was applied the firmness was of 16,46 lbs and for **RDM** the value was of 16,91 lbs. After being stored for 12 days in different temperature conditions (Table 5), fruit firmness suffered modifications like: the fruits stored at 27 °C registered a decrease in firmness of 62,75 % for **control**, those stored at 17 °C a decrease of 28,98 % and the fruit stored at 4 °C a decrease in percentage of 28,14 %. The experimental variant were **Elite** fertilizer was applied had the following influence in regarding the fruit firmness: a decrease of 61,48 % in fruit firmness was established after being stored at 27 °C, 24,61 % for the fruit stored at 17 °C and 27,70 % for those stored at 4°C. For the fruit were **RDM** treatment was applied the firmness decreased with a percentage of 54,58 % after the fruits were stored at 27 °C, with 23,83 % for fruit kept at 17 °C and with 26,79 % after the fruit were stored at 4°C.

Experimental results regarding maturity starch-iodine test (AI) in BBCH 81 vegetation stage.

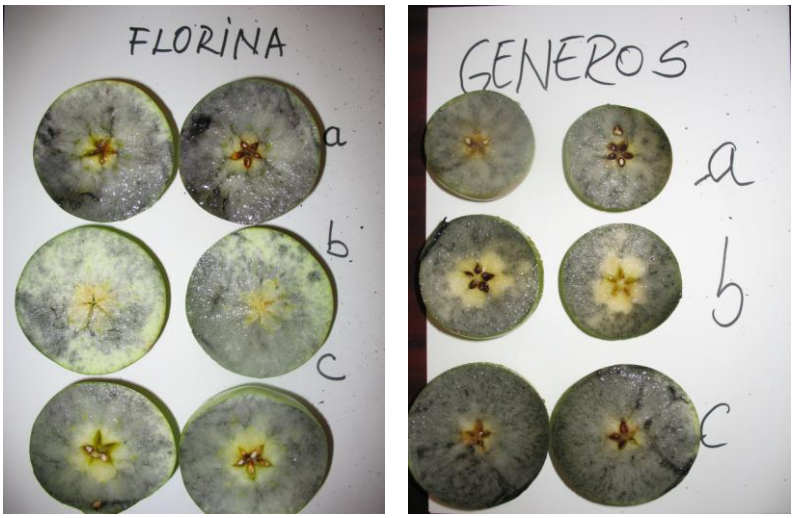

Figure 2. Maturity starch-iodine test (AI) at harvest – BBCH 81 vegetation stage

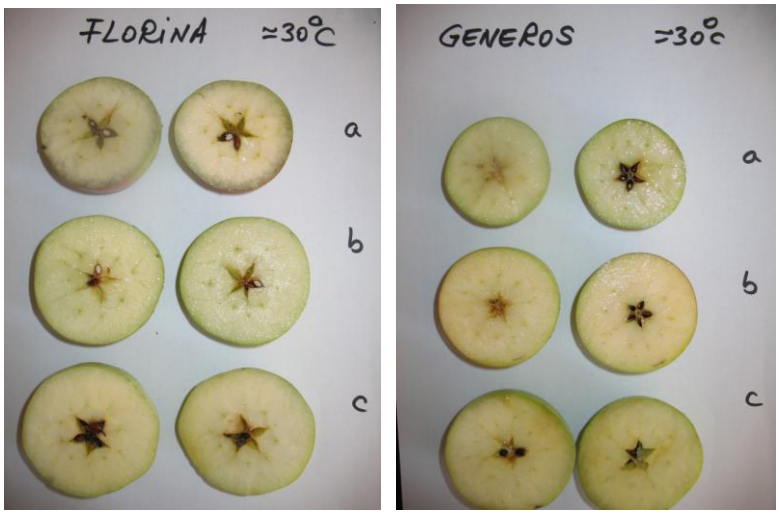

Figure 3. Maturity starch-iodine test (AI) – fruit stored at 27 °C

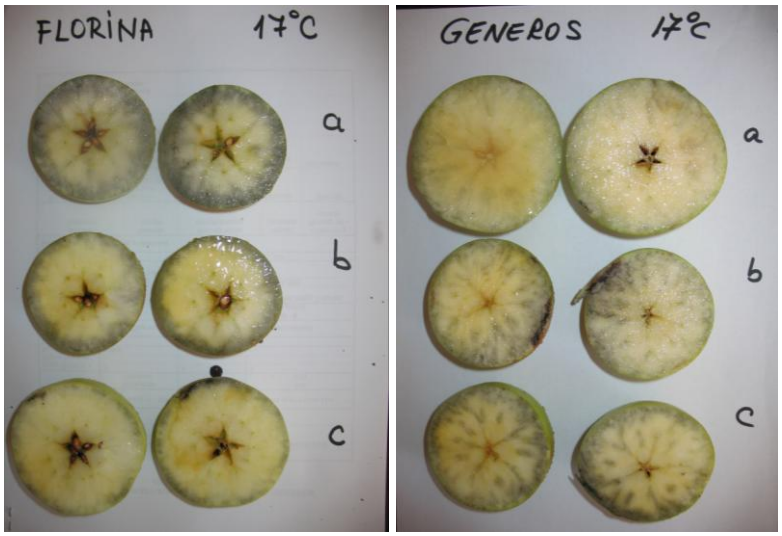

Figure 4. Maturity starch-iodine test (AI) – fruit stored at 17 °C

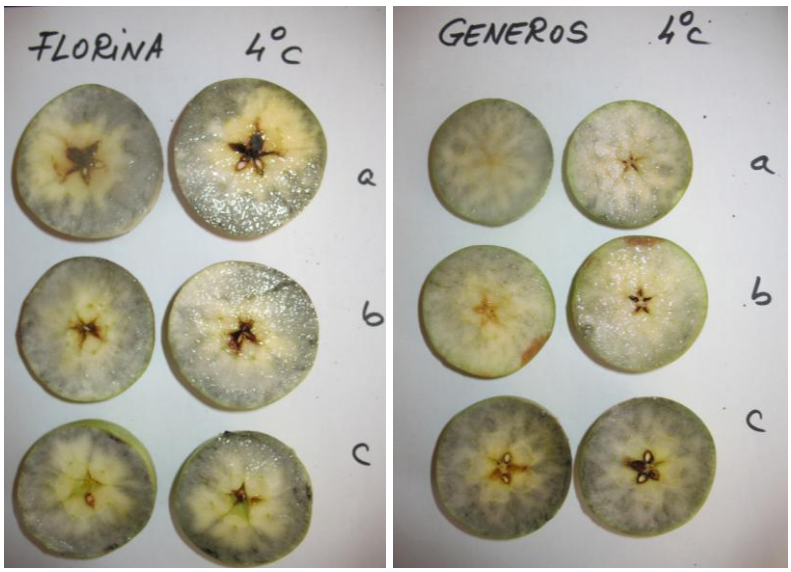

Figure 5. Maturity starch-iodine test (AI) – fruit stored at 4 °C

By comparing the charts of the maturity starch-iodine test results can be seen that at harvesting all the fruit had maximum starch content (Figure 2).

After their exposure to a temperature of 27 °C in the maturity starch-iodine test can be seen that the soluble carbohydrate content increases and there are no visible surfaces that present starch iodide (Figure 3). In regarding the experimental variant were the fruit was stored at 17 °C for 12 days (Figure 4) for **control** (calcium chloride) in Florina cases an increase of starch can be observed. In Generos case the highest increase in starch content (Figure 5) can be observed were RDM was applied for fruit stored at 4 °C.

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Volatile Organic Compounds Emission from *Betula verrucosa* under Drought Stress

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Abstract

In the nature, plants are under a multitude of different stress factors. In response to biotic or abiotic stress plant elicited biogenic volatile organic compounds (BVOC). Plants emission patterns change both quantitatively and qualitatively as well in riposte to damage by biotic or abiotic stress. In the present work we focus our study to the emission of volatile organic compounds from *Betula verrucosa* under drought stress. Solid-phase micro extraction (SPME) technique have been used for trapping of the BVOC followed by GC-MS desorption. The results have been show that drought induced a high emission of lipoxygenase pathway products.

Introduction

During the past hundred years, there has been an increasing concern about the potential future impact of drought on global climate change. It is predicted that the warming effect of climate change will lead to significant changes in the frequency and quantity of precipitation (Saetersdal et al., 1998). Plants have frequently evolved in habitats where drought occurs, and so have developed multiple strategies to cope with drought stress. Tolerance strategies can be divided into resistance mechanisms, which enable plants to survive dehydration (drought stress causes stomata closure and reduced CO_2 diffusion into leaves, limiting photosynthesis), and avoidance mechanisms, which are growth habits that prevent the exposure of plant to osmotic stress (reduced normal growth and crop production in all species) (Chaves et al., 2011). However, plants have a variety of physiological and

biochemical responses to stress at cellular and whole organism levels. From them, we are focused on emission of volatile secondary metabolites.

Birch is a widespread tree in the temperate and boreal zones of Europe. The trees are fast growing tolerant to spring frost, low temperatures in general, and nutrient deficiency, though their ecological amplitude is limited by shade intolerance and short life span.

In the physiological conditions some plants emit BVOCs constitutively (Holopainen et al., 2010; Holopainen and Gershenzon, 2010).

Birch (*Betula* spp.) emits a variety of C_{10} monoterpenes, C_{15} sesquiterpenes, and also aliphatic and aromatic compounds even if they are not affected by stress factors (Pääkkönen et al., 1998; Vuorinen et al., 2007). The composition of the emitted volatile depends on abiotic or biotic stress factors. (Hakola et al., 1998; Pääkkönen et al., 1998; Schade and Goldstein, 2003; Schurgers et al., 2009; Vuorinen et al., 2005). In stressful conditions, birch could emit different quantities of BVOCs depending on the stress factors. For examples, applied heat stress at different day and night periods determined emission of isoprene, changing in sesquiterpenes and monoterpenes pattern. As well have been shown a high emission of green leaves volatiles (Z)-3-Hexenyl acetate (Ibrahim et al., 2010), (Laothawornkitkul et al., 2009). Some BVOCs, like β-ocimene and α-farnesene, are emitted in a higher quantity when birch is exposed to a pathogen (*Marssonina betulae*) (Vuorinen et al., 2007). Higher quantity of methylsalicilate, (Z)-ocimene, (E)-β-ocimene, (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and linalool have been also detected after 72 h feeding by *Epirrita autumnata* larvae (Vuorinen et al., 2007).

In this work, we studied the emission of volatile organic compounds from *Betula verrucosa* under a long period of drought stress and after recovery.

Materials and methods

One year old seedlings of *Betula verrucosa*, provided by a local nursery (a generous gift from Arad Forestry Department) have been used. Plants have been cultivated in 3 liters pots filled with commercial soil (AGRO CS, Lucenic, Slovakia), fertilized with a universal fertilizer (Compo Gbh, Munster, Germany) and watered daily.

To study the response to drought stress three plants have been chose as control and three plants for drought experiment. The pots have been covered with plastic foils to exclude the influence of water evaporation from soil. Response to drought was followed for 11 days (up to relative soil water content was below 10 %) and then the seedlings was re-watered.

The water status of each pot was checked each day by measuring weight of the seedlings pots. Every day, to each control plant has been watered until full pot capacity.

The volatile organic compounds measurements have been performed using a custom-built system (Figure 1-for picture). The airflow in the measurement chamber was 1 L and all tubing in the system was made of Teflon. Light was provided by 2 fluorescent lamps at a level of light intensity at 300 μmol m^{-2} s^{-1}.

Figure 1. Custom-built system for measuring volatile organic compounds

Solid Phase Micro Extraction (SPME) was performed to analyze plant volatiles. The fiber used for the absorption of the volatile components was polydimethylsiloxane/divinylbenzene (PDMS/DVB), thickness 65 μm Supelco Company (Bellefonte, PA, USA). The fiber was conditioned before use for 30 minutes, as recommended by the manufacturer.

Volatile organic compounds were captured by placing the coated fiber extraction phase in the measurement chamber, with the plant, for 10 minutes followed by desorption in gas chromatograph injector. All measurements of plant volatiles have been done at room

temperature of 25 °C. Background air samples were collected from the empty chamber before the measurements and were subtracted from the emission samples of the plants.

BVOC samples were analyzed using a gas-chromatograph Agilent Technology 7820A (Agilent Scientific, USA) coupled with mass spectrometer MSD 5975, using a method described previously (Copolovici et al., 2009). The compounds were identified based on NIST library and on the retention time of standard compounds and the concentration of alpha-pinene, sabinene, 3-(Z)-1-hexen-1-ol and 3-(Z)-1-hexen-1-ol acetate were calculated based on with external authentic standards consisting of known amount of those compounds.

Results and discussion

For identification of different mono and sesquiterpenes emitted by the plants, a solid phase microextraction followed by gas-chromatography mass spectrometer (GC-MS) analyses has been employed.

A mixture of different compounds (monoterpenes, LOX products and sesquiterpene) has been used to calibrate the GC-MS device. We used a method which has been already standardized for determinations of different terpenes and LOX by Copolovici et al. (2009). A typical chromatogram is presented in Figure 2.

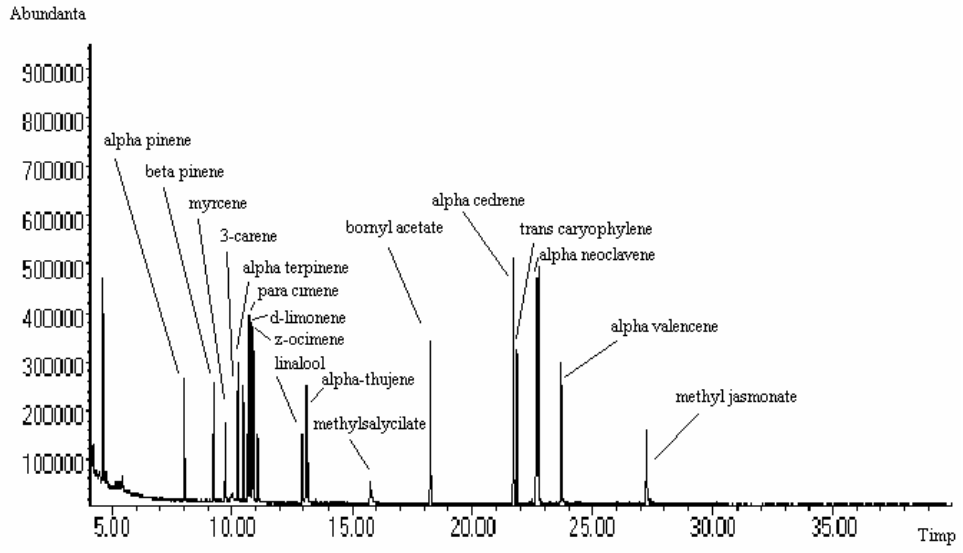

Figure 2. Typical chromatogram for standard mixture

The method provides a good separation of different compounds which could be found in stress plants emission. Table 1 presents analytical parameters of calibration curves for 17 compounds including detection and determination limits, correlation coefficients and range of

linearity. As well for all compounds Kovacs indexes have been calculated and they are in a good accordance to those presented in the literature.

Table 1 Analytical parameters obtained by the GC-MS method for all the tested BVOC standards

Substance	Corr. coefficient	Det. limit (nmol/L)	Deter. Limit (nmol/L)	Target ion (m/z)	Qualifiers (m/z)	Kovacs Indices
Alpha-pinene	0.999	28.91	43.35	93	92, 91	937
Betha-pinene	0.989	39.62	98.89	93	41, 69	979
myrcene	0.991	13.72	37.14	41	93, 69	992
3-carene	0.992	25.99	55.75	93	91, 77	1012
alpha terpinene	0.993	3.51	4.66	93	121, 91	1019
para-cimene	0.993	1.53	2.63	119	134, 91	1027
D-limonene	0.988	0.66	1.18	68	67, 93	1032
Z-ocimene	0.994	4.66	10.45	93	92, 91	1040
linalool	0.996	12.18	32.72	71	43, 81	1000
Alpha-tujene	0.995	2.90	7.39	81	110, 41	1008
methylsalycilate	0.998	1.37	3.14	120	92, 152	1097
bornyl acetate	0.999	1.02	1.63	95	43, 93	1089
Alpha-cedrene	0.999	1.55	3.23	119	93, 105	1020
trans-caryophyllene	0.999	1.00	2.41	41	69, 93	1026
alpha-neoclovene	0.999	1.11	2.50	189	161, 204	1059
Alpha-valencene	0.999	0.59	0.75	191	204, 105	1099
methyl jasmonate	1.000	1.72	4.08	83	41, 151	947

The method has been used to follow the response of *Betula Verrucosa* plants exposed to drought stress. We measured the emission of volatile secondary metabolites for plants exposed to drought for 11 days followed by two days of recovery.

Only two green leaves volatiles and two monoterpens have been increased emission due to drought stress: α – pinene, sabinene, 3-(*Z*)-hexene-1-ol, and 3-(*Z*)-hexene-1-ol-acetate. (Figure 3). The other compounds remained to a very low level at the detection limit of the device.

Both green leaves volatiles (GLV) emissions followed the same trend, increasing after one day and reached a maximum level after 4-5 day of drought (Figure 3). The GLV emission decreased drastically after the plants have been re-watered. The same trend have been found by Simpraga et al. (2011). GLV function as a fast and efficient airborne signaling way to pass stress information onto neighboring trees (Niinemets, 2010).

Lipoxygenase (LOX) pathway products are induced in a variety of plant species during different stress conditions in a process where free octadecanoid fatty acids (linoleic acid = 18:2 and linolenic acid = 18:3) are released from plant membranes by phospholipases. LOX activity produces 9- or 13-hydroperoxylinoleic or -linolenic acid or a mixture of both.

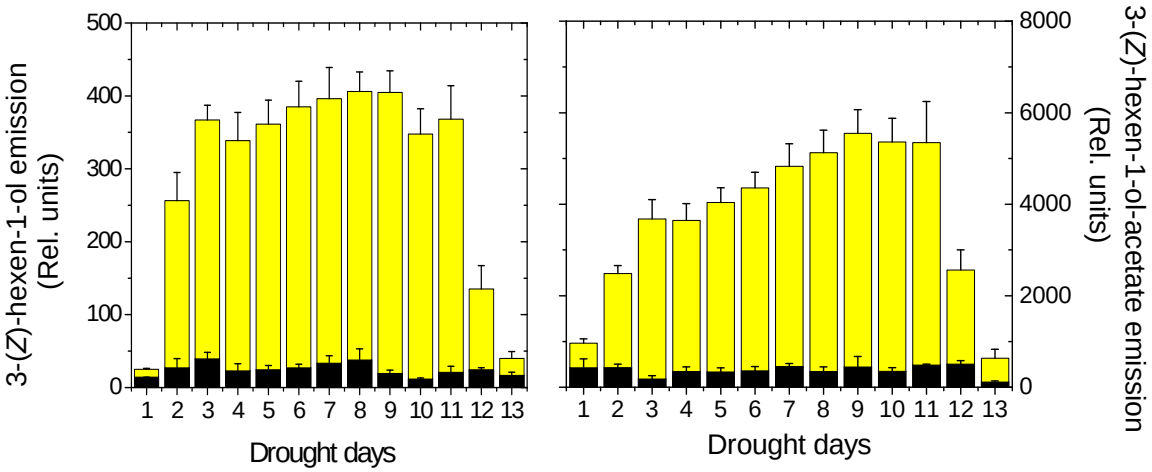

Figure 3. The emission of green leaves volatiles from *B. verrucosa*

A hydroperoxide lyase then catalyzes the breakdown of 13-hydroperoxylinole(n)ic acid to a C6-compound, *(Z)*-3-hexenal, and a C12-product (12-oxo-(*Z*)-9-dodecenoic acid). (*Z*)-3-hexenal can give rise to (*Z*)-3-hexenol, (*E*)-2-hexenol, (*E*)-3-hexenol or (*E*)-2-hexenal in consequent reactions (Copolovici et al., 2012; Liavonchanka and Feussner, 2006; Porta and Rocha-Sosa, 2002).

The emissions of α – pinene and sabinene increased after the first two days of drought stress treatment (Figure 4).

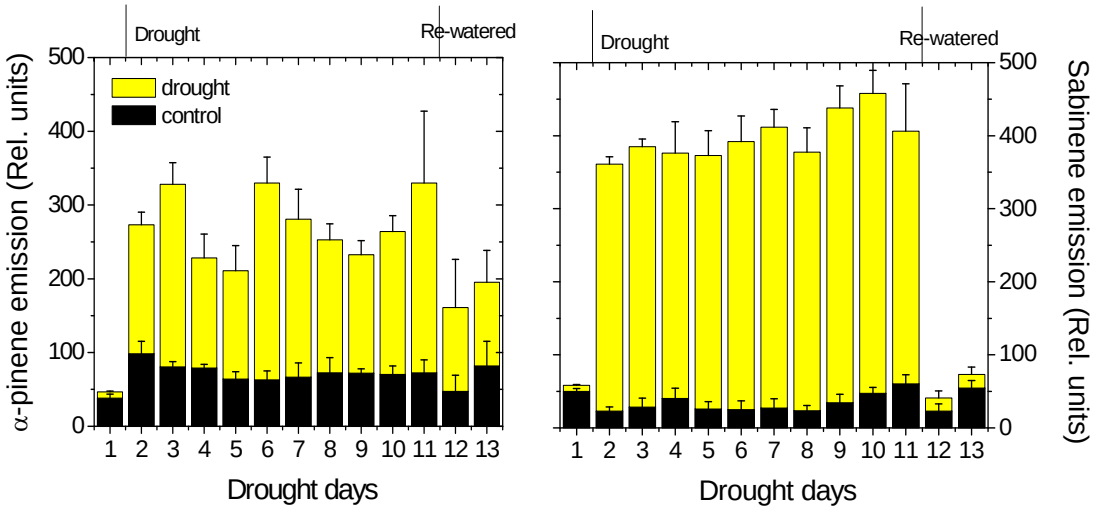

Figure 4. The emission of monoterpenes from *B. verrucosa*

The emission of α – pinene and sabinene were increasing after two days of drought followed by a decreasing in emissions. This type of trend has been found in other studies and could be due to the usage of non-specific storage pool(s) (Demarcke et al., 2010). Other hypothesis can be that monoterpenes could be stored in specialized organs in leaves and/or stems (Penuelas and Llusia, 2003).

Conclusions

We found that only some of terpenes and green leaves volatiles emission have been increased due to drought stress: α - pinene, sabinene, 3 - (Z)-hexen-1-ol and 3 - (Z)-hexen-1-ol-acetate.

Acknowledgements

This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCS-UEFISCDI, roject number PN-II-RU-TE-2011-3-0022. We also thank to Arad Forestry Department for the gift of *Betula Verucossa* seedlings.

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The relationship between the civil juristic document and the contract

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Abstract

In this paper ,we are debating the problem of the relationship between the civil juristic document and the contract .The civil juristic document was defined as ... „a manifestation of will with the intention of producing juristic effects, meaning the birth, change and cancel of an concrete civil juristic relation" or … "the manifestation of will occurred for the realization of juristic effects materialized in the creation, the change, the transfer or the canceling of an concrete civil juristic relation" (Art.1166 C.civ.). The contract was defined [1]in the doctrine as the agreement of a will between two or more persons for the purpose of producing juristic effects, in other words giving birth, changing, transferring or canceling juristic relations. The contract is the main form of the juristic act, and the new civil code made the classification from more points of view (art.1171-1177) in synallagmatic and unilateral contracts, contracts with onerous title and contracts with costless title, contracts with immediate or successive fulfillment, named or no-named contracts, consensual, solemn and real contracts, etc.

Keywords: the synallagmatic and unilateral contracts, contracts with onerous title and contracts with costless title, contracts with immediate or successive fulfillment ,named or no-named contracts, consensual, solemn and real contracts.

The contracts can be classified from more points of view. Each one of the classifications made by the doctrine has a theoretical and practical interest. Most frequently, the classification of a contract included in a certain category obligates the contract to produce certain juristic consequences that are different from the ones that are created by the contracts that belong to other categories.

The new civil code regulates deliberately some of them, for example: sale and purchase, location, society, mandate, bailment, transaction etc. As we will see some

[1] To see art.1166 from the New civil code

classifications of the contracts are enumerated in the texts of code (art. 1171-1177), other classifications are the result of the juristic doctrine activity.

I. The civil juristic document was defined as ... „a manifestation of will with the intention of producing juristic effects, meaning the birth, change and cancel of an concrete civil juristic relation" or … "the manifestation of will occurred for the realization of juristic effects materialized in the creation, the change, the transfer or the canceling of an concrete civil juristic relation" (Art.1166 C.civ.)

The contract was defined in the doctrine as the agreement of a will between two or more persons for the purpose of producing juristic effects, in other words giving birth, changing, transferring or canceling juristic relations.

The comprehension domain. As it may be observed from the analysis of the two juristic categories – the juristic act and the contract – results:

a) the civil juristic act is a manifestation of will occurred in the purpose of producing juristic effects, while the contract is an agreement (agreement of will) occurred to produce juristic effects;

b) the manifestation of will in the case of the civil juristic act is non circumstantiated under the aspect of the number of the civil right subjects, which means that, in this case, the will can be unilateral (manifested by only one person) or we can find ourselves in the presence of an agreement of will (a will that is manifested by two or more persons that, together, create the agreement of their will);

c) in the case of the contracts we discuss only the agreement of a will that occurs between two or more persons in the purpose of ;

d) according these elements, results that civil juristic act has a bigger comprehensive domain than the contract, because between the civil juristic act and the contract exists a gender (a whole) – species (party) relation, the whole namely the gender is the civil juristic act and the species, the party is the contract.

The main form of the civil juristic act is the contract. This results from the following specifications:

a) as a domain of comprehension, the contract almost covers the comprehension domain of the civil juristic act;

b) the bilateral juristic acts (the contracts) have a much bigger frequency in the daily juristic life than the unilateral civil juristic acts; people may realize or not what they do, but they are concluding every day an impressive number of contracts. For example, every day

people buy the necessary things nourishment and we have, through extrapolation to all of his needs, the approximate image of the impressive number of the contracts that he concludes;

c) among the civil juristic acts, the contract represents the juristic category of civil right without which the juristic operations between the civil right subjects cannot be conceived;[2]

d) a large number of legal texts are assigned for the contract, this is why there is a much wider domain of juristic rules in comparison with the other civil juristic acts.

It can be observed that the juristic regulations concerning the contract, trough extrapolation, are applied in some cases to the civil juristic act. Even from the way of defining the civil juristic act by the specialized literature – the definition, on one side, of his various species, and on the other side, the general definition of this act – results that in the outlining of the civil juristic act, we start from the consecrated juristic regulations of his most important species – the convention (the contract) – and other species as the offer, the testament etc.

II. The classification of the contracts

The importance of the classification

The contracts can be classified from more points of view. Each one of the classifications made by the doctrine has a theoretical and practical interest. Most frequently, the classification of a contract included in a certain category obligates the contract to produce certain juristic consequences that are different from the ones that are created by the contracts that belong to other categories.

The new civil code regulates deliberately some of them, for example: sale and purchase, location, society, mandate, bailment, transaction etc. As we will see some classifications of the contracts are enumerated in the texts of code (art. 1171-1177), other classifications are the result of the juristic doctrine activity.

II.I. Synallagmatic contracts and unilateral contracts

Depending of their content, the contracts are classified in synallagmatic contracts and unilateral contracts.

The synallagmatic contracts. The synallagmatic contracts are the contracts that give birth to some mutual obligations between the parties. Every party **of** the contract assumes obligations and of course, and receives rights as well.

[2] See LIVIU POP " *Tratat of the civil law",*Ed.Universul Juridic,Bucureşti 2011,pag.122.

The specific of the synallagmatic contract is the mutual and interdependent character of the party's obligations. This means that every party has at the same time the quality of creditor and the quality of debtor. In comparison with the other party: the obligation that costs one of the parties has a juristic cause in the mutual obligation of the other party; they cannot exist one without the other, they are interdependent.[3] The idea of cause, that explains the interdependency of the obligations in the synallagmatic contracts, must be understood in a bivalent way, as a manifestation of the idea of the purpose when the contract is concluded and during the existence and the execution of the contract.

The most relevant example of the synallagmatic contracts is the sell – purchase contract. The seller takes the responsibility to transfer the right of propriety of the soled object and to hand it over, and the buyer has the obligation to pay the price. This example demonstrates that in the synallagmatic contracts:

- parties assume obligations, but they also earn rights;
- the obligations of the parties are mutual, meaning that all the parties from this type of contract have obligations;

The obligations of the parties are in connection, meaning that to one obligation of one party it corresponds a certain obligation of another party; for example, to the obligation of the seller to transfer the right of propriety of the soled good corresponds the obligation of the buyer to pay the price or to the obligation of the seller to hand over the soled good corresponds the obligation of the buyer to receive the soled good.

The unilateral contracts.

The unilateral contracts are the contracts that born obligations only for one of the parties, the other party is the holder of some correlative rights. The new Civil Code in the article 1171 stipulates: "The contract is unilateral when one or more persons have obligations towards one or more persons, without the last one to have obligations". One party is only debtor and one party is only creditor. Thus in a donation contract the giver is only a debtor and the acceptor is only a creditor.

[3] To see VALERIU STOICA ,"*The civil law and the civil contracts",*Ed. Editas,Bucureşti 2011,pag.206;

II.II. Contracts with onerous title and contracts with costless title

This is the classification of the contracts that considers the purpose of the parties in the moment of their conclusion.

Contracts with onerous title. These contracts are those ones in which each of the parties intends an advantage, a conscription in exchange, meaning the creation of an own patrimonial interest. The head quarter for this is the article 1172, new Civil Code, that stipulates „The onerous contract is the contract in which every party wants to receive an advantage". This includes the location contract, the change contract, life annuity contract etc. There are two types of onerous contracts: commutative and aleatory.

The onerous title contract is commutative when the obligation of one party is the equivalent of the obligation of the other party. It is characterized through the fact that the mutual labor conscriptions to which the parties obligate themselves are equivalent, and the dimension of the owed labor conscriptions by the parties is sure and their value is known from the moment of the contract's conclusion. We can observe that the contracts with onerous title have a commutative character[4].

The aleatory contract. According to the regulations of article 1173, paragraph 2 of the new Civil Code, a contract is aleatory when the equivalent depends, for one of the parties, by an uncertain event. Thus the advantages that will be obtained are unknown because the parties have obligations, one towards the other, that depend on a future and uncertain event concerning the production or at least the moment of the production of it. The event represents for every party, at the same time a chance to win and a risk to lose. An example of this type of contract is the insurance contract, life annuity contract, the maintenance contract etc.

II.III. The charity contracts or with onerous title

The new Civil Code, article 1172, paragraph 2, stipulates: "The costless contract or charity contract is the contract that in which one of the parties wants to obtain, without an equivalent, an advantage for the other". Thus, one of the parties has the obligation to obtain for the other a patrimonial advantage without receiving something in exchange. Are parts of this category: donation, bailment, mandate, guarantee etc.

These contracts are divided in: liberality and costless service contracts or disinterested contracts.

[4] See GABRIEL BOROI,"*The new civil code"*Ed.Hamangiu,Bucureşti 2010,pag.238;

a) The liberalities are those onerous contracts through which one of the parties transfers a right from his patrimony in the patrimony of the other party without receiving an equivalent. One of the parts becomes poor and the other rich. The object of this type of contract is the labor conscription of giving. Through liberalities we mean the donation contracts. We want to mention that enter, in the wide category of liberties, some unilateral juristic acts, example the legatee.

b) Contracts of costless services or disinterested contracts are those contracts through which one party has the obligation to do a service without becoming poorer and neither in the purpose of the enrichment of the other party: costless mandate, the costless bailment, the costless loan.

At the base of the difference between these contracts with onerous title and those with costless title are two criteria: an objective and a subjective. The objective criteria consist in the existence of mutual advantages for both parties in the onerous contracts and the lack of any advantage for one of the parties in the costless contracts. The subjective criteria consist in the cause or the purpose of concluding the contract. In the onerous contracts, the parties give their consent for concluding the contract having the intention of obtaining an equivalent in exchange of the labor conscription that is obligated. On the contrary, in the contract with costless title, the party that has the obligation is doing it in the purpose or with the intention of obtaining an advantage for someone else, without claiming, under juristic aspect, nothing in exchange. Therefore, the subjective element consists in the intention of liberality.

II.IV. Contracts with immediate or successive fulfillment

These types of contracts are those classified according their execution.

Contracts with immediate execution (instantaneous) are those contracts whose execution is made immediately after their conclusion; normally the object of the obligation is labor conscription. Contracts with successive execution are those contracts whose execution takes place in time, as permanent labor conscription, for example the renting contract or as some successive labor conscriptions, for example the providing contracts. The importance of this classification consists in the followings:

- for the non execution from guilt of the contracts with immediate execution operates the resolution and therefore, is abolished with retroactive effect, while the

non execution by guilt of the contracts with successive execution operates the annulment and are abolished only for the future[5];

- some contracts with successive execution can be annulled through the will of any party, for example the location contract without a term, or only through the will of one party, for example the renting contract or the bailment contract;
- normally, the suspension of the obligations is put only in the contracts with successive execution;
- this classification presents an importance even in the domain of supporting contractual risks, according to the rules that govern this domain.

II.V. Named contracts and no-named contracts

Named contracts are the contracts that have a special regulation, that correspond to some economical operations. Ex: the selling contract, the location contract, the change contract etc. This category of contracts was known even in the Roman law, it was called *nova negotio* (new juristic acts) containing:

- *do ut des* = I give you so you can give me;
- *do ut facis* = I give you so you make me;
- *facio ut des* = I make you so you can give me;
- *facio ut facias* = I make you so you can make me[6].

The importance of this classification consists in the fact that parties do not have to stipulate all the relation's implications in which they enter, because these are regulated by the law, only if they violate the regulations – suppletive of course – of the law, while, in the case of the no-named contracts, the parties have to stipulate the clauses the refer to all the implications of such relations.

II.VI. Consensual contracts, solemn contracts and real contracts

This classification has at it's base the criteria of their way of valid creation (art.1174, new code civil).

[5] To see CONSTANTIN STĂTESCU,CORNELIU BÎRSAN, "*The civil contracts",* Ed.Hamangiu,Bucureşti 2010,pag.178;

[6] See LIVIU POP " *Tratat of the civil law",*Ed.Universul Juridic,Bucureşti,2011,pag. 336.

A. Consensual contracts. Are those contracts that are concluded through the simple agreement of the parties (solo consensu), without no other formality. In our law, the consent is a principle that has some exceptions to, deliberately stipulated by the law for some contracts. The existence of these exceptions is determined by the necessity of protecting the interests of the parties and of third parties and other times, for the defense of a public interest. Therefore, regarding the parties, the obligation of respecting some formal conditions is disposed by the law to win their attention on the importance of their decisions and offers them time to think; otherwise, the respect for a certain form, that consists, normally, in the writing of a document, means precision and clearness in the establishment of the contract's effects and of the parties responsibility, offering to the third parties, that want to contract one of them, the possibility to know for sure the existent juristic relation. Other times, the written form of the contract is to serve the public interest consisting in the necessity of knowing, by some authorities of the State, all the changes that have occurred in the juristic situation of some goods of great importance for the society, as the real estates.

B. The solemn contracts. The solemn contracts are those contracts for which for conclusion to be valid is needed that the will agreement of the parties to wear a certain form or to be enclosed by certain solemnities foreseen by the law. The simple will agreement is not sufficient to have the value of a contract. Not respecting the form or the formalities foreseen by the law is penalized with the absolute nullity of the contract. The solemn contracts are: the donation (art. 1011/1033 Civ. c), the mortgage contract (art. 1772 Civ. c), conventional subrogation agreed by the debtor (art. 1107 p. 2 Civ. c), the selling-buying of the lands (art. 46 from the Law nr. I8.1991). All these contracts must be concluded under the form of authentic document. Exception is the donation of mobile goods, when the solemnity that must be respected consists in the material handing of the good or the goods from the giver to the receiver.

C. The real contracts. Are those contracts for which forming, besides the will agreement of the parties, the material handing of the thing that is the object of one of the party's carrying-out is also needed. The juridical doctrine includes in this category: the loan of consumption, storage, the mortgage contract and the transportation contract. All these contracts are considered concluded only from the moment of the handing of the good at which it refers. Considering those shown above, within the literature of specialty has been said that in reality we wouldn't find ourselves in front of the presence of different categories of civil contracts. This because the handing of the good, object of the contract, from a party to the other, would be a necessary solemnity for the conclusion of the contract. There,for, the solemn contracts

may be grouped in: authentic contracts and real contracts. For one, the solemnity consists in an authentic document, and for the other, in the material handing of the good, object of the contractual carrying-out.

Conclusions

Most frequently, the classification of a contract included in a certain category obligates the contract to produce certain juristic consequences that are different from the ones that are created by the contracts that belong to other categories.

The new civil code regulates deliberately some of them, for example: sale and purchase, location, society, mandate, bailment, transaction etc. As we will see some classifications of the contracts are enumerated in the texts of code (art. 1171-1177), other classifications are the result of the juristic doctrine activity. Depending of their content, the contracts are classified in synallagmatic contracts and unilateral contracts.

The synallagmatic contracts. The synallagmatic contracts are the contracts that give birth to some mutual obligations between the parties. Every party of the contract assumes obligations and of course, and receives rights as well.

The specific of the synallagmatic contract is the mutual and interdependent character of the party's obligations. The unilateral contracts are the contracts that born obligations only for one of the parties, the other party is the holder of some correlative rights. The new Civil Code in the article 1171 stipulates: "The contract is unilateral when one or more persons have obligations towards one or more persons, without the last one to have obligations". One party is only debtor and one party is only creditor. Thus in a donation contract the giver is only a debtor and the acceptor is only a creditor.

Contracts with onerous title. These contracts are those ones in which each of the parties intends an advantage, a conscription in exchange, meaning the creation of an own patrimonial interest. The head quarter for this is the article 1172, new Civil Code, that stipulates „The onerous contract is the contract in which every party wants to receive an advantage". This includes the location contract, the change contract, life annuity contract etc. There are two types of onerous contracts: commutative and aleatory.

The onerous title contract is commutative when the obligation of one party is the equivalent of the obligation of the other party. It is characterized through the fact that the mutual labor conscriptions to which the parties obligate themselves are equivalent, and the dimension of the owed labor conscriptions by the parties is sure and their value is known from

the moment of the contract's conclusion. We can observe that the contracts with onerous title have a commutative character.

The aleatory contract. According to the regulations of article 1173, paragraph 2 of the new Civil Code, a contract is aleatory when the equivalent depends, for one of the parties, by an uncertain event. Thus the advantages that will be obtained are unknown because the parties have obligations, one towards the other, that depend on a future and uncertain event concerning the production or at least the moment of the production of it.

The new Civil Code, article 1172, paragraph 2, stipulates: "The costless contract or charity contract is the contract that in which one of the parties wants to obtain, without an equivalent, an advantage for the other". Thus, one of the parties has the obligation to obtain for the other a patrimonial advantage without receiving something in exchange. Are parts of this category: donation, bailment, mandate, guarantee etc.

These contracts are divided in: liberality and costless service contracts or disinterested contracts.

Contracts with immediate execution (instantaneous) are those contracts whose execution is made immediately after their conclusion; normally the object of the obligation is labor conscription. Contracts with successive execution are those contracts whose execution takes place in time, as permanent labor conscription, for example the renting contract or as some successive labor conscriptions, for example the providing contracts. Named contracts are the contracts that have a special regulation, that correspond to some economical operations. Ex: the selling contract, the location contract, the change contract etc.

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Advantages of PTR-MS and PTR-TOF-MS techniques for measuring volatile organic compounds (VOCs)

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The Proton Transfer Reaction Mass Spectrometer (PTR-MS) was developed by the University of Innsbruck (Austria) in the mid 1990s (Hansel et al., 1995; Lindinger et al., 1998). The device uses the chemical ionization to protonize volatile compounds with a proton affinity higher than H_2O (691 kJ/mol) (Blake et al., 2009). The major components of ambient air have a lower proton affinity than H_2O (e.g. N_2, O_2, CO_2, etc), thus the primary ion H_3O^+ only reacts with traces gases like volatile organic compounds. Therefore, PTR-MS technique can detect a large list of compounds such as aldehydes (e.g. acetaldehyde), ketones (e.g. acetone), aromatic compounds (e.g. benzene), alcohols (e.g. methanol and ethanol), sulphur compounds (e.g. dimethyl sulfide), unsaturated compounds (e.g. isoprene and α pinene), nitriles (e.g. acetonitrile), esters, acids and compounds with multiple functional groups (e.g. methyl salicylate). PTR-MS is a very reliable instrument for real-time trace gas monitoring. Since its release to the market, the instrument has improved its sensitivity and limit of detection achieving the pptv (parts per trillion by volume) level.

PTR-MS technique uses a method for soft ionization of molecules in the gas phase: pure water vapor enters in the ion source hollow cathode, where it is protonized to H_3O^+. Then, the protons are transported to a drift tube where they meet a react with the air sample. The ionization of trace gases is very effective because of the high purity (~ 99 %) and much larger concentration of H_3O^+ compared to trace gases. In addition, the molecule fragmentation is minor compared to other ionization methods like electron impact ionization. This creates mass spectra trustable and easily interpretable.

PTR-MS device performs the separation of protonized compounds by mass by a quadrupole mass filter. This technique is limited by its low mass resolving power and different compounds within one nominal mass could not be discriminated. For example, at nominal mass 69, protonated furan (m/z = 69.03349) and protonated isoprene (m/z = 69.06988) would appear as a single signal.

This problem was further solved after the development of time-of-flight (TOF) mass detection technique. The first PTR-TOF-MS prototype was presented in 2007, and commercially released in 2008. In contrast to quadrupole PTR-MS instruments, the time-of-flight version produces continuous mass spectra. This is because TOF detection is based on the time (a continuous variable) that molecules take in "flying" a known distance helped by repulsive voltages. Thanks to this advanced mass detection system; molecules of different molecular mass hit the detector at different times. Then, it is possible to discriminate different peaks within multipeaks (isobaric compounds) and take in account the decimals of the molecular masses to identify the compounds.

Unfortunately, isomeric compounds (molecules with the same molecular formula but different chemical structures) cannot be distinguished. These compounds are of special interest to plant biology, perfume industry and air quality and other complementary techniques like VOC trapping and further gas chromatography analysis must be performed to detect the range of isomeric compounds. The same problem occurs with geometric isomerism.

In PTR-MS technology, the primary ion can also be switched to NO^+ or O_2^+ to investigate the range of mass spectrum near to the H_3O^+ signal, because when H_3O^+ is the primary ion, the signal is saturated at the neighboring masses to m/z = 19 (H_3O^+) and m/z = 37 (water cluster $H_5O_2^+$).

In conclusion, the quadrupole and TOF technologies developed in these last years have widen the possibilities of gas trace analysis; in particular for animal and plant biology, medicine, food chemistry, atmospheric chemistry, etc. For example, the on-line data acquisition permits to monitories animal and plant responses through volatile compound emissions (e.g. herbivory, light, exposition to compounds, stresses or metabolic reactions). Also, the high time resolution (smaller to 100 ms in PTR-TOF-MS) of analysis allows using these instruments for eddy-covariance emission and deposition flux measurements setups (where 10 Hz data is needed).

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Natural protected areas. Definition, classification and some examples

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Under the legislation of Romania and management objectives assigned, regarding natural protected areas, natural habitats, wild flora and fauna the following categories are accepted:

- Scientific reserve;
- Natural reserve
- National park;
- Natural park;
- Natural monument;
- Biosphere reserve;
- Wetland of international importance (eg. Ramsar site);
- Natural World Heritage Site.

The total surface of protected areas in Romania, included in Law no. 5/2000 regarding national territory planning for national protected areas is 1.234.710 hectares (5.18 % of the country land). Biosphere reserves, national and natural parks represent an area of 1.132.176 hectares. Together with 134 natural reserves and natural monuments cumulate an total area of 129. 643 ha.

The natural protected area is described as „A clearly defined geographical space, recognized, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values" (UNEP-WCMC, 2008).

A particular and important category from the protected areas is the category of national and natural parks which covers a major part from the total protected areas.

These parks can be defined as follows:

National Parks are protected areas with the aim of the protection and conservation of representative samples from biogeographical national area. A national park includes natural elements with special value like: physical-geographical, flora, fauna, hydrological, geological,

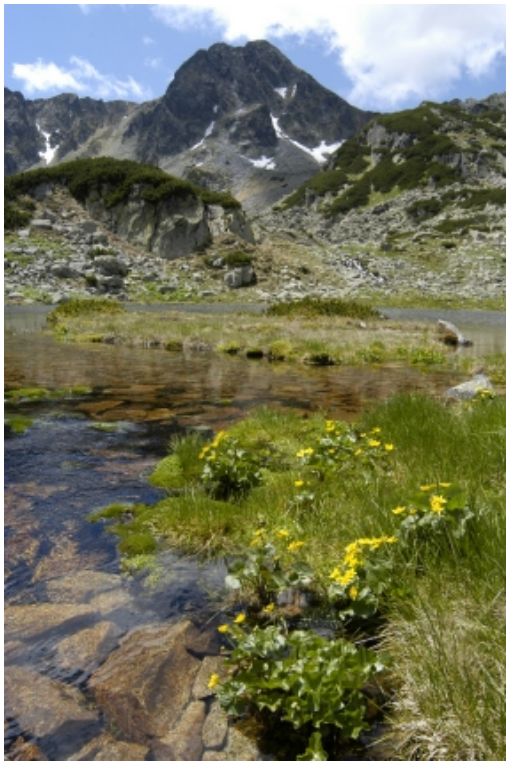
paleontological, speleological, pedological, etc. It can be visited for scientific, educational, recreational and tourist purpose. In Romania there are 13 national parks covering an area of approx. 316.000 ha. The oldest national park established in 1935 is Retezat National Park.

Natural parks are protected areas whose goals are the protection and preservation of the landscape where the interaction of human activities and nature over time has created a distinct area with significant landscape and/or culture value, often with high biological diversity.

There are a total number of 15 natural parks in Romania with an area of 562 000 hectares. Cefa Natural Park from Bihor County is the latest natural park established which was founded in 2010.

Retezat National Park

It is the first National Park founded in 1935 on the initiative of Professor Alexandru Borza with the aim of preservation of floral and faunal heritage and specific landscape of high mountains. Retezat National Park is located in the Retezat Mountains in Hunedoara County, Romania. It has an area of 38.138 hectares with altitude between 800 and 2509 m and contains more than 40 peaks over 2200 meters.



Bucura peak, Retezat National Park (© Tudor Predescu)

In the middle of Retezat Mountains is a special protected area called “Gemenele (Twins) Scientific Reserve”. This is the first strictly protected area with scientific character where all type of activities like mining, grazing, hunting, fishing, fruit collection, hiking or camping are entirely forbidden. In this area a number of rare and endemic species of vegetation like virgin and natural beech, mixed beech-fir-spruce and spruce forests, alpine and subalpine shrubs and meadows are protected.

In National Retezat Park there are 1200 species of plants and a high mountain rare fauna like wolves, brown bear, wild boar, Eurasian lynx, European wildcat, chamois, roe deer and red deer. The park also is populated by small carnivore species such as Eurasian badger and Eurasian otter, birds as golden eagle and grouse, diffrent species of fish (trout) and some rare invertebrates.

Rhododendron in Retezat National Park (© Tudor Predescu)

In the park, the research activity is conducted in one laboratory located in “Gemenele Scientific Reserve” near to the “Black lake” and in “Pietrele” and “Rotunda” cottages.

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The Dezna Valley

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"Dezna Valley" river is the right affluent of the White Cris River. It springs from Apuseni foothills and after crossing two depressions goes into the White Cris River below Sebis town. The Dezna valley has a length of about eight km and a width of four km being cultivated with corn and grains. Upstream of Sebis town we meet a lot of villages like: Donceni, Buhani, Dezna, Laz, Zugău, Rănuşa and Moneasa, villages located in the valley, each with its own attractions.

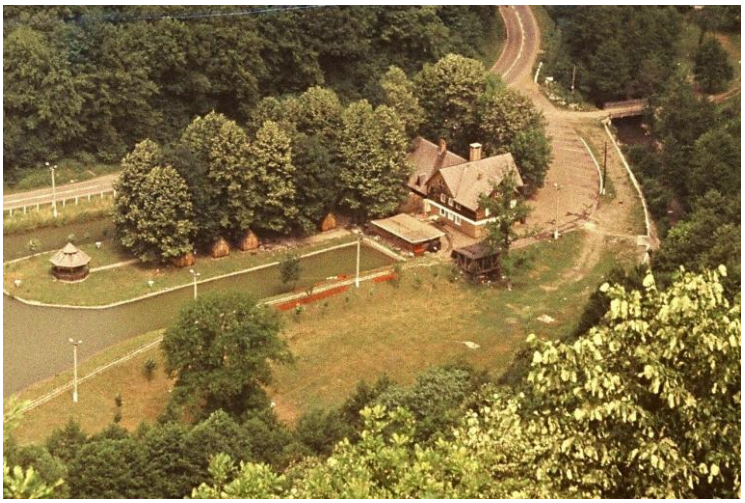
The most important historical monument from Dezna is the fortress located up on Ozoiu hill with an altitude of 390 meters. According to a legend in 1817, Ladislau Nagy de Peretseny says that the name of the fortress derives from the name of the Dacian king Decebal. The construction was built in XIIIth century and dominates the region and the access road to the core of "Codru-Moma" Mountains. The article of Mrs. Academician Cornelia Bodea, published in "Hotarul" revue, describes the turmoil that has undergone Dezna fortress.

Dezna Fortress

Another notable monument is Orthodox Church, situated in the center of the village. Historian Marki says that the church was built on the foundations of a Roman building early

in feudal era. The church has a remarkable Byzantine painting dating from the XVIIth – XVIIIth – century.

At the exit of Dezna village to Moneasa direction, on the right side we find the fishery with public house fish, built in 1971. Here is a nice place where you can eat fresh fish and more other dishes.



The Fishery restaurant from Dezna

In Dezna, also works a famous sanatorium for neuromotor recovery, founded and administrated by doctor Barsan. His fame has exceeded the borders of the country due to the outstanding results obtained.

Dezna village has a camp for students that hosts in every summer hundreds of children from all over the country. The camp and boarding school are installed in a castle built in neoclassical style in the first half of the nineteenth century. From Dezna, the valley splits into Zugău Valley and Moneasa Valley. The first is less populated, while the second valley is recognized, especially for Moneasa resort.

Landscapes become more and more attractive and unique after crossing Rănuşa village which covers a distance of 3 km. The Rănuşa-Moneasa Depression is relatively small but cultivated with corn and wheat by locals.

Moneasa village lies over a distance of 6-7 km along the valley. It consists of Moneasa village and Moneasa resort, and now also with a new district, where are many recently built houses for tourism purpose. The resort offers some attractions like: trout fishery, a camp school, and ruins from an old metalworking place. The same type of ruins can be seen at Raştirata village due to the fact that the whole valley was an important metalworking place in the past.

Therapeutic baths from Moneasa were mentioned by Magyar Matias Sandor in 1880. Since than it was known that are recommended in neurosis, gynecological, and other health problems. In the valley that goes to a cave are ruins of "Pavilion No.1" where until 1989 were made different balneary recovery procedures. In 1970 the resort has grown considerably and held modern treatment facilities like “Moneasa” treatment base. The resort was visited by approximately 1500 people per each series of treatment.

Pavilion no.1 Moneasa

Moneasa Hotel

Zugău Valley, wild and picturesque hides many natural beautiful places and animals such as: roe deer, red deer, lynx, brown bear, wild boar, grouse, etc. Here also springs Zugău River.

The spring of Zugău river

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Institute of Technical and Natural Sciences Research-Development-Innovation of "Aurel Vlaicu" University present at the fair "ECOMEDIU"

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Between 16 to 18 October 2013, Institute of Technical and Natural Sciences Research-Development-Innovation of "Aurel Vlaicu" University attended the XIth "ECOMEDIU" fair with the specific field of environmental protection such as: technologies and equipment for drinking water preparing, technologies and utilities for waste water processing, urban, industrial and special waste management, projecting and execution of ecological landfills for urban and rural wastes, utilities for investigation, analyzing and quality control of water, air, ground, urban hygiene equipment and many other green technologies and products.

49 exhibitors from different countries (Romania, Hungary, France, Croatia, etc.), gathered in an attractive exhibition with stands where were exposed products and technologies dedicated to a cleaner environment.

The representatives of Institute of Technical and Natural Sciences Research-Development-Innovation of "Aurel Vlaicu" University took part with a stand where were exposed and promoted different products obtained by our research team. They also visited and held interesting discussions with participants from different companies and public institutions and visitors regarding clean technologies, energy efficiency, which can lead to technological and economic progress, but also to a cleaner and healthy environment for all of us.

During these three days of exhibition took place an International Conference whose agenda included 45 presentations divided into three days with different topics:

Day 1. Protection of water, air and soil - 16.10.2013;

Day 2. Recovery, recycling and/or waste sorting - 17.10.2013;

Day 3. Biodiversity - 18.10.2013.

The rich program of this conference, supported by local and international experts, assisted by translators, brought to the attention of the participants the latest legal studies and examples of best practice in the above mentioned areas.

Among the presentations of experts from Romania, Italy, Germany, Ukraine was communicated the presentation entitled: “Identification and role in atmosphere of volatile compounds emitted by plants under stress” by Dr. Lucian Copolovici – Deputy Director of Institute of Technical and Natural Sciences Research-Development-Innovation of “Aurel Vlaicu” University.

Emission of volatile organic compounds as a signal of plant stress

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Plant volatiles

During growth and development plants produce around 100 000 chemical products out of which 1700 are known to be volatile (Dicke and Loreto, 2010). Plant volatile organic compounds (VOCs) are released by various plant organs such as flowers (Colquhoun et al., 2013), fruits (Laothawornkitkul et al., 2009), roots (Köllner et al., 2004; Crespo et al., 2012) and leaves (Owen et al. 2001). Among all the VOC-s, isoprene (Laothawornkitkul et al., 2008; Sharkey et al., 2008; Darbah et al., 2010; Li and Sharkey, 2013), mono- and sesquiterpenes (Chen and Pawliszyn, 2003; Martin et al., 2003; Tholl, 2006; Chen et al., 2011; Rajabi Memari et al., 2013), fatty acid cleavage products known also as green leaf volatiles (GLV-s)(Hatanaka, 1993) and aromatic volatiles (e.g., methyl benzoate and methyl salicylate) (Dudareva et al., 2000; Cardoza et al., 2002; Dudareva et al., 2004; Zhao et al., 2010; Holopainen et al., 2013) are the most studied ones. The rate of plant volatile release can depend on environmental drivers including temperature (Guenther et al., 1993; Bertin et al., 1997; Staudt and Bertin, 1998; Niinemets et al., 2010b; Hu et al., 2013) and light (Guenther et al., 1993; Staudt et al., 1997; Niinemets et al., 2010b; Kesselmeier and Staudt, 1999; Hu et al., 2013; Monson, 2013) and atmospheric CO_2 concentration (Vourinen et al., 2004; Räisänen et al., 2008; Velikova et al., 2009; Rasulov et al., 2009; Monson, 2013; Sun et al., 2012) and endogenous drivers including circadian rhythms (Wilkinson et al., 2006; Loivamäki et al., 2007), leaf age (Mayrhofer et al., 2005; Guenther et al., 2006; Sun et al., 2009; Niinemets et al., 2010a; Sun et al., 2012) and plant age (Shiojiri and Karban, 2006).

Plant stress factors and defenses

During the growth, plants have to cope with various abiotic and biotic stress factors (Mittler, 2006; Loreto and Schnitzler, 2010; Niinemets, 2010a; Niinemets, 2010b; Copolovici et al., 2011). Among these, herbivores (Fatouros et al., 2012), plant viruses (Eigenbrode et al., 2002) and pathogens (Huang et al., 2012) are the key biotic stressors, while temperature

(Velikova and Loreto, 2005; Possell and Loreto, 2013; Sun et al., 2013), drought and flooding (Rennenberg et al. 2006; Kreuzwieser and Rennenberg, 2013), light (Loreto et al. 2006), ozone (Beauchamp and Wisthaler, 2005; Pinto et al., 2010; Calfapietra et al., 2013) and nutrient availability (Lopéz-Bucio et al., 2003) are the main abiotic stress factors. In addition, the severity of stress can be importantly modified by elevated atmospheric CO_2 concentrations (Vourinen et al., 2004; Räisänen et al., 2008; Calfapietra et al., 2013; Sun et al., 2013).

Multiple stress factors can affect plants´ resistance simultaneously or consecutively. In canopy top high leaf temperature and radiance can cause leaf necrosis, which in turn endangers the survival of low-growing trees (Valladares and Pearcy, 1995). Meanwhile in areas of low soil nutrient availability development of root system of young seedlings is blocked and seedlings experience drought stress (Oliet *et al*., 2013). Additionally soil nitrogen content affects frost injury of plants, because nitrogen contributes the biosynthesis of anti-freeze proteins in apoplast and prevents ice crystal formation (Lambers et al. 2008), otherwise breakage of cell membranes triggers immediate release of GLVs (Copolovici *et al*., 2012).

Plants have developed physical and chemical defense systems to protect themselves against stressors. Among physical defense systems, spines, thorns and hardened leaves play a major role in several plants species (Milewski *et al.*, 1991; Cooper and Ginnet, 1998; Hanley *et al.*, 2007), while enhanced investment in waxes and secondary plant metabolites is a common direct defense response across plants (Halitschke *et al.*, 2000; Arimura *et al.*, 2005; Leitner *et al.*, 2005; Howe and Schaller, 2008; Kessler and Heil, 2011). In contrast, stress-induced volatile compounds that can be attractants of predators and parasitoids belong to indirect defense, when the attacked plant is „calling for help“ against herbivores (Dicke, 1994; Dicke *et al.* 2009; Dicke and Baldwin, 2010; Holopainen and Gershenzon, 2010; Fatouros *et al.* 2012). There are numerous recent studies demonstrating that indirect chemical defense systems do increase the fitness of attacked plants (for reviews see Dicke and Baldwin, 2010; Trowbridge and Stoy, 2013), and thereby constitute an important rapidly induced defense system.

Induced stress volatiles

Volatiles are biosynthesized mainly via four biochemical pathways: the lipoxygenase pathway for green leaf volatiles (GLV-s) (Hatanaka, 1993), shikimic acid pathway for aromatic volatiles (Paré and Tumlinson, 1996), methylerythritol pathway (MEP) for isoprene and monoterpenoids (Pichersky *et al.*, 2006; Rajabi Memari *et al.*, 2013) and mevalonic acid

pathway (MVA) for volatile sesquiterpenoids (Taveira *et al.*, 2009; Rajabi Memari *et al.*, 2013; Rosenkranz and Schnitzler, 2013) (Figure 1).

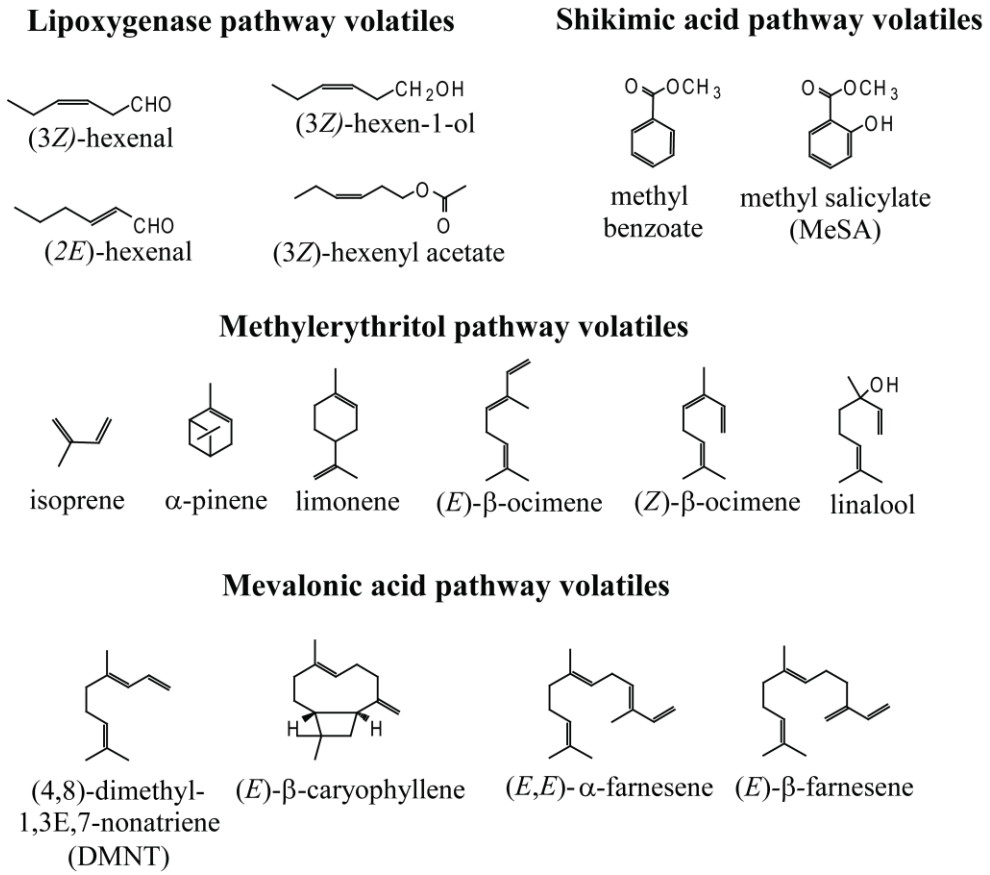

Figure 1. Molecular structures of plant volatiles in the emission of a stressed plant. Fatty acid cleavage products known also as green leaf volatiles (GLV-s) are biosynthesized via lipoxygenase pathway (Hatanaka, 1993), aromatic volatiles such as methyl salicylate (MeSA) are volatile products of shikimic acid pathway (Dudareva et al., 2000), isoprene and monoterpenoids are produced via methylerythritol (MEP) pathway (Pichersky et al., 2006), homoterpene (4,8)-dimethyl-1,3E,7-nonatriene (DMNT) and sesquiterpenes are produced via mevalonic acid (MVA) pathway (Taveira et al., 2009).

Typical stress emissions consist of green leaf volatiles such as (*E*)-2-hexenal, (*Z*)-3-hexenol, (*Z*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate, volatile mono- and sesquiterpenoids such as linalool, ocimene isomers, farnesene isomers, (*E*)-*β*-caryophyllene, methyl jasmonate, methyl salicylate (MeSA), and methanol (Geervliet et al., 1997; Lerdau and Gray, 2003; Holopainen, 2004; Baldwin et al., 2006; Maffei, 2010; Raghava et al., 2010; Copolovici et al., 2011; Spinelli et al., 2011; Copolovici et al., 2012; Pinto-Zevallos et al., 2013). Any stress factor can potentially change the rate of volatile release and alter the bouquet of VOCs and thereby affect the relationships between living organisms (Dicke and Baldwin, 2010; Holopainen and Gershenzon, 2010; Niinemets et al., 2013). For example, leaf damage increases the emission of sesquiterpenes and increases alkaloid content of nectar, and hence,

can alter pollinator preference (Adler et al., 2006; Theis et al., 2009). In addition, (Z)-3-hexenol, which is a signaling volatile of herbivore attack or mechanical wounding, induces the stress reaction in neighboring intact plants (Wei and Kang, 2011). On the other, the bouquet of volatiles can importantly depend on stressed plant species (Llusià et al., 2010a; Llusià et al., 2010b; Holopainen et al., 2013; Llusià et al., 2013) or a stressor (Känanste et al., 2009, Takabayashi et al., 1991).

Role of volatile organic compounds in plant-plant-, plant-insect- or plant-insect-environment relationships

The question of why plants emit VOC-s has been posed over and over again, and the role of many volatiles in abiotic stress tolerance, including thermotolerance of photosynthesis and reduced oxidative stress, has been highlighted (Sharkey and Singsaas, 1995; Singsaas et al., 1997; Loreto et al., 1998; Loreto and Velikova, 2001; Velikova et al., 2004; Copolovici et al., 2005; Llusià et al., 2005; Velikova et al., 2005; Vickers et al., 2009; Possell and Loreto, 2013; Sun et al., 2013). Moreover in the 21th century global climate is predicted to change drastically (IPPC, 2007). For example today we know that water availability affects the content and emission of secondary metabolites in plants and different plant species respond to water deficit differently (Kainulainen et al., 1992; Turtola et al., 2003; Peñuelas et al., 2009; Lusebrink et al., 2011; Kännaste et al., 2013). Yet in future in relation to climate change the existence and prolongation of drought may increase the attack of pines of low vitality by the mountain pine beetle *Dendroctonus ponderosae* MPB (Lusebrink et al., 2011).

VOC-s are essential in plant-plant and plant-insect (Baldwin et al., 2002; Duhl et al., 2008; Dicke and Baldwin, 2010; Fatouros et al., 2012; Holopainen et al., 2013; Trowbridge and Stoy, 2013). In plant-herbivore interactions, the volatiles can act as attractants or repellents to herbivores (Laothawornkitkul et al., 2008; Loivamäki et al., 2008). For instance, the monoterpene α-pinene released by wounded Scots pine (*Pinus sylvestris* L.) acts as attractant to large pine weevil (*Hylobius abietis*), and thus, previous damage of a conifer can increase herbivory damage. Yet attraction of *H. abietis* can be reduced by repelling limonene (Nordlander, 1991). Due to the increasing emissions of allylisothiocyanate heat stressed *Brassica nigra* plants may become attractive to spezialized feeders of Brassicaceae (Figure 2) (Mithen, 2001).

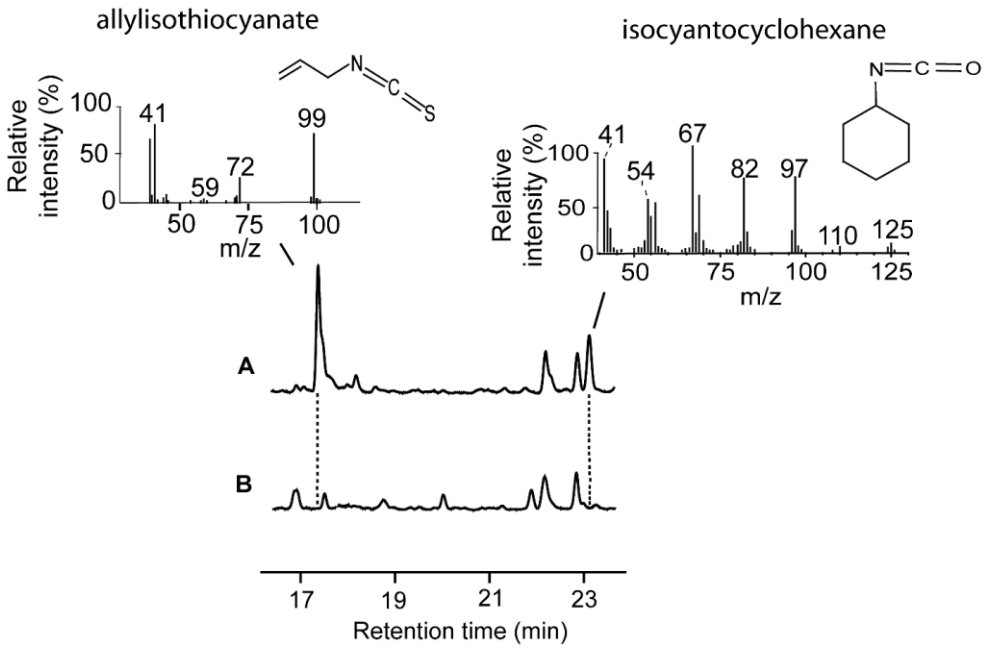

Figure 2. Volatile glucosinolate biodegradation products of *Brassica nigra* released at leaf temperature of 25 ^{o}C (A) and 44 ^{o}C (B).

For example volatiles emitted from *Tetranychus urticae*-infested lima bean (*Phaseolus lunatus* L.) can activate genes encoding pathogenesis-related proteins and phenylalanine ammonia-lyase in leaves of non-infested neighboring plants', as well GLV-s can serve as signal compounds in plant-plant communication (Arimura et al., 2001; Bate, Rothstein, 1998). This kind of info "sharing" depends on the diffusion and convection of the volatile info between the sender and the receiver plant (Baldwin et al., 2002).

Conclusions

Overall, this information summarized here emphasizes the rich spectrum of stress-triggered volatile emissions and underscores the importance of volatiles in stress responses, stress tolerance and plant interactions with other plants and organisms. While a lot of basic information on plant volatile emissions has accumulated during the last years, we still lack quantitative understanding of how the emission rate scales with the severity of stresses, how far the stress-elicited volatiles travel in the atmosphere and what are the relationships between the strength of the emitted signal and receiver plants' and other receiver organisms' responses. There is encouraging evidence that the strength of the emission signal can be quantitatively related to the severity of both abiotic and biotic stresses (Niinemets et al., 2013) and we argue that future work should be devoted towards filling these important gaps in knowledge.

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