The emission of volatile organic compounds from *Quercus robur* plants affected by *Phylloxera quercus* and temperature

[1]Andreea Pag, [1]Daniel Tomescu, [1]Adina Bodescu, [2]Astrid Kännaste, [2]Ülo Niinemets, [1,3]Lucian Copolovici

[1] Institute of Technical and Natural Sciences Research-Development of Aurel Vlaicu University, Arad, Romania; [2] Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu 51014, Estonia; [3] Faculty of Food Engineering, Tourism and Environmental Protection, Aurel Vlaicu University, Arad, Romania.
Corresponding author: L. Copolovici, lucian.copolovici@emu.ee

Abstract. In this study, green leaves volatiles (GLV) and isoprene emissions were studied in *Quercus robur* leaves subjected to biotic stress (different infection degree of *Phylloxera quercus*) combined with abiotic stress (temperature). Results obtained have been shown that GLV emission rates from *Q. robur* infected leaves are increasing along with the number of leaves infected spots compared to control. A cumulative effect of abiotic and biotic stress has been noticed.
Key Words: green leaf volatiles, isoprene emission, quantitative responses, volatile organic compounds, *Phylloxera quercus*, pedunculate oak.

Introduction. Terrestrial vegetation is a key player in the carbon and water cycles being a key player for Earth's climate.

Multiple stress factors can affect plants resistance simultaneously or consecutively. Abiotic stress is defined as any factor exerted by the environment on the optimal functioning of an organism. An abiotic stress can be considered heat, cold, freezing, drought salinity, flooding or oxidizing agents, light, ozone, nutrient availability. Beside those, biotic stress can be caused by plant viruses, pathogens and/or herbivores.

Since plants cannot flee from the stressful environment, they have to develop strategies to adapt or defend themselves. Two main strategies are adopted by plants in order to defend themselves: physical (spines and thorns) (Hanley et al 2007) and chemical (waxes and secondary plant metabolites) (Arimura et al 2005; Kessler & Heil 2010). One strategy which is used in response to biotic or/and abiotic stress by plants is to elicit a variety of volatile organic compounds (VOC) into the atmosphere (Bertin & Staudt 1996; Copolovici & Niinemets 2010). VOCs represent a large carbon loss which can be up to similar to 10% of that fixed by photosynthesis under stressful conditions some tropical ecosystems (Penuelas & Llusia 2003).

VOC play important roles in atmospheric chemistry as they are involved in photochemical ozone formation. Indirectly VOCs also affect Earths' climate as they are precursors of secondary organic aerosols (Hallquist et al 2009). For predicting future climate change, the disturbance of photosynthetic capacity or transpiration cause by stressful environment are of high importance (Hallquist et al 2009). Accurate prediction of VOC emissions is highly relevant for reliable simulation of a number of atmospheric properties, including chemical reactivity and clearness (Arneth & Niinemets 2010; Claeys et al 2004; Peñuelas & Staudt 2010; Spracklen et al 2008). VOC are involved in photochemical ozone formation (Bates et al 2000; Kleindienst et al 2007) and high concentrations of atmospheric ozone can potentially cause reduction in the photosynthetic process, growth and biomass accumulation (Moretti et al 2010). High concentrations of VOC emissions could contribute to the complex processes associated with global warming (Penuelas & Llusia 2003).

Volatiles are biosynthesized mainly via four biochemical pathways: the lipoxygenase pathway for green leaf volatiles (GLV) (Hexenal, Hexenyl acetate) (Hatanaka 1993; Matsui 2006), shikimic acid pathway for aromatic volatiles (methyl salicylate) (Paré & Tumlinson 1999), methylerythritol pathway (MEP) for isoprene and monoterpene (Limonene, Linalool) (Lichtenthaler et al 1997) and mevalonic acid pathway (MVA) for volatile sesquiterpene (caryophyllene, farnesene) (Arimura et al 2009).

Phylloxera quercus is found in southern Europe, North Africa and the Middle East. In Europe, it is confirmed from Italy, France and Spain (Blackman & Easop 2008).

P. quercus are thiny insects, 0.2-0.5 mm, that feed on the vim of the pedunculate oak (*Quercus robur*) leaves (Lubiarz 2007). *P. quercus* is related to aphids (Herbert et al 2008; Lubiarz 2007). Many *Phylloxera* species can cause plant galls (Lubiarz 2007; Oliveira et al 2011). Oak phylloxera doesn't cause galls, but causes, small necrotic yellow to brown feeding injury spots and leaf distortions (Jackson 2014). More susceptible to the infection are young trees which are weakened by their feeding activity and subsequent leaf loss, in combination with other environmental stress factors, the disease can lead to tree decline (Hallquist et al 2009; Lubiarz 2007). In Iraq, *P. quercus* is reported to kill young oaks during years of severe infestation. It is appreciated that phylloxera can be a threat to the growth of the wine industry all over the world (Herbert et al 2008; Jackson 2014).

P. quercus infection reduces the rate of growth. However, quantitative relationships between these physiological modifications and the degree of infection have not been studied. As the shape of stress severity vs. plant response can differ for various stresses (Beauchamp et al 2005; Niinemets 2010; Niinemets et al 2010a, b), predicting stress responses requires understanding of quantitative relationships between stress severity and plant physiological response (Niinemets et al 2010).

Pedunculate oak constitutively emits isoprenoid compounds, monoterpenes and sesquiterpenes (Lehning et al 2001; Pearse et al 2013).

In the present work we study the emission of green leaves volatiles and isoprene from *Q. robur* under biotic stress (*P. quercus*) combined with abiotic stress (temperature).

Material and Method. Oak leaves were infested gradually with *P. quercus*. In order to estimate the degree of *P. quercus* infection, both sides of the leaf were photographed. Different numbers of leaf infected spots were obtained from 10 to 40 per 8 cm^2.

In order to assess the cumulative effect of both biotic and abiotic stress, already infested leaves were subjected to a temperature of 35° C for 90 minutes.

In order to investigate the relationship between both type of stress factors applied and the emission of volatile organic compounds, the number injury spots were counted and correlated with GLV emission. Green leaves volatiles (GLV) and isoprene emission rates from *Q. robur* leaves infected with various degree of *P. quercus* were measured using a custom-made gas-exchange system described by (Copolovici & Niinemets 2010). Isoprene and GLV emission rates have been measured using a GC-MS method described previously by (Copolovici et al 2009).

Results and Discussion. Infection with *P. quercus* led to a reduction of isoprene emission from 20.1 ± 0.5 nmol m^{-2} s^{-1} (average ± SE) in healthy leaves of *Q. robur* to 2.5 ± 0.5 nmol m^{-2} s^{-1} in heavily infected leaves (40 spots), and a strong negative correlation between isoprene emission rate and percentage of leaf infection was observed (Figure 1).

The same trend has been shown by Anderson et al (2000) in oak wilt *Ceratocystis fagacearum* infected with *Quercus fusiformis* and by Niinemets et al (2013) in *Q. robur* infected by *Erysiphe alphitoides*. This reduction could be associated with the general effect on dimethylallyl diphosphate (DMADP), the pool size of the substrate for isoprene synthesis (Possell & Hewitt 2011; Rasulov et al 2009).

Emission of a variety of compounds was induced by infection, including lipoxygenase pathway products as (Z)-3-hexenol, (E)-2-hexenal, (Z)-3-hexenyl acetate and 1-hexanol) also called green leaves volatiles (GLV). In healthy leaves the GLV emission rate have been under the detection limit while in infected leaves the GLV emission rates increasing along with the number of leaves infected spots. It has been shown a positive correlation between GLV emission rate and the number of leaves infected spots (Figure 2.). Usually fungal emissions are dominated by alcohols, aldehydes benzenoids, and ketones (Penuelas et al 2014). As a result of an attack by pathogens, specific elicitor molecules are generated by chemical or physical damage to plant cell walls and cellular membranes which can be followed by the release of free fatty acids

from cell membranes, and their peroxidation by lipoxygenase enzymes (Liavonchanka & Feussner 2006).

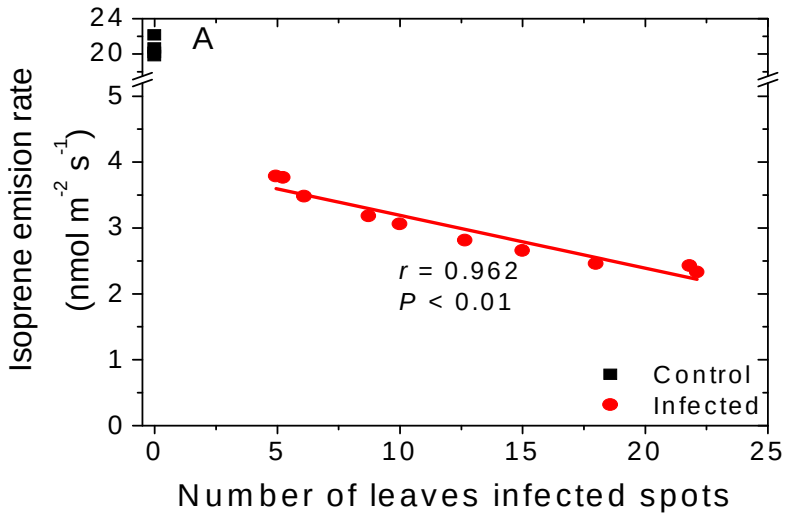

Figure 1. Correlations of isoprene emission rates with the number of infected spots of *Phylloxera quercus* in *Quercus robur* leaves.

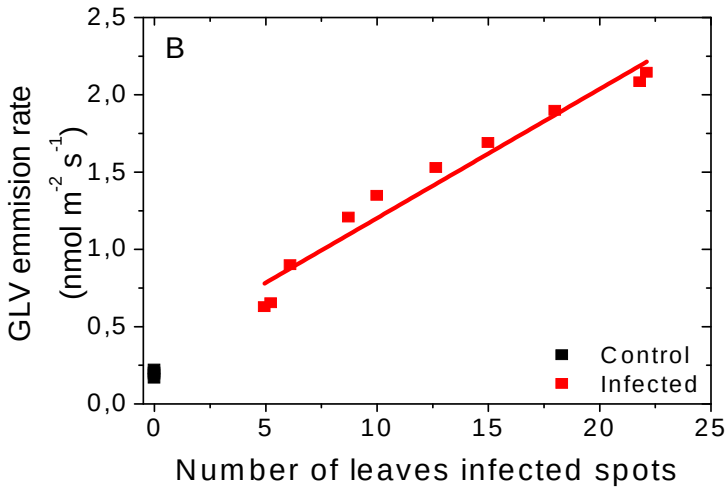

Figure 2. Correlation between lipoxygenase pathway volatile (various C_6 alcohols, aldehydes and derivatives, also called green leaf volatiles (GLV)) from *Q. robur* leaves with the number of infected (*Phylloxera quercus*) spots on the leaves.

In order to investigate both biotic (*P. quercum*) and abiotic (temperature) stress, infected leaves were subjected at 35°C for 90 minutes. Data obtained have been shown a cumulative effect of both effects (Figure 3).

The data obtained have been shown a cumulative effect of abiotic stress (temperature) and biotic stress (Figure 3). A priming effect of temperature has been found on green leaves volatiles emissions which are in accordance with our previous study, where we have shown a quantitative relationship between stress dose (number of larvae feeding) vs. GLV emissions (Copolovici et al 2011).

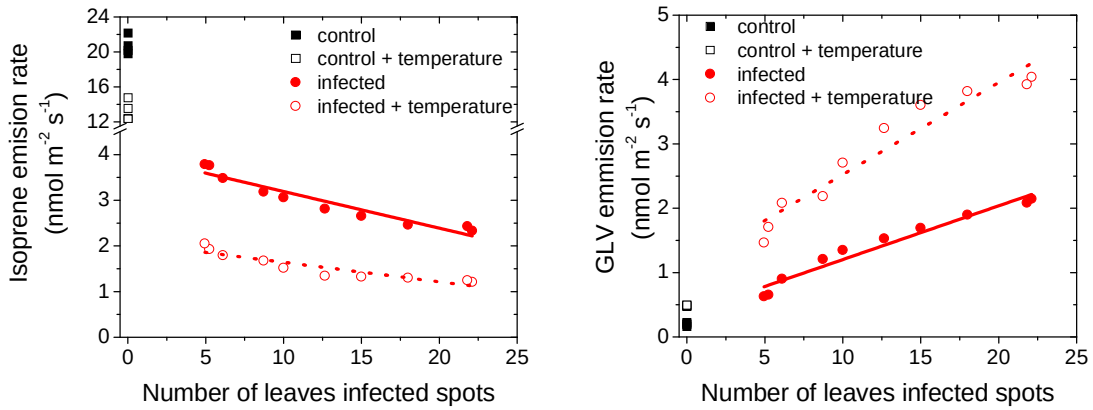

Figure 3. Isoprene emission rate (A) and GLV emission rate (B) from *Q. robur* leaves under temperature stress depending on the number of leaves infected spots

Conclusions. The results indicate a significant increase in the concentration of lipoxygenase compounds (GLV) along with increasing number leaf infected spots, while isoprene concentration decreases linearly.

It has been demonstrated that GLV emissions are quantitatively related to the stress dose.

The results have been show that the infection with *P. quercus* combined with high temperature induced a high emission of GLV and a decrease in emission of isoprene.

Acknowledgements. Funding for this study has been provided by the Romanian National Authority for Scientific Research, CNCS – UEFISCDI (project number PN-II-RU-TE-2011-3-0022).

References

Anderson L. J., Harley P. C., Monson R. K., Jackson R. B., 2000 Reduction of isoprene emissions from live oak (*Quercus fusiformis*) with oak wilt. Tree Physiol, pp. 1199-1203.

Arimura G., Kost C., Boland W., 2005 Herbivore-induced, indirect plant defences. Biochim Et Biophysica Acta-Molecular and Cell Biology of Lipids 1734:91-111.

Arimura G., Matsui K., Takabayashi J., 2009 Chemical and molecular ecology of herbivore-induced plant volatiles: proximate factors and their ultimate functions. Plant and Cell Physiology 50:911-923.

Arneth A., Niinemets U., 2010 Induced BVOCs: how to bug our models? Trends in Plant Science 15:118-125.

Bates M. S., Gonzalez-Flesca N., Sokhi R., Cocheo V., 2000 Atmospheric volatile organic compound monitoring. Ozone induced artefact formation. Environmental Monitoring and Assessment 65:89-97.

Beauchamp J., Wisthaler A., Hansel A., Kleist E., Miebach M., Niinemets U., Schurr U., Wildt J., 2005 Ozone induced emissions of biogenic VOC from tobacco: relationships between ozone uptake and emission of LOX products. Plant Cell and Environment 28:1334-1343.

Bertin N., Staudt M., 1996 Effect of water stress on monoterpene emissions from young potted holm oak (*Quercus ilex* L.) trees. Oecologia 107:456-462.

Blackman R. L., Easop V. F., 2008 Book review. Journal of Natural History 42:2169-2171.

Claeys M., Graham B., Vas G., Wang W., Vermeylen R., Pashynska V., Cafmeyer J., Guyon P., Andreae M. O., Artaxo P., Maenhaut W., 2004 Formation of secondary organic aerosols through photooxidation of isoprene. Science 303:1173-1176.

Copolovici L., Niinemets U., 2010 Flooding induced emissions of volatile signalling compounds in three tree species with differing waterlogging tolerance. Plant Cell and Environment 33:1582-1594.

Copolovici L., Kännaste A., Niinemets U., 2009 Gas chromatography-mass spectrometry method fordetermination of monoterpene and sesquiterpene emissions from stressed plants. Studia Universitatis Babes-Bolyai Chemia 54:329-339.

Copolovici L., Kannaste A., Remmel T., Vislap V., Niinemets U., 2011 Volatile emissions from *Alnus glutinosa* induced by herbivory are quantitatively related to the extent of damage. Journal of Chemical Ecology 37:18-28.

Hallquist M., Wenger J. C., Baltensperger U., Rudich Y., Simpson D., Claeys M., Dommen J., Donahue N. M., George C., Goldstein A. H., Hamilton J. F., Herrmann H., Hoffmann T., Iinuma Y., Jang M., Jenkin M. E., Jimenez J. L., Kiendler-Scharr A., Maenhaut W., McFiggans G., Mentel T. F., Monod A., Prãcvãt A. S. H., Seinfeld J. H., Surratt J. D., Szmigielski R., Wildt J., 2009 The formation, properties and impact of secondary organic aerosol: current and emerging issues. Atmos Chem Phys 9:5155-5236.

Hanley M. E., Lamont B. B., Fairbanks M. M., Rafferty C. M., 2007 Plant structural traits and their role in anti-herbivore defence. Perspectives in Plant Ecology, Evolution and Systematics 8:157-178.

Hatanaka A., 1993 The biogeneration of green odour by green leaves. Phytochemistry 34:1201-1218.

Herbert K. S., Hoffmann A. A., Powell K. S., 2008 Assaying the potential benefits of thiamethoxam and imidacloprid for phylloxera suppression and improvements to grapevine vigour. Crop Protection 27:1229-1236.

Jackson R. S., 2014 Grape species and varieties wine science. Fourth edition. Academic Press, San Diego, pp. 21-67.

Kessler A., Heil M., 2010 The multiple faces of indirect defences and their agents of natural selection. Functional Ecology 25:348-357.

Kleindienst T. E., Lewandowski M., Offenberg J. H., Jaoui M., Edney E. O., 2007 Ozone-isoprene reaction: re-examination of the formation of secondary organic aerosol. Geophysical Research Letters 34:L01805.

Lehning A., Zimmer W., Zimmer I., Schnitzler J. P., 2001 Modeling of annual variations of oak (*Quercus robur* L.) isoprene synthase activity to predict isoprene emission rates. Journal of Geophysical Research 106:3157-3166.

Liavonchanka A., Feussner I., 2006 Lipoxygenases: occurrence, functions and catalysis. J Plant Physiol 163:348-57.

Lichtenthaler H. K., Schwender J., Disch A., Rohmer M., 1997 Biosynthesis of isoprenoids in higher plant chloroplasts proceeds via a mevalonate-independent pathway. FEBS Lett 400:271-4.

Lubiarz M., 2007 Life cycle and number dynamics of *Phylloxera* sp. on pedunculate oak (*Quercus robur*.) in industrial and protected areas. Aphids and Other Hemipterous Insects 13:137-144.

Matsui K., 2006 Green leaf volatiles: hydroperoxide lyase pathway of oxylipin metabolism. Current Opinion in Plant Biology 9:274-280.

Moretti C. L., Mattos L. M., Calbo A. G., Sargent S. A., 2010 Climate changes and potential impacts on postharvest quality of fruit and vegetable crops: a review. Food Research International 43:1824-1832.

Niinemets U., 2010 Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: past stress history, stress interactions, tolerance and acclimation. Forest Ecology and Management 260:1623-1639.

Niinemets Ü., Copolovici L., Hüve K., 2010a High within-canopy variation in isoprene emission potentials in temperate trees: implications for predicting canopy-scale isoprene fluxes. Journal of Geophysical Research 115:G04029.

Niinemets Ü., Monson R. K., Arneth A., Ciccioli P., Kesselmeier J., Kuhn U., Noe S. M., Peñuelas J., Staudt M., 2010b The leaf-level emission factor of volatile isoprenoids: caveats, model algorithms, response shapes and scaling. Biogeosciences 7:1809-1832.

Niinemets U., Kannaste A., Copolovici L., 2013 Quantitative patterns between plant volatile emissions induced by biotic stresses and the degree of damage. Front Plant Sci 4:262.

Oliveira D. C., Santos Isaias R. M., Moreira A. S. F. P., Magalhaes T. A., de Lemos-Filho J. P., 2011 Is the oxidative stress caused by *Aspidosperma* spp. galls capable of altering leaf photosynthesis? Plant Science 180:489-495.

Paré P. W., Tumlinson J. H., 1999 Plant volatiles as a defense against insect herbivores. Plant Physiology 121:325-331.

Pearse I. S., Gee W. S., Beck J. J., 2013 Headspace volatiles from 52 oak species advertise induction, species identity, and evolution, but not defense. Journal of Chemical Ecology 39:90-100.

Penuelas J., Llusia J., 2003 BVOCs: plant defense against climate warming? Trends in Plant Science 8:105-109.

Peñuelas J., Staudt M., 2010 BVOCs and global change. Trends in Plant Science 15:133-144.

Penuelas J., Asensio D., Tholl D., Wenke K., Rosenkranz M., Piechulla B., Schnitzler J. P., 2014 Biogenic volatile emissions from the soil. Plant Cell Environ 37:1866-1891.

Possell M., Hewitt C. N., 2011 Isoprene emissions from plants are mediated by atmospheric CO_2 concentrations. Global Change Biology 17:1595-1610.

Rasulov B., Copolovici L., Laisk A., Niinemets Ü., 2009 Postillumination isoprene emission: in vivo measurements of dimethylallyldiphosphate pool size and isoprene synthase kinetics in aspen leaves. Plant Physiology 149:1609-1618.

Spracklen D. V., Bonn B., Carslaw K. S., 2008 Boreal forests, aerosols and the impacts on clouds and climate. Philosophical Transactions of the Royal Society of London A 366:4613-4626.

Received: 28 January 2015. Accepted: 26 February 2015. Published online: 31 March 2015.
Authors:
Andreea Pag, Institute of Technical and Natural Sciences Research-Development of Aurel Vlaicu University, Elena Dragoi str., no. 2, 310330 Arad, Romania
Daniel Tomescu, Institute of Technical and Natural Sciences Research-Development of Aurel Vlaicu University, Elena Dragoi str., no. 2, 310330 Arad, Romania
Adina Bodescu, Institute of Technical and Natural Sciences Research-Development of Aurel Vlaicu University, Elena Dragoi str., no. 2, 310330 Arad, Romania
Astrid Kännaste, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, 51014 Tartu, Estonia
Ülo Niinemets, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, 51014 Tartu, Estonia
Lucian Copolovici, Faculty of Food Engineering, Tourism and Environmental Protection, “Aurel Vlaicu” University, Elena Dragoi str., no. 2, 310330 Arad, Romania, e-mail: lucian.copolovici@emu.ee

How to cite this article:
Pag A., Tomescu D., Bodescu A., Kännaste A., Niinemets Ü., Copolovici L., 2015 The emission of volatile organic compounds from *Quercus robur* plants affected by *Phylloxera quercus* and temperature. Ecoterra 12(1):94-99.