Content of *Trans*-resveratrol in Leaves and Berries of Interspecific Grapevine (*Vitis* sp.) Varieties

Petra BÁBÍKOVÁ[1], *Naděžda VRCHOTOVÁ*[2], *Jan TŘÍSKA*[2] *and Marie KYSELÁKOVÁ*[1]

[1]*Department of Viticulture and Enology, Faculty of Horticulture, Mendel University of Agriculture and Forestry in Brno, Lednice, Czech Republic;* [2]*Laboratory of Environmental Analytical Chemistry, Institute of Systems Biology and Ecology of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic*

Abstract: The aim of this project was to study changes in the content of *trans*-resveratrol in berries and leaves of grapevine (*Vitis* sp.) infested by fungal diseases, especially by *Botryotinia fuckeliana* Whetzel, called as grey mildew, *Plasmopara viticola* (Berk. & M.A. Curtis) Berl & De Toni, called downy mildew and *Uncinula necator* (Schw.) Burr, called powdery mildew. In our experiments two white and two blue varieties were used. Contents of *trans*-resveratrol were determined in healthy and infested leaves and in healthy berries. Infested leaves of white varieties contained more *trans*-resveratrol than those of blue varieties. The content of *trans*-resveratrol in berries was lower than that in leaves.

Keywords: *trans*-resveratrol; HPLC; wine

It is known that after the infestation with *Botryotinia fuckeliana* Whetzel., the grapevine is capable to synthesise greater amounts of phytoalexin *trans*-resveratrol, which serves as a natural protection against infection. In European cultural grapevine *Vitis vinifera* L. the contents of resveratrol were repeatedly reported. However, as a result of crossing European and American grapevine species, some new varieties were obtained, which showed a better resistance to fungal diseases. As they are products of crossing of different species, they are called interspecific varieties.

Although phenolic substances are routinely synthesised during the processes of plant growth, the synthesis of some of them is induced also by stress factors caused for example by fungal diseases or UV radiation (Beckman 2000). From the chemical point of view, these compounds are derivatives of amino acids phenylalanine and tyrosine (Naczk & Shahidi 2004). The group of phenolic substance involves a number of compounds ranging from simple phenols, phenolic acids and their derivatives to coumarins, flavonoids and stilbenes, tannins and lignins (Merken & Beecher 2006). The roles of these compounds within the plants are very different: they protect them against pests and UV radiation, attract pollinators, function as antioxidants, and endow sensory properties and colour to their fruit. They are important not only for plants but also for humans as they often show high antioxidative properties and also positively influence the cardiovascular system (Jackson 1994; Revilla & Ryan 2000; Nikfardjam *et al.* 2006).

These phenolic substances are not distributed uniformly within the plant organism and they are typical only for some parts of the plant organism: insoluble phenols occur in cell walls while soluble ones are present in cell vacuoles. Lignin

Supported by the Internal Grant Agency (Project No. 5/2008/591), by the Czech Science Foundation (Grant No. GA 525/06/1757) and by the Research Intention of ISBE AS CR (Project No. AV 0Z60870520).

and hydroxycinnamic acids are present above all in ligneous structures where they increase the mechanical strength of plant tissues (Scalbert 1993; Baucher *et al.* 1998). Stilbenes belong to the groups of non-flavonoid phenolic substances and just these compounds are considered to be the carriers of resistance against fungal diseases. Increased amounts of stilbenes are synthesised not only in plants infested with fungal diseases (above all with *Botryotinia fuckeliana* Whetzel. *Plasmopara viticola* Berl. &. de Toni, and *Uncinula necator* Burr.), but also under the effect of UV radiation and some other stressors (Langcake & Pryce 1976).

It is known that after the infestation with *Botryotinia fuckeliana* the grapevine plants can increase the synthesis of *trans*-resveratrol as a natural means of protection in the neighbourhood of the infested spot so that a further spread of the infection is prevented (Langcake 1981). Recently, the main attention has been paid to resveratrol. This compound can occur either in *cis* or *trans*-form. In grapes, *trans*-resveratrol is considered to be the main stilbene derivative. It is present above all in skins of berries and also in leaves and grape stalks (Mikeš *et al.* 2008) and during the process of maceration it passes into wine. The occurrence of *trans*-resveratrol in leaves was described in papers (Adrian *et al.* 2000; Okuda & Yokotsuka 1996) dealing with the synthesis of resveratrol under the effects of UV radiation, aluminium chloride and/or mechanical damage. *Cis*-resveratrol does not occur in grapes (Jeandet 1991) and its presence in wine is explained as a result of photochemical isomerisation of *trans*-resveratrol and/or as a product of organic viticulture (Jeandet 1995). There are also glycosides of resveratrol in plant material called piceids. These glycosides were identified in grape skins and berries (Sun *et al.* 2006). Grapevines, grapevine wine and also other foodstuffs of plant origin contain great amounts of various flavonoids and phenolic compounds. Their content may be as high as several grams per kg of plant material (Burns *et al.* 2000). However, the health function for humans and the protective one for plants fulfils only one isomer, viz. *trans*-resveratrol, and for that reason the aim of this study was to estimate its contents in interspecific wine grape varieties approved in the territory of the Czech Republic and to follow changes in its contents in berries and leaves of grapevine plants infested with fungal diseases.

MATERIAL AND METHODS

Berries and leaves were sampled in the vineyard of the Department of Viticulture and Oenology, Faculty of Horticulture, Mendel University of Agriculture and Forestry in Brno, in the research station Mendeleum in 2006.

Samples. Two white varieties were selected for this study: Hibernal (Siebel 7053 × Riesling clone 239) and Malverina (Rakish × Merlan). The former one was selected in German town Geisenheim and its acreage in the Czech Republic is approximately 10.6 hectares. The latter one was selected in Moravia (by Mádl, Kraus, Michlovský, Peřina, and Glos) and its acreage is 6.2 hectares.

The blue variety Laurot (Merlan × Fratava) was also selected in Moravia (by Mádl, Kraus, Michlovský, Peřina, and Glos) and is grown on 3.5 ha of vineyards, while Cerason (MI-5-100) (Merlan × Fratava) is a new breeding, which has not yet been registered in the National List of Varieties of the Czech Republic (Pavloušek 2007).

Both healthy and infested leaves were sampled (on 22nd August and on 3rd October) and also healthy berries (on 3rd and 23rd October). As 2006 vintage was very favourable and the berries were not infested, only healthy ones were sampled. Prior to processing, all samples were deep frozen.

Processing of leaves. Frozen leaves were freeze-dried. Weighed samples of freeze-dried material (0.25 g) were extracted for 30 min in 90% methanol in shade at room temperature and thereafter centrifuged at 3500 rev/min for 10 minutes. The sediment was rinsed twice with methanol and centrifuged. Supernatants were pooled, the total volume was recorded and the sample was stored in a freezing box until the HPLC measurement.

Processing of berries. Frozen berries were homogenised in 90% methanol, extracted for 30 min, and centrifuged under the same condition as leave samples. This was followed by the second extraction for 30 min and centrifugation. The sediment was rinsed with 90% methanol and centrifuged. Supernatants were pooled, the total volume was recorded and the sample was stored in a freezing box until the HPLC measurement.

Reagents and solvents. *Trans*-resveratrol was obtained from Sigma-Aldrich (Prague, Czech Republic). *o*-Phosphoric acid was obtained from Fluka (Sigma-Aldrich, Prague, Czech Republic). Acetonitrile and methanol were purchased from Merck (Prague, Czech Republic).

Chromatographic methods. Extracts were analysed using an HPLC apparatus (Hewlett Packard 1050) with a diode array detector (DAD) in a column Phenomenex Luna C18(2) (3 μm, 2 × 150 mm). The volume of injected sample was 5 μl.

Mobile phase A: acetonitrile:*o*-phosphoric acid: water (5:0.1:94.9), mobile phase B: acetonitrile: *o*-phosphoric acid:water (80:0.1:19.9). For separation, the gradient from 0% to 45% of mobile phase B within 55 min was used and thereafter from 45% to 100% of B within 10 min; the flow rate was 0.25 ml/min. Column temperature was 25°C. *Trans*-resveratrol was detected at 315 nm.

RESULTS AND DISCUSSION

Samplings were performed on two different dates. It was expected that the date of sampling (i.e. the degree of ripeness) could influence the content of resveratrol in berries and also in leaves. López (López *et al.* 2001) analysed five samples of Spanish wines originating from different regions and detected *trans*-resveratrol only in one of them.

Our research was focused on the detection of *trans*-resveratrol. As can be seen in Table 1, the biosynthesis of *trans*-resveratrol was increased in infested parts of the plant. It is also important that in infested leaves of white varieties the content of *trans*-resveratrol was higher than in blue varieties. This difference was studied also by Romero-Pérez *et al.* (2001). Interesting results were published also by Navarro *et al.* (2008) who studied contents of various compounds (including *trans*-resveratrol) in skins of berries of varieties Tempranillo, Bobal, Cabernet Sauvignon, and Crujidera. The authors demonstrated significant differences in contents of *trans*-resveratrol between individual varieties. However, their results were not corroborated in our study. We observed the significant differences in resveratrol contents in samples of individual varieties; however, the content in blue varieties was higher.

As results from the data presented in Table 1 show, infested leaves of white varieties (Hibernal, Malverina) contained much more *trans*-resveratrol than those of blue varieties (Laurot, Cerason). On the second date of the harvest, contents of *trans*-resveratrol were increased both in healthy and infested leaves but values recorded for white varieties were still higher than those in blue ones. A similar study with infected leaves was performed by Bernard *et al.* (2006). The authors infected leaves with spores of *Plasmopara viticola* (Berk. & M.A. Curtis) Berl & De Toni and found in fresh leaves as much as 40 μg/g of *trans*-resveratrol.

The statistical analysis revealed by Anova, that white or blue berries showed to be a very important trait. Results obtained by variance analysis indicated (Figure 1) that the effect of the date of harvest was highly significant, especially when comparing the sum of values recorded for white varieties with that of blue ones. Above all, the second sampling the content of *trans*-resveratrol was markedly increased. While on the first date of sampling (22nd August) the effect of variety colour was not significant, it was statistically highly significant on the second one (3rd October). Results of the evaluation of the health condition and variety colour on the content of *trans*-resveratrol (Figure 2) also corroborated their highly significant effect only in leaves of white varieties; in blue varieties

Table 1. Content of *trans*-resveratrol in leaves of interspecific hybrids (mg/kg of fresh matter)

		Sampled on	
		22nd August	3rd October
Hibernal	healthy leaves	0.96	6.84
	infested leaves	8.88	14.51
Malverina	healthy leaves	1.20	11.90
	infested leaves	8.08	13.06
Laurot	healthy leaves	1.91	5.79
	infested leaves	2.63	6.41
Cerason	healthy leaves	1.57	4.69
	infested leaves	1.56	5.95

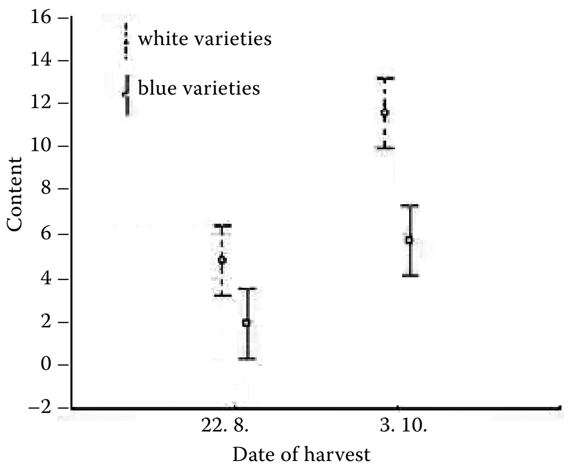

Figure 1. Content of *trans*-resveratrol in leaves as dependent on colour of grapes and date of leaf sampling

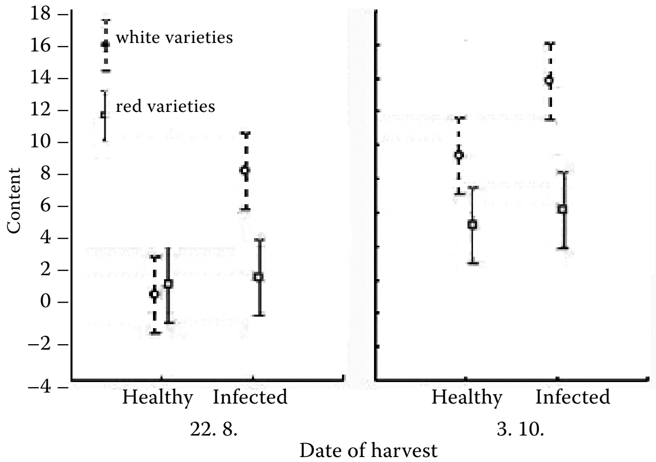

Figure 2. Content of *trans*-resveratrol in leaves as dependent on colour, health condition and date of sampling

Table 2. Content of *trans*-resveratrol in berries of interspecific hybrids (mg/kg of fresh matter)

Berries	Sampled on	
	22nd August	3rd October
Hibernal	0.23	0.14
Malverina	0.31	0.25
Laurot	0.65	0.53
Cerason	1.33	0.54

the effect of these factors was insignificant. If the variety colour was not involved into the variance analysis, the effect of infestation (band also of the variety) showed to be insignificant. On the other hand, the effect of the date of sampling was statistically highly significant.

Table 2 presents the results of analyses of berries sampled on two different dates. In contradistinction to leaves, the effect of sampling was not significant; however, it was even slightly reduced on the second date of sampling (Table 2). Berries of white varieties contained less *trans*-resveratrol than those of blue ones. Leaves and berries were sampled on different dates because of leaf fall occurred before the fruit fully ripened. The effect of the date of sampling of berries was statistically insignificant.

CONCLUSIONS

In grapevine plants the content of *trans*-resveratrol increases above all in cases of their infestation with fungal diseases. Interspecific varieties obtained recently by means of hybridisation show a higher degree of resistance than plants of the European cultural grapevine *Vitis vinifera* L. Our analyses demonstrated that, in case of infestation, these more resistant plants also synthesised greater amounts of *trans*-resveratrol then healthy plants. It was surprising that infested leaves of white varieties contained more *trans*-resveratrol than those of blue ones. The content of *trans*-resveratrol in leaves was influenced also by the date of sampling. In berries, the content of *trans*-resveratrol was influenced only slightly and higher values were recorded in blue varieties. Although the content of *trans*-resveratrol in interspecific hybrids was not so high that it would be the cause of their resistance. It could be a great advantage that it is not necessary to use the chemical protection in a such extent as in case of European varieties. This fact reduces the possible contents of pesticide residues in grapes, juice and wine so that the health importance of these varieties is higher.

References

Adrian M., Jeandet P., Breuil A., Levite D., Deborg S., Bessis R. (2000): Assay of resveratrol and derivative stilbenes in wine by direct injection high performance liquid chromatography. American Journal of Enology and Viticulture, **51**: 37–41.

Baucher M., Monties B., van Montagu M., Boerjan W. (1998): Biosynthesis and genetic engineering of lignin. Critical Reviews in Plant Science, **17**: 125–197.

Beckman C.H. (2000): Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants. Physiological and Molecular Plant Pathology, **57**: 101–110.

Bernard J.-D.J., Pezet R., Tabacchi R. (2006): Rapid analysis of stilbenes and derivatives from downy mildew-infected grapevine leaves by liquid chromatography-atmospheric pressure photoionisation mass spectrometry. Journal of Chromatography A, **1112**: 263–268.

Burns J., Gardner P.T., O'Neil J., Crawford S., Morecroft L., McPhail D.B., Lister C., Matthews D., MacLean M.R., Lean M.E.J., Duthie G.G., Crozier A. (2000): Relationship among antioxidant activity, vasodilatation and phenolic content of wines. Journal of Agricultural and Food Chemistry, **48**: 220–230.

Jackson R.S. (1994): Wine Science. Academic Press, New York.

Jeandet P., Bessis R, Gautheron B. (1991): The production of resveratrol (3,5,4'-trihydroxystilbene) by grape berries in different developmental stages. American Journal of Enology and Viticulture, **42**: 41–46.

Jeandet P., Bessis R., Sbasghi M., Meunier P., Trolat P. (1995): Resveratrol content of wines of different ages: Relationship with fungal disease pressure in the vineyard. American Journal of Enology and Viticulture, **46**: 1–4.

Langcake P. (1981): Physiology. Plant Pathology, **18**: 213.

Langcake P., Pryce R.J. (1976): The production of resveratrol by *Vitis vinifera* and other members of *Vitaceae* as a response to infection or injury. Physiology and Plant Pathology, **9**: 77–85.

López M., Martínez F., Del Valle C., Orte C., Miró M. (2001): Analysis of phenolic constituents of biological interest in red wines by high performance liquid chromatography. Journal of Chromatography A, **922**: 359–363.

Merken H.N., Beecher G.R. (2000): Liquid chromatographic method fort he separation and quantification of prominent flavonoid aglycones. Journal of Chromatography A, **897**: 177–184.

Mikeš O., Vrchotová N., Tříska J., Kyseláková M., Šmidrkal J. (2008): Distribution of major polyphenolic compounds in vine grapes of different cultivars growing in south Moravian vineyards. Czech Journal of Food Sciences, **26**: 182–189.

Naczk M., Shahidi F. (2004): Extraction and analysis of phenolics in food. Journal of Chromatography A, **1054**: 95–111.

Navarro S., León M., Roca-Pérez L., Boluda R., García Ferriz L., Pérez- Bermúdes P., Gavidia I. (2008): Characterization of Bobal and Crujidera grape cultivars, in comparison with Tempranillo and Cabernet Sauvignon: Evolution of leaf macronutrients and berry composition during grape ripening. Food Chemistry, **109**: 182–190.

Nikfardjam M S., Pour M.L., Avar P., Figler M., Ohmacht R. (2005): Polyphenols, anthocyanins and *trans*-resveratrol in red wines from Hungarian Villány region. Food Chemistry, **98**: 453–462.

Okuda T., Yokotsuka K. (1996): *Trans*-resveratrol concentrations in berry sins and wines from grapes in Japan. American Journal of Enology and Viticulture, **47**: 93–99.

Pavloušek P. (2007): Encyklopedie révy vinné. Computer Press, Brno,.

Revilla E., Ryan J.M. (2000): Analysis of several phenolic compounds with potential antioxidant properties in grape extracts and wines by high performance liquid chromatography-photodiode array detection without sample preparation. Journal of Chromatography A, **881**: 461–469.

Romero-Pérez A.I., Lamuela-Raventóz R.M., Andrés-Lacueva C., de la Torre-Boronat M.C. (2001): Method for the quantitative extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content. Journal of Agricultural and Food Chemistry, **19**: 210–215.

Scalbert A. (1993): Polyphenolic Phenomena. INRA Editions, Paris: 143–147.

Sun B., Ribes Q.M., Leandro M.C., Belchior A.P., Spranger M.I. (2006): Stilbenes: Quantitative extraction from grape skins, contribution of grape solids to wine and variation during wine maturation. Analytica Chimica Acta, **563**: 382–390.

Corresponding author:

Ing. Petra Bábíková, DiS., Mendelova zemědělská a lesnická univerzita v Brně, Zahradnická fakulta,
Ústav vinohradnictví a vinařství, Valtická 337, 691 44 Lednice, Česká republika
tel.: + 420 519 367 254, fax: + 420 519 367 222, e-mail: petraba@centrum.cz