Agrovoc descriptors: vitis vinifera, wine grapes, polyphenols, infection, ultraviolet radiation, chemicals, stress
Agris category code: H20, F60, Q04

COBISS code 1.01

The influence of virus diseases on grape polyphenols of cv. 'Refošk'[a]

Irma TOMAŽIČ[1], Urška VRHOVŠEK[2], Zora KOROŠEC-KORUZA[3]

Recived April 30, 2003; accepted September 9, 2003.
Delo je prispelo 30. aprila 2003; sprejeto 9. septembra 2003.

ABSTRACT

External stimuli such as microbial infections, ultraviolet radiation, and chemical stressors can modulate the synthesis of polyphenols in the plants. Cv. 'Refošk' was used to show the influence of the GLRaV-1 and rugose wood (RW) on the polyphenols in grape. The infection shifted polyphenols from seeds to grape skins but had no impact on anthocyanins.

Key words: grapevine, *Vitis vinifera*, virus diseases, polyphenols

IZVLEČEK

VPLIV VIRUSNIH BOLEZNI NA VSEBNOST POLIFENOLOV GROZDJA SORTE 'REFOŠK'[b]

Zunanji dejavniki, kot so okužbe z mikrobi, ultravijolično sevanje in kemični dejavniki vplivajo na tvorbo polifenolov v rastlinah. Na primeru sorte 'Refošk' smo poskušali ugotoviti vpliv GLRaV-1 in bolezni razbrazdanja lesa (RW) na vsebnost polifenolov v grozdju. Bolezni vplivata na prerazporeditev polifenolov iz pečk v kožice, nimata pa vpliva na antociane grozdja.

Ključne besede: vinska trta, *Vitis vinifera*, virusne bolezni, polifenoli

---

[a] The article is a part of a doctoral dissertation (justification July 5, 2002), supervisor Ph. D. Assistant Professor. Zora Korošec-Koruza

[1] Ph. D. Nova Gorica Polytechnic, Vipavska 13, p.p. 301, SI-5000, Nova Gorica, irma.tomazic@p-ng.si

[2] Assist. Prof., Ph. D. Agricultural Institute of Slovenia, Hacquetova 17, SI-1001, Ljubljana,

[3] Assist. Prof., Ph. D. University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Jamnikarjeva 101, p.p. 2995, SI-1111, Ljubljana

[b] Prispevek je del doktorske disertacije (zagovor 5. jul. 2002), mentorica doc. dr. Zora Korošec-Koruza

1 INTRODUCTION

Polyphenols have attracted much attention recently due to their role in prevention of illnesses such as heart diseases and diseases of cardiovascular system whose causes are in the oxidation of LDL (low density lipoproteins) (Rice-Evans et al., 1997). The anti-viral functioning of polyphenols is very well known (Serkedjieva and Ivancheva, 1999). Diseases and even ageing can occur due to molecular damages caused by harmful free radicals – side metabolic products, which however, can be neutralized by antioxidants. Oxidants comprise the vitamins E and C as well as polyphenols (Whitehead et al., 1995; Hu et al., 2000), which are present in grape and wine in large amounts.

Polyphenols play a vital role in the growth and propagation of plants and protect plant tissue from damage (Gutmann and Feucht, 1994). They neutralise free radicals and thus protect biologically vital molecules from oxidation. Polyphenols are a part of a complex immunity system, which can be acquired in the tissues under stress (Feuch, 1994). The plants cannot, contrary to animals, synthesize antibodies but they can produce numerous substances - phytoalexins. Those are secondary metabolites, which inhibit and kill pathogenic organisms (Bennett and Wallsgrove, 1994). In addition, polyphenols protect plants against insects and herbivorous mammals (Harborne, 1995).

The response to fungus diseases and bacterial diseases in grapevine has been well investigated (Robert et al., 2001). The species of the genus *Vitis*, which contain larger amounts of polyphenols (e.g. *Vitis rotundifolia*) are more resistant to infections caused by downy mildew (*Plasmopara viticola* Berl. & de Toni) and when infected they produce larger amounts of polyphenols than varieties which are more susceptible (Dai et al., 1994). A positive correlation between the content of catechin in grape and resistance to grey mould (*Botrytis cinerea* Pers.) has been discovered. (Goetz et al., 1999). In grape infected by powdery mildew (*Oidium tuckeri* Berk.) the synthesis of *trans*-resveratrol increases especially due to the synthesis of anthocyanins (Piermattei et al., 1999).

However, little do we know about the influence of viruses on the content of polyphenols in plants. Some authors (Kaur et al., 1989; Kaur et al., 1991; Baruah and Chowfla, 1994) suggest that there are higher contents of polyphenols present in healthy plants. Higher contents of secondary metabolites in healthy plants protect the plants from infection. On the contrary, Kumar (1991), Sharma and Chowfla (1991) as well as Suresh et al., (1991) state that there are higher amounts of total polyphenols in virus infected plants.

The first researcher to investigate the influence of viruses on the content of grapevine polyphenols was Borgo (1991). According to his discoveries there were lower amounts of total polyphenols present in the grape cultivars ‘Merlot’, ‘Cabernet Franc’ and ‘Cabernet Sauvignon’, which were infected with the GLRaV-3 than in those which were not infected and therefore healthy. The ripening of the grape of infected vine was delayed because there was less sugar in the infected vine than in the healthy one. Later, Guidoni et al., (1997) also discovered lower contents of polyphenols in the skins of ‘Nebiolo’ grape which was infected with the GLRaV-3 and GVA than in those which were not infected. The amounts of polyphenols in the grape skins of cv.

‘Grignolino’ infected with GLRaV-1 and GVA were lower than the contents of polyphenols in the grape skins of healthy ones.

2 MATERIAL AND METHODS

In the vintage of 2000 we aimed to investigate the influence of the GLRaV-1 infection and rugose wood disease on the contents of polyphenols in the skins and seeds of cv. ‘Refošk’ grafted on ‘SO4’. Three plants with rougose wood symptoms, three plants infected with GLRaV-1 and five healthy plants were chosen for analysing. The crop was measured by counting the bunches of grapes, measuring the yield weight per vine, and determining the contents of sugar and titrable acids.

Spectrophotometric methods

To control the grape and wine quality the spectrophotometric methods are still applied since they are efficient, repeatable, and in comparison with the HPLC methods, cheaper. They provide accurate information on the content and type of polyphenols (Vrhovšek et at., 2001). The extracts for spectrophotometric analyses of polyphenols were gathered according to the method created by Mattivi et al. (2002). The two extractions, from the seeds and from the skins, were performed separately. The published spectrophotometric methods were applied (Di Stefano et al., 1989) in conditions optimised by Rigo et al. (2000). The following parameters were defined:

<u>Total polyphenols</u>: They were defined with the application of the Folin-Ciocalteu method (Sigleton and Rossi, 1965), under optimal conditions previously described by Di Stefano and Guidoni (1989).

<u>Proanthocyanidin</u>: They were defined through the transformation into cyanidin (Di Stefano et al., 1989). Such a method is applied especially when determining high molecular proanthocyanidins.

<u>Index of vanillin</u>: The method designed by Broadhurst and Jones (1978), is based on the fact that especially low-molecular flavonoids (catechin and low-molecular proanthocyanidins) in a acid environment react with vanillin and produce a coloured product which features maximum absorbance at 500 nm. In grape the reaction between catechin and proanthocyanins and vanillin is measured under conditions described by Di Stefano et al. (1989).

<u>Total anthocyanins</u>: Anthocyanins were determined according to maximum absorbance in visible range (536 - 540 nm) against a blank (solution: ethanol/water/HCl 70:30:1) (Di Stefano et al., 1989).

HPLC analysis of the anthocyanin profile in the grape skins

The extracts intended for the HPLC analysis were prepared according to the method applied in the Dipartamento Laboratorio Analisi e Ricerche, Istituto Agrario di San Michele, in Italy, where the samples were analysed (Mattivi, 2001). With the HP 1090 Liquid Chromatograph (Agilent, Palo alto, CA) the following anthocyanins were analysed:

- delphinidin-3-monoglucoside,
- cyanidin-3-monoglucoside,
- petunidin-3-monoglucoside,
- peonidin-3-monoglucoside,
- malvidin-3-monoglucoside
- acylated form of anthocyanins, their *p*-coumarat and malvidin-3-caffeoylmonoglucoside.

3 RESULTS AND DISCUSSION

Spectrophotometric analysis

Total polyphenols (mg/kg (+) catechin): The content of total polyphenols in seeds does not depend on the sanitary status of the grapevine. The content of total polyphenols is higher in seeds when the content of polyphenols in skins is lower, consequently, the total polyphenols in seeds and skins reach similar values in both infected and healthy vines (Table 1). It can be concluded that viral infections do not affect the synthesis of polyphenols but have effects on the distribution of polyphenols in a grape berry.

Table 1: Effect of GLRaV-1 and rugose wood disease on extractable polyphenols of grapevine skins and seeds of cv. 'Refošk'.

		Healthy (-)	Vines infected with GLRaV-1	Rugose wood symptoms RW
	Skins	686.4 (598.5)[a]	657.3	741.3
Total phenols	Seeds	738.2 (619.7)	618.3	543.3
mg/kg (+) catechin	**Total**	**1428.6 (1218.2)**	**1275.6**	**1284.6**
Proanthocyanidins	Skins	723.2 (628.2)	761.0	874.0
mg/kg (+) cyanidin	Seeds	759 (647.0)	590.0	523.3
	Total	**1483.0 (1275.2)**	**1351.0**	**1397.3**
	Skins	217.8	290.0	307.3
Index of vanillin	Seeds	751.8	695.3	603.3
mg/kg (+) catechin	**Total**	**969.6**	**985.3**	**910.6**
	Skins	619.4	503.3	574.0
Total anthocyanins mg/kg				

[a] without the outlier

Among the healthy samples there is one outlier. The sample which had a high amount of polyphenols differed from others in that its yield was very low. The yield weight influences the sugar content in grape (Fig 1) which is a precursor of acetil-CoA from which polyphenols are synthetized (Taiz and Zeiger, 1998; Heldt, 1997). In our research sugar had impact on the total polyphenols content which can explain 71 % variability among the samples (Fig 2). The vines infected with the GRLaV-1 had on average by 36 % lower yield. The vines with rugose wood had by 34 % lower yield than the healthy ones do (Tomažič et al., 2000) Consequently, the content of polyphenols is affected. It is better to reduce the yield with ampelotechnique and thus improve the quality of grape. To establish the true influence of viruses on polyphenols the experiment should be rerun in the grapevine producing the same yield but having a different sanitary status.

Proanthocyanidins (mg/kg cyanidin): The contents of proanthocyanidins did not differ between the healthy and infected vines. And it was obvious that in the vines infected with a virus the proanthocyanidins were shifted from seeds to skins.

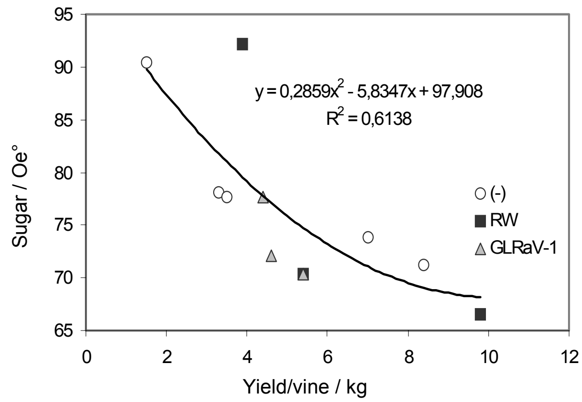

Figure 1: Effect of crop/vine on grape sugar content of healthy and infected vines of cv. 'Refošk'.

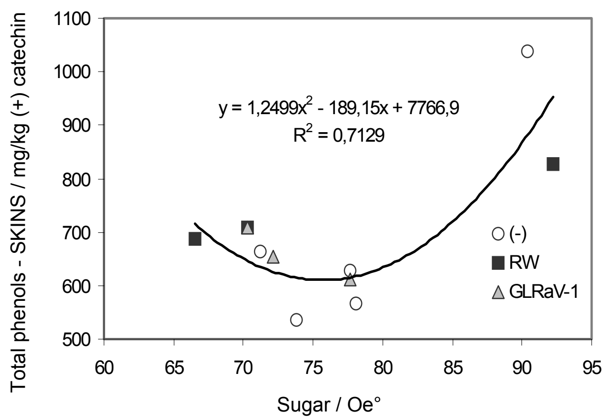

Figure 2: Effect of grape sugar content on total phenols of grapevine skins of healthy and infected vines of cv. 'Refošk'.

<u>Index of vanilline (mg/kg (+) catechin):</u> With the index of vanilline no differences between the healthy and infected vines (in the content of low-molecular proanthocyanins) were noted. The repositioning of proanthocyanidins between skins and seeds is evident. The infected vines, compared to healthy ones, exhibited more low-molecular proanthocyanidins in the skins, and less in the seeds.

<u>Total anthocyanins (mg/kg):</u> The content of total anthocyanins in healthy grapevine does not differ from the content in infected ones. However, Mannini et al. (1998) discovered lower contents of anthocyanins in cv. 'Grignolino' infected with the GLRaV-1 and GVA, and in cv. 'Nebiolo' infected with the GLRaV-3 and GVA, than in the healthy cultivars. Less polyphenols were detected also in the grapevine infected with powdery mildew (Piermattei et al., 1999). The occurrence of lower contents of anthocyanins in grape of infected vine can be ascribed to the competition of the enzymes for the two substrates (*p*-coumaroyl-CoA and malonyl-CoA) which are essential in both the formation of anthocyanins and the production of resveratrol (Jeandet et al., 1995). It is interesting to note that the content of total anthocyanins is the lowest in the vine infected with the GLRaV-1, which causes reddening of the

leaves. Guidoni et al. (1997) measured higher contents of anthocyanins in the leaves of the vine infected with the GLRaV-1 and GVA than in the leaves of the healthy vine. They explain the event by eventual disturbance in transition of anthocyanins from leaves to berries.

HPLC analysis of the anthocyanins profile (mg/kg of berries)

In the analysis of the anthocyanins profile no differences were noted between the healthy and infected vines. Guidoni et al. (1997) who observed the alterations in the anthocyanins profile during the time of ripening of cv. 'Nebiolo' discovered no significant differences among individual anthocyanidins. They discovered that the accumulation of anthocyanins occurred faster in the healthy vines than in ones infected with the GLRaV-3 and GVA. The profile of anthocyanins is genetically determined and is often used in cultivar classifications (Mattivi et al., 1989; Bucelli et al., 1995). This, however, would not be possible if the profile tended to change due to viral infections.

4 CONCLUSIONS

The examination of the influence of viruses on grape polyphenols shows that viral infections have different effects on the plant than the infections caused by other pathogenic organisms which induce higher production of polyphenols. Pannazio and Roggero (1998) stated that there had not been a case of a plant acquiring resistance against systemic virus infection. Antioxidants are produced in viral infections only when the infected tissue becomes hypersensitive. Hypersensitivity does not disturb replication but it causes the decay of the tissue around the infection and thus limits the expansion into other cells. The infected tissue produces salicylic acid which induces the accumulation of lipid prooxidants through free radicals of salicylic acid. They induce the expression of defence genes (Anderson et al., 1998). It is also possible that in the place of infection oxidation conditions occur due to the reduction in antioxidative enzymes activities. This triggers the accumulation of free radicals and the peroxidation of lipids what leads to the tissue decay. When the production of oxidized derivatives is enlarged the genes which code antioxidative enzymes are induced to limit the decay of the tissue around the necrosis (Fodor et al., 1997).

The outcomes of the research show that the GLRaV-1 infection and roguse wood cause the polyphenols to shift from seeds into the grape skins but the content of total polyphenols in seeds and skins stays the same. In the previous research only the total contents of polyphenols contained in both seeds and skins together were analysed, therefore the differences between the content in seeds and the content in skins were not discovered. The content of polyphenols in grape is affected also by sugar whose content depends on the yield weight. To achieve a more accurate determination of the virus influence on the content of polyphenols it would be necessary to rerun the experiment in the grapevine with the same yield weight. The content of total anthocyanins in the grape skins does not differ between the infected and healthy ones analysed in the research. This, however, does not comply with the results of the two viruses GLRaV-3 and GVA (Manini et al., 1998). It could be ascribed to the specific activity of individual viruses. Virus diseases do not affect the anthocyanins profile in grape therefore the use of the anthocyanins profile in the cultivar classifications has been proved to be correct.

5 ACKNOWLEDGMENT

We would like to express our thanks to Dr. F. Mattivi who enabled us to carry out the analysis in the laboratory Dipartamento Laboratorio Analisi e Ricerche, Istituto Agrario di San Michele, in Italy, and to Mr. D. Tonon for his help in the conduct of the analysis.

6 REFERENCES

Anderson M.D., Chen Z., Klessing F. 1997. Possible involvement of lipid peroxidation in salicylic acid-mediated induction of PR-1 gen expression. Phytochemistry, 47, 4: 555-566.

Baruah B.P., Chowfla S.C. 1994. Physico-chemical changes in healthy and Turnip mosaic virus-ordinary strains infected cauliflower. Indian J. Hill Farm., 7, 1: 57-61.

Bennett R.N., Wallsgrove R.M. 1994. Secondary metabolits in plant defence mechanisms. New Phytol., 127: 617-633.

Borgo M. 1991. Influenza delle virosi dell'accartocciamento fogliare della vite su alcuni parametri della produzione. Riv. Vitic. Enol., 44, 2: 21-30.

Broadhurst R.B., Jones W.T. 1978. Analysis of condensed tannins using acidified vanillin. J. Sci. Food. Agric., 29: 788-794.

Bucelli P., Faviere V., Giannetti F., Gigliotti A. 1995. Valutazione di alcuni componenti fenolici in cultivar di vite a bacca nera in Toscana. Riv. Vitic. Enol., 48, 1: 39-51.

Dai G.H., Andary C., Cosson-Mondolot L., Boubals D. 1994. Poliphenols and resistance of grapevines to downy mildew. Acta hortic. 381: 763-766.

Di Stefano R., Guidoni S. 1989. La determinazione dei polifenoli totali nei mosti e nei vini. Vignevini, 1/2: 47-52.

Di Stefano R., Cravero M.C., Gentilini N. 1989. Metodi per lo studio dei polifenoli dei vini. Enotecnico: 83-89.

Feucht W. 1994. The localization of phenols at the cellular and tissue level. Acta hortic., 381: 803-815.

Fodor J., Gullner G., Ádám A.L., Barna B., Kömives T., Király Z. 1997. Local and systemic response of antioxidants to Tobacco mosaic virus infection and salicylic acid in tobacco. Role in systemic acquired resistance. Plant Physiol., 114: 1443-1451.

Goetz G., Fkyerat A., Métais N., Kunz M., Tabacchi R., Pezet R., Pont V. 1999. Resistance factors to grey mould in grape berries: identification of some phenolics inhibitors of *Botrytis cinerea* stilbene oxidase. Phytochemistry, 52: 759-767.

Guidoni S., Mannini F., Ferrandino A., Argamante N., Di Stefano R. 1997. The effect of Grapevine leafroll and Rugose wood sanitation on agronomic performance and berry and leaf phenolic content of Nebbiolo clone (*Vitis vinifera* L.). Am. J. Enol. Vitic., 48, 4: 438-442.

Guidoni S., Mannini F., Ferrandino A., Argamante N., Di Stefano R. 2000. Effect of virus status on leaf and berry phenolic compounds in two wine grapevine *Vitis vinifera* cultivars V: Proceedings of the Fifth International Symposium on Grapevine Physiology. Bravdo, B.A. (ur.). Acta hortic., 256: 445-452.

Gutmann M., Feucht W. 1994. Histochemical localization of catechins and procyanidins. Acta hortic., 381: 797-801.

Harborne J.B. 1995. Plant polyphenols and their role in plant defence mechanisms. V: Polyphenols 94, Palma de Mallorca (Spain), May 23-27, 1994. Paris, INRA: 19-26.

Heldt H.W. 1997. Plant biochemistry & molecular biology. Oxford, New York, Tokyo, Oxford University Press: 522 str.

Hu H., Forsey R.J., Blades T.J., Barratt M.E.J., Parmar P., Powell J.R. 2000. Antioxidants may contribute in the fight against ageing: an in vitro model. Mech. Ageing Dev. 121: 217-230.

Jeandet P., Sbaghi M., Bessis R., Meunier P. 1995. The potential relationship of stilbene (resveratrol) synthesis to anthocyanin content in grape berry skins. Vitis, 34, 2: 91-94.

Kaur G., Raheja K.R., Ahuja K.L., Singh M., Labana K.S. 1989. Physico-chemical changes associated with Peanut clump virus in *Arachis hypogaea* L. Biochem. Physiol. Pflanzen, 185: 269-276.

Kaur G., Gill C.K., Rataul H.S., Raheja R.K. 1991. Biocemichal changes in Soybean (*Glycine max* L.) cultivars infected with Yellow mosaic virus. Biochem. Physiol. Pflanzen, 187: 357-371.

Kumar N.N.U. 1991. Effect of Mosaic virus on physiology of mulberry (*Morus alba* L.). Ad. Plant Sci., 4, 1: 183-185.

Mannini F., Gerbi V., Credi R. 1998. Heat-treated V. virus-infected grapevine clones: agronomical and enological modifications. V: Proceedings of the international symposium on the importance of varieties and clones in the production of quality wine, Kecskemet, 24-28 August 1997.- Acta hortic., 473: 155-163.

Mattivi F, Scienza A., Failla O. 1989. *Vitis vinifera*: a chemotaxonomic approach - anthocyanins in the skin. V: Proc. of the 5th International Symposium on Grape Breeding. Vitis, Special Issue 1990: 119-133.

Mattivi F., Zulian C., Nicolini G., Valenti L. 2002. Wine, biodiversity, technology and antioxidants. V: Proc. of an April 26-29, 2001 New York Academy conference, Alcohol and Wine in Health and Disease. Annals New York Academy of Sciences, 975: 37-56.

Mattivi F. 2001. ″HPLC analiza profila antocianov grozdja″. Dipartamento Laboratorio Analisi e Ricerche, Istituto Agrario di San Michele, Italia (personal communication).

Pennazio S., Roggero P. 1998. Systemic acquired resistance against plant virus infections: a reality? J. Plant Pathol., 80, 3: 179-186.

Piermattei B., Piva A., Castellari M., Arfelli G., Amati A. 1999. The phenolic composition of red grapes and wines as infected by *Oidium tuckeri* development. Research Note. Vitis, 38, 2: 85-86.

Rice-Evans C.A., Miller N., Paganga G. 1997. Antioxidant properties of phenolic compounds. Trends in plant sci., 2, 4: 152-159.

Rigo A., Vianello Fabio V., Clementi G., Rossetto M, Scarpa M., Vrhovšek U., Mattivi F. 2000. Contribution of proanthocyanidins to the peroxy radical scavenging capacity of some Italian red wines. J. Agric. Food Chem., 48: 1996-2002.

Robert N., Ferran J., Breda C., Coutos-Thévenot P., Boulay M. 2001. Molecular characterization of the incompatible interaction of *Vitis vinifera* leaves with *Psevdomonas syringae* pv. *pisi*: Expression of genes coding for stilbene synthase and class 10 PR protein. Eur. j. plant pathol., 107: 249-261.

Serkedjieva J., Ivancheva S. 1999. Antiherpes virus activity of extracts from the medicinal plant Geranium sanguineum L. J. Ethnopharmacology, 64, 1: 59-68.

Singleton U.L. Rossi J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic., 16: 144.

Sharma P.N., Chowfla S.C. 19991. Some metabolic changes in *Amaranthus caudatus* L. infected with Amaranthus mosaic virus. Plant Dis. Res., 6, 1: 61-62.

Suresh G.L., Govinda-Rao N., Gopal-Singh B. 1991. Physiological changes in french bean infected by Bean Common Mosaic virus. J. Res. APAU, 19, 2: 79-81.

Taiz L. 1998. Plant Physiology. Sunderland, Sinauer Associates Inc. Publishers, Massachusetts: 792 str.

Tomažič I. 1999. Virus identification and virus impact on grapevine (*Vitis vinifera* L.) ampelographic characteristic. Master of science thesis. Ljubljana, Univ. v Ljubljani, BF: 96 str.

Vrhovšek U., Mattivi F., Waterhouse A.L. 2001. Analysis of red wine phenolics: comparison of HPLC and spectrophotometric methods. Vitis 40 (2): 87-91.

Whitehead T.P., Robinson D., Allaway S., Syms J., Hale A. 1995. Effect of red wine ingestion on the antioxidant capacity of serum. Clin. Chem., 41, 1: 32-35.