TRANS-RESVERATROL CONTENT IN GRAPE CANE AND ROOT OF DIFFERENT SCION-ROOTSTOCK COMBINATIONS

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The trans-resveratrol content of *Vitis vinifera L.* cv. 'Cabernet Sauvignon' and cv. 'Italian Riesling' grafted on five rootstocks was analysed from matured cane and root during 3 years in field conditions. In the past decade resveratrol, one of the stilbene molecules of grape, has been intensively studied and has shown its various promising bioactivities. Resveratrol has been detected in several grape organs, but the highest concentrations occur in cane and in root. Grafts used in viticulture have genetically different root and shoot systems. HPLC-DAD method was used to determine the trans-resveratrol content of the ethanolic extracts of grape canes and roots. Trans-resveratrol contents of the roots ranged from 9.3 to 219.1 mg/kg fresh weight and of the canes from 6.7 to 207.9 mg/kg fresh weight. The concentration of trans-resveratrol was affected mainly by the year in both cases of the scion varieties and we observed significant differences between scion-rootstock combinations ($p<0.05$) under the same cropping conditions. Consequently, it was found that grape canes and roots vary considerably with respect to trans-resveratrol contents depending on the year. The rootstocks have effect on the trans-resveratrol content within the years in the case of 'Italian Riesling' and 'Cabernet Sauvignon'.

Keywords: stilbene, *Vitis sp.*, genotype, root-system, cane, plant organs.

Trans-Resveratrolgehalte in Rebholz und -wurzeln verschiedener Edelsorten/Unterlagen-Kombinationen. Über drei Jahre wurden die trans-Resveratrolgehalte in reifem Holz und und in Wurzeln von *Vitis Vinifera L.*-Weinreben der Sorten 'Cabernet Sauvignon' und 'Welschriesling' im Freiland auf fünf verschiedenen Unterlagen untersucht. In den letzten Jahrzehnten wurde Resveratrol, ein Stilben-Molekül von Weintrauben, intensiv studiert, und es zeigte zahlreiche aussichtsreiche bioaktive Eigenschaften. Resveratrol wurde bereits in verschiedenen Organen von Weintrauben nachgewiesen, aber in den höchsten Konzentrationen findet es sich im Rebholz und in den Wurzeln. Mittels HPLC-DAD wurden die trans-Resveratrolgehalte der ethanolischen Extrakte von Rebholz und -wurzeln bestimmt. Der trans-Resveratrolgehalt betrug bei den Wurzeln zwischen 9,3 und 219,1 mg/kg (Frischgewicht), beim Rebholz 6,7 bis 207,9 mg/kg (Frischgewicht). Die Konzentrationen von trans-Resveratrol wurden bei beiden Edelsorten vom Jahrgang beeinflusst. Ein signifikanter Unterschied wurde zwischen den Edelsorten/Unterlagen-Kombinationen festgestellt ($p<0,05$). Rebholz und -wurzeln variieren sehr stark hinsichtlich ihrer trans-Resveratrolgehalte abhängig vom Jahrgang. Innerhalb der einzelnen Jahrgänge wirken sich bei den Sorten 'Cabernet Sauvignon' und 'Welschriesling' die Unterlagen auf den trans-Resveratrolgehalt aus.
Schlagwörter: Stilbene, *Vitis sp.*, Genotyp, Wurzelsystem, Rebholz, Pflanzenorgane

Resveratrol (3,5,4'-trihydroxystilbene) is a low-molecular-weight non-flavonoid phenolic compound, a stilbene molecule. Its putative medical benefits have been intensively studied during the last decade. This antioxidant agent is thought to fend off cardiovascular diseases and cancer, to influence Alzheimer's diseases and ageing, and to prevent bone loss and diabetes (Tosun and Inkaya, 2009). Resveratrol is naturally produced by plants as self-defence agent in response to biotic or abiotic stress or it can be synthesized constitutively without elicitors (Jeandet et al., 2010), so it can be phytoalexin and also phytoanticipine. It has been identified in more than twenty plant families, including edible species like grape (*Vitis vinifera* Linné), peanut (*Arachis hypogaea* Linné) and blueberries (*Vaccinium spp.*). The fresh grape berry skin contains about 50 to 100 μg/g resveratrol, in red wine usually the concentration ranges from 1.5 to 3 mg/l. (Chun-Fu et al., 2013) As the root of Japanese knotweed (*Polygonum cuspidatum* Siebold & Zucc.), the cane of several *Vitis* species contains resveratrol in significant amounts, in mg/g magnitude. Grape cane may be a great, stable (Zhang et al., 2011) and economic (Rayne et al., 2008) source of resveratrol. Limited data are available on the grape root resveratrol content. The resveratrol content of the vine-stock is influenced by the growing conditions (Bavaresco, 2002). The cane and the root are usually genetically different in viticulture, since the phylloxera epidemics, graftings have been used. According to our knowledge there is no information on the fact that the rootstock does or does not play a role for the resveratrol content of grape organs, except of the leaves (Bavaresco and Zamboni, 1990). The objective of this study was to evaluate the trans-resveratrol content of the canes and roots of two *V. vinifera* L. varieties grafted on five different rootstocks and investigate the impact of the scion-rootstock interaction in this context. The field-experiment was conducted over three years.

MATERIALS AND METHODS

PLANT MATERIAL AND FIELD CONDITIONS

V. vinifera L. cv. 'Cabernet Sauvignon' red wine grape variety and cv. 'Italien Riesling' white wine grape variety were used as scions and Fercal, Georgikon 28 (G28), Ruggeri 140 (R140), Teleki 5C (T5C) and Teleki-Kober 5BB (5BB) as rootstocks.

Samples were collected at the Experimental Vineyard of the Georgikon Faculty University of Pannonia located in Cserszegtomaj (46°49'45" N/17°15'16"E; 110 m altitude) in the Balatonfelvidék wine region of Hungary. 'Cabernet Sauvignon' was planted on different rootstocks in 2004. The 'Italian Riesling' plot was established on the same five rootstock varieties in 1992. The cultivation methods of the experimental plots were the same and there were no differences in pruning and bud load. Prior to the dormant season of 2013 in 2012, the growing degree days (GDD) were 1709 °C and the rainfall (RF) was 293 mm. In 2013 GDD was 1433 °C and RF was 726 mm and in 2014 year it was 1460 °C and 757 mm, respectively.

SAMPLE COLLECTION

The samples were collected in the dormant season, in the second week of February of 2013, 2014 and 2015. Root samples (approximately 100 g fresh weight/vines) were collected from ten vines from each scion-rootstock combination. The ten samples of one scion-rootstock combination were cut in pieces, pooled and ground. Cane samples were collected from the middle of the cane, in every case the fifth node was excised. Each cane sample comprised plant material from 40 vines per scion-rootstock combination. Per vine-stock two pieces of one node section were taken. Per scion-rootstock combination three biological repeats were carried out. All samples were collected from sound grafts and kept at 4 °C. The sample processing was carried out within two weeks after collection.

SAMPLE PROCESSING

Sample preparation and extraction was performed according to the method of Rayne et al. (2008) with minor modifications. The pooled root samples of one scion-rootstock combination were divided for three technical repeats before further processing. 100 to 120 g of plant material from each sample were treated with liquid nitrogen, then ground with Moulinex AR100 mill (Groupe SEB, Ecully, France) and kept at -70 °C in the dark till further processing. 1 g of the fresh sample powder (particle size <1 mm) was extracted in three steps in 8 ml ethanol-water (80:20 v/v) with 10 min ultrasound treatment of 38 kHz (Realsonic RS-26, Realtrade, Budapest, Hungary) and mixing (VWR-IKA VMS-C4, IKA Werke, Staufen im Breisgau, Germany) at room temperature. The mixture was centrifuged at 5500 g at 2 °C for 10 min and filtered. The combined supernatants were evaporated under a nitrogen stream at 35 °C (RV06-ML, HB4 basic, IKA Werke, Staufen im Breisgau, Germany). The dried residue was re-dissolved in 1 ml of extracting solution and kept at -20 °C in the dark. The solution was filtered (pore size 0.45 µm cellulose filter) prior to HPLC injection.

CHEMICALS AND REAGENTS

All chemicals and the trans-resveratrol (3,5,4'-trihydroxy-trans-stilbene) standard (>99 % purity) were obtained from VWR (Radnor, PA, USA).

CHROMATOGRAPHIC METHODS

The analyses of cane samples were performed according to Kállay and Török (1997) using HP1050 HPLC (Hewlett Packard, Palo Alto, CA, USA) connected to an adjustable wavelength detector and HP 3396 integrator. The analytical column Lichrospher 100 CN 4x250 mm (Merck, Darmstadt, Germany) was thermostated at 30 °C. Separation was carried out under isocratic conditions using water-acetonitrile-methanol (90:5:5 v/v) as an eluent. The flow rate was 1 ml/min, the injection volume was 20 µl and the wavelength of detection was 306 nm.

HPLC-DAD analyses of root samples were conducted on Agilent 1100 chromatograph (Agilent, Santa Clara, CA, USA) coupled with a diode array detector (DAD G1315B), a model G1379A degasser, G1311A quaternary pump, G1313A autosampler and Agilent Chemstation Rev.B.04.03 SP1 data collection software. The analytical column Zorbax Eclipse XDB-C8 4.6 × 150 mm (Agilent, Santa Clara, CA, USA), was thermostatically controlled at 40 °C. The mobile phase "A" was water with 0.5 % formic acid and the mobile phase "B" was methanol. The flow rate was 0.5 ml/min and the injection volume was 5 µl. Gradient elution was performed from 7.5 % to 55 % eluent "B" in 37 minutes with the following gradient program: 0 ▸ 7.0 min 7.5 % B; 7.5 ▸ 37.0 min 7.5 % ▸ 55 % B; 37.0 ▸ 42.0 min 55 % B; 42.0 ▸ 56.0 min 55 % ▸ 7.5 % B. The UV detector was set at 320 nm for resveratrol and at 362 nm reference wavelength for testing the purity of peaks. Trans-resveratrol has a maximum absorption at 320 nm and no absorption at 362 nm counter to other phenolic compounds with similar retention time.

Quantification was performed using external 5-point calibration curve (0.1, 1.0, 10.0, 40.0, 100.0 mg/l). The assay for resveratrol standard was linear in the range 0.10 to 100 mg/l with a high correlation coefficient (r = 0.999). The slope of the calibration curve was 0.0076

ppm/mAUs. The limit of detection was 0.33 mg/l and the limit of quantification was 1.11 mg/l. The precision was determined with root sample injected 10 times, and expressed as relative standard deviation, which was 3.28 %. The inter-day precision was 2.8 % (n = 3). Accuracy was determined by standard addition (5, 10 and 40 mg/l) to a root sample (33.92 mg/l), the recovery rate was 95.52 to 98.94 %.

STATISTICAL ANALYSIS

Data were analysed by two-way analysis of variance (ANOVA) with SPSS 15. The Levene's test of equality of error variances for testing of the homogeneity of variances was performed and it was not significant. The normal distribution was provable in case of the data per year, but was not provable over the three-year period. In the latter case the "n" was big enough to make the Duncan test and to use the Partial Eta Squared (PES). In order to compare the two scions Student's t-test was performed and SD5 % values were calculated. In order to evaluate the rootstock's effect in each year in case of two scions 12 independent Duncan Post Hoc test was performed. $P<0.05$ was considered statistically significant. In order to characterize the intensity of the investigated effects, the data of three years, two scions and five rootstocks was evaluated together, the PES was determined.

RESULTS AND DISCUSSION

TRANS-RESVERATROL CONCENTRATION OF GRAPE CANES

The 'Italian Riesling' canes had at least 10 times higher trans-resveratrol contents than the 'Cabernet Sauvignon' for all the five rootstocks after the arid 2012 year. The results were quite contrary in the other years and there was smaller but still significant difference between the scions (Fig. 1).

This difference could have been caused by the different weather conditions of the years. In each investigated year there were significant differences ($p<0.05$) in the trans-resveratrol content between scion-rootstock combinations (Table 1). The ranking of the rootstocks according to resveratrol content over the years is not constant. Various studies have been conducted with different grape species and varieties in different countries. Aaviksaar et al. (2003) measured 100 to 4700 mg trans-resveratrol content per kg dry weight in different grape hybrid samples originated from Estonian vineyards. Karacabey et al. (2012) and Rayne et al. (2008) measured about 3000 mg/kg (dry weight) trans-resveratrol in 'Pinot noir' canes in Canada. In 'Cabernet Sauvignon' canes Zhang et al. (2011) observed 1237 mg/kg (fresh weight) trans-resveratrol in China, Gorena et al. (2014) 2407 mg/kg (dry weight) in Chile. The variation of stilbene concentrations measured in grape organs can be high in different studies. Ji et al. (2014) showed that the trans-resveratrol concentration in *Vitis amurensis* Ruprecht organs differed significantly in different seasons. Even the post-pruning storage before the analysis of the cane samples has an effect on the measurement results (Vergara et al., 2012). Trans-resveratrol concentration in the berry is also significantly affected by the variety and by the elevation (Bavaresco et al., 2007). According to our results rootstocks affect the concentration of the trans-resveratrol in grape canes. We have observed significant differences ($p<0.05$) among the rootstocks within both scion and each examined years (Table 1).

TRANS-RESVERATROL CONCENTRATION OF GRAPE ROOTS

The trans-resveratrol concentrations are different in the roots of the grafts as per rootstock genotype (Table 2), but differences by the scion were also measured (Fig. 2).

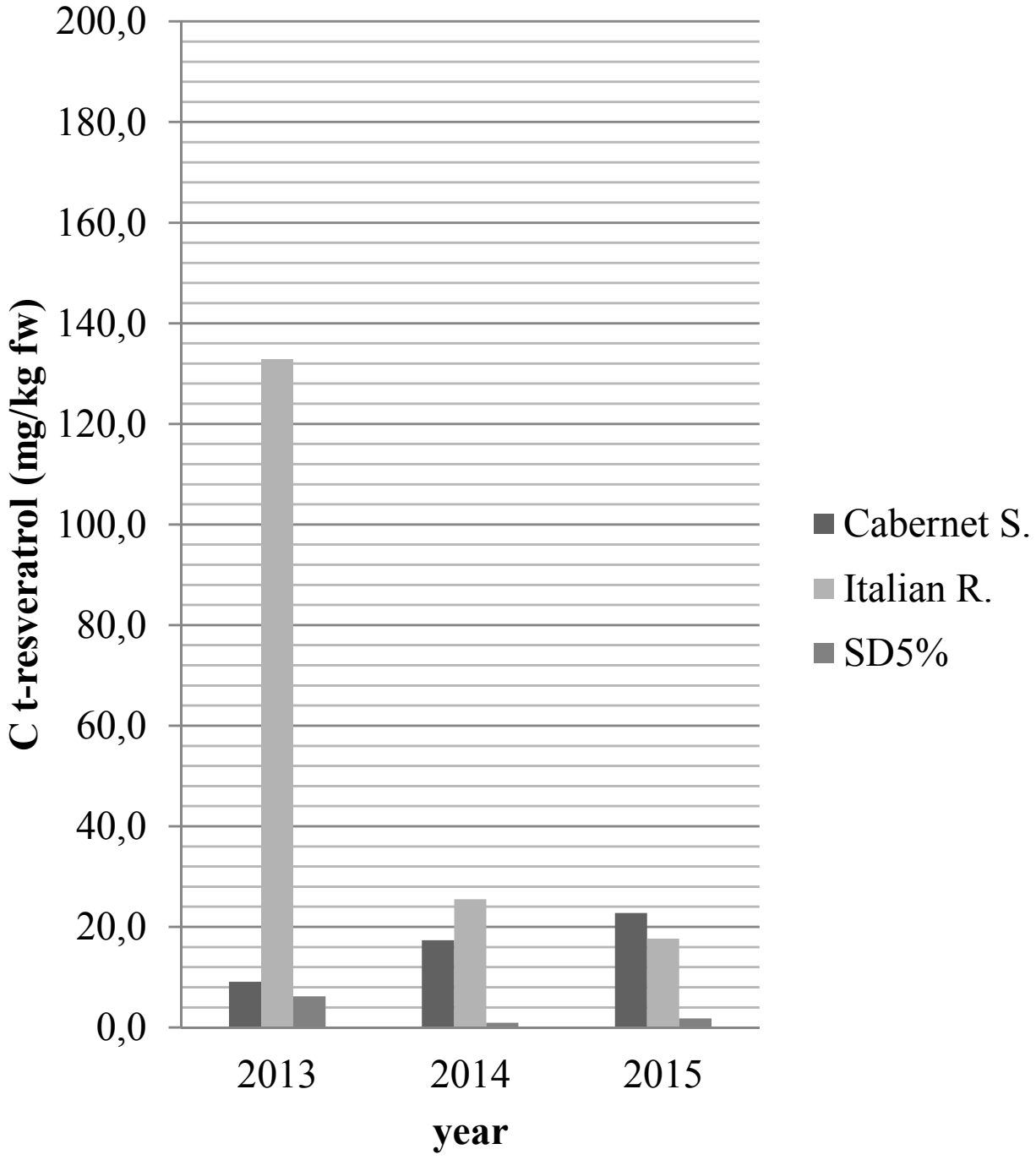

Fig. 1: Trans-resveratrol content of canes of 'Cabernet Sauvignon' and 'Italian Riesling' in three consecutive years (SD5% shows the smallest significant difference ($p<0.05$) between the two scions within a year. This value is derived from Student's t-test.)

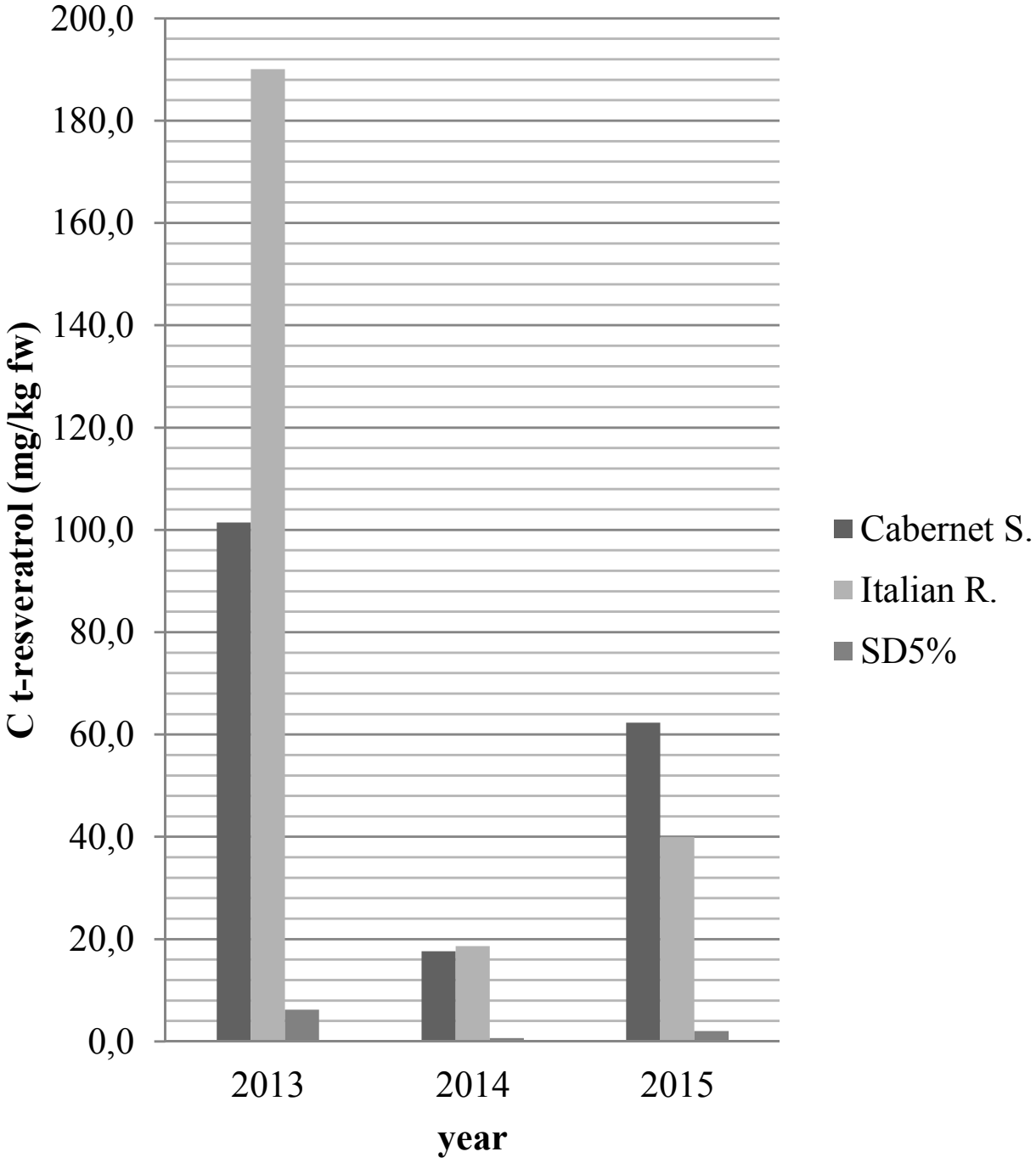

Fig. 2: Trans-resveratrol content of roots of 'Cabernet Sauvignon' and 'Italian Riesling' in three consecutive years (SD5% shows the smallest significant difference ($p<0.05$) between the two scions within a year. This value is derived from Student's t-test.)

Table 1: Production of resveratrol in the canes of 'Cabernet Sauvignon' and 'Italian Riesling' grafted on 5 different rootstocks

Trans-resveratrol concentration (mg/kg fresh weight, mean ± S.D.)						
Rootstock	2013		2014		2015	
	Cabernet S.	Italian R.	Cabernet S.	Italian R.	Cabernet S.	Italian R.
T5C	6.7b ± 2.4	72.8b ± 3.6	21.1d ± 2.3	19.3b ± 1.6	21.7c ± 1.1	26.8a ± 7.5
R140	9.1ab ± 0.8	90.5bc ± 26.5	10.4b ± 0.7	23.6c ± 0.8	17.6b ± 1.2	15.3bc ± 0.9
G28	8.6b ± 1.1	189.5a ± 21.0	13.5bc ± 3.2	33.3a ± 1.8	17.3b ± 0.2	21.9ac ± 4.3
5BB	11.7a ± 1.6	207.9a ± 12.8	14.7c ± 1.5	28.4d ± 1.4	18.2bc ± 3.4	11.6b ± 2.0
Fercal	9.4ab ± 0.2	103.7c ± 1.6	27.1a ± 1.2	23.0c ± 1.1	39.1a ± 2.5	12.6b ± 2.7

Samples were collected in February 2013, 2014 and 2015. Data are the means of 3 replicates. Concentrations within a column having the same letters are not significantly different at $p<0.05$.

Table 2: Production of resveratrol in the roots of 'Cabernet Sauvignon' and 'Italian Riesling' grafted on 5 different rootstocks

Trans-resveratrol concentration (mg/kg fresh weight, mean ± S.D.)						
Rootstock	2013		2014		2015	
	Cabernet S.	Italian R.	Cabernet S.	Italian R.	Cabernet S.	Italian R.
T5C	80.1b ± 10.0	216.4c ± 7.5	15.5c ± 1.1	17.4c ± 1.8	51.7b ± 1.7	39.2cd ± 1.1
R140	149.1a ±11.7	219.1c ± 13.7	12.5b ± 1.3	9.3b ± 0.7	59.0c ± 1.5	43.4d ± 1.3
G28	89.7b ± 6.7	157.3b ± 15.3	27.6a ± 1.2	20.9d ± 1.8	64.1c ± 6.4	58.4a ± 8.4
5BB	142.1a ± 6.3	186.3a ± 7.2	18.2d ± 0.6	26.6a ± 1.4	89.3a ± 2.4	33.3c ± 2.3
Fercal	46.4c ± 2.1	171.2ab ± 21.9	14.3bc ± 0.8	18.9cd ± 1.8	47.4b ± 2.5	25.4b ± 1.7

Samples were collected in February 2013, 2014 and 2015. Data are the means of 3 replicates. Concentrations within a column having the same letters are not significantly different at $p<0.05$.

The 'Italian Riesling' produced higher trans-resveratrol concentration in the root than the 'Cabernet Sauvignon' in 2013 with all the five rootstocks. In 2015 the inverse was observed. The data suggest that interaction exists between scion and rootstock driven by weather conditions of the year. As for the canes in each investigated year there were significant differences between scion-rootstock combinations. The Fercal rootstocks gave the lowest trans-resveratrol content with 'Cabernet Sauvignon' in all the three years and with 'Italian Riesling' in 2013 and 2015 (Table 2).

In literature fewer data are available about the grape root resveratrol content. In roots of *V. vinifera* cv. 'Cabernet Sauvignon' Wang et al. (2010) measured approx. 5 mg/kg (fresh weight) trans-resveratrol, and in *V. amurensis* roots Ji et al. (2014) measured 61 to 123 mg/kg (dry weight). Bavaresco et al. (2003) measured 15.5 mg/kg (fresh weight) trans-resveratrol in the roots of 'Chardonnay' on Kober 5BB. Our findings of trans-resveratrol concentrations in grape roots are comparable to the values of *V. amurensis* roots.

EFFECT OF YEAR, SCION AND ROOTSTOCK GENOTYPE

The trans-resveratrol concentration of canes of all examined combinations was significantly higher in 2013 than in the following two years. In case of roots these values in all the three years differed significantly ($p<0.05$) According to our knowledge long-term field experiments have not been conducted yet in this topic. The influence of the months during a year to the trans-resveratrol content of *V. amurensis* roots and vines was observed by Ji et al. (2014). The rootstock-effect in this field has been little investigated. Bavaresco and Zamboni (1990) found that the rootstocks have an influence on the resveratrol content of the grape leaves. Studies reported the impact of the rootstocks on the

vine yield as well as its quality, including the polyphenol content of the berries (Brighenti et al., 2012; Kocsis et al., 2012). The trans-resveratrol content of the cane is mainly influenced by the year (PES = 0.945) and the scion (PES = 0.932) and the rootstock has moderate effect (PES = 0.716). In case of the roots the main effect was also the year (PES = 0.989) and both the scion (PES = 0.791) and the rootstock (PES = 0.768) have less influence.

CONCLUSION

The results of this work show that the canes and the roots are rich in trans-resveratrol and its amount is influenced by the year and the rootstock genotype in case of 'Cabernet Sauvignon' and 'Italian Riesling'. The year has inductive effect so the stilbene content of matured canes and dormant roots is affected by the weather conditions of the prior active vegetative and generative period. The trans-resveratrol content of grape canes and roots highly depend on the cultivar genotypes. In spite of the strong effect of the scion and yearly variable weather conditions the grape rootstocks have effect on trans-resveratrol content in field condition.

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REFERENCES

Aaviksaar, A., Haga, M., Pussa, T., Roasto, M. and Tsoupras, G. 2003: Purification of resveratrol from vine stems. Proceedings of the Estonian Academy of Sciences, Chemistry 52: 155-164.

Bavaresco, L. 2002: Role of viticultural factors on stilbene concentrations of grapes and wine. Drugs under Experimental and Clinical Research 29 (5-6): 181-187.

Bavaresco, L., Pezzutto, S., Fornaroli, A. and Ferrari, F. 2003: Grapevine iron-chlorosis occurrence and stilbene root concentration as affected by the rootstock and arbuscular mycorrhizal infection. Acta Horticulturae 603: 665-670.

Bavaresco, L., Pezzutto, S., Gatti, M. and Mattivi, F. 2007: Role of the variety and some environmental factors on grape stilbenes. VITIS-Journal of Grapevine Research 46: 57-61.

Bavaresco, L. and Zamboni, M. 1990: Influence of the rootstock and potassium fertilizer on phytoalexin synthesis in Pinot blanc grown in a calcareous soil. VITIS-Journal of Grapevine Research 29: 295-299.

Brighenti, A., Rufato, L., Kretzschmar, A., Würz, D., Duarte, A., Brighenti, E., Lages, S., Malinovski, L. and da Silva, A. 2012: Effect of Different Rootstocks on Productivity and Quality of 'Cabernet Sauvignon'Grapevine Produced in High Altitude Regions of Santa Catarina State, Brazil. Acta Horticulturae 931: 379-384.

Chun-Fu, W., Jing-Yu, Y., Fang, W. and Xiao-Xiao, W. 2013: Resveratrol: botanical origin, pharmacological activity and applications. Chinese Journal of Natural Medicines 11: 1-15.

Gorena, T., Saez, V., Mardones, C., Vergara, C., Winterhalter, P. and von Baer, D.2014: Influence of post-pruning storage on stilbenoid levels in Vitis vinifera L. canes. Food Chemistry 155: 256-263.

Jeandet, P., Delaunois, B., Conreux, A., Donnez, D., Nuzzo, V., Cordelier, S., Clément, C. and Courot, E. 2010: Biosynthesis, metabolism, molecular engineering, and biological functions of stilbene phytoalexins in plants. Biofactors 36: 331-341.

Ji, M., Li, Q., Ji, H. and Lou, H. 2014: Investigation of the distribution and season regularity of resveratrol in

Vitis amurensis via HPLC–DAD–MS/MS. Food Chemistry 142: 61-65.

Kállay, M. and Török, Z. 1997: Determination of resveratrol isomers in Hungarian wines. Horticultural Science Kertészeti Tudomány 29 (3-4): 78-82.

Karacabey, E., Mazza, G., Bayindirli, L. and Artik, N. 2012: Extraction of bioactive compounds from milled grape canes (*Vitis vinifera*) using a pressurized low-polarity water extractor. Food and Bioprocess Technology 5: 359-371.

Kocsis, L., Tarczal, E. and Kállay, M. 2012: The effect of rootstocks on the productivity and fruit composition of Vitis vinifera L.'Cabernet Sauvignon' and 'Kékfrankos'. Acta Horticulturae 931: 403-412.

Rayne, S., Karacabey, E. and Mazza, G. 2008: Grape cane waste as a source of trans-resveratrol and trans-viniferin: High-value phytochemicals with medicinal and anti-phytopathogenic applications. Industrial Crops and Products 27 (3): 335-340.

Tosun, I. and Inkaya, A.N. 2009: Resveratrol as a health and disease benefit agent. Food Reviews International 26: 85-101.

Vergara, C., von Baer, D., Mardones, C., Wilkens, A., Wernekinck, K., Damm, A., Macke, S., Gorena, T. and Winterhalter, P. 2012: Stilbene levels in grape cane of different cultivars in southern Chile: determination by HPLC-DAD-MS/MS method. Journal of Agricultural and Food Chemistry 60: 929-933.

Wang, W., Tang, K., Yang, H.-R., Wen, P.-F., Zhang, P., Wang, H.-L. and Huang, W.-D. 2010: Distribution of resveratrol and stilbene synthase in young grape plants (*Vitis vinifera* L. cv. Cabernet Sauvignon) and the effect of UV-C on its accumulation. Plant Physiology and Biochemistry 48: 142-152.

Zhang, A., Fang, Y., Li, X., Meng, J., Wang, H., Li, H., Zhang, Z. and Guo, Z. 2011: Occurrence and estimation of trans-resveratrol in one-year-old canes from seven major Chinese grape producing regions. Molecules 16: 2846-2861.

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