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Resveratrol and piceid isomers concentrations in grapevine shoots, leaves, and tendrils

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Abstract

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The objective of this study was to evaluate the levels of *cis-* and *trans*-isomers of resveratrol and piceid contained in the shoots, leaves and tendrils of six grapevine varieties and three locations processed under two different drying conditions. The highest *trans*-resveratrol content was found in the shoots; *trans*-piceid was contained in lesser amounts (7%) and *cis*-forms only in very small amounts (~1%). In leaves, both forms of piceid were dominant, while in tendrils *trans-* and *cis*-forms of piceid were dominant in samples dried in the laboratory oven at 40°C. Pinot Noir differed from other varieties with a high *trans*-resveratrol amount. Growing location affected *trans*-resveratrol levels. Our results suggest that the trimmed clippings might be used as a valuable and inexpensive source of stilbenes. Clippings preserved by drying might be further processed to nutraceuticals or as an additive to the feed.

Keywords: phenolic stilbenes; vine trimming; vine clippings

Stilbenes are phenolic compounds comprising two aromatic rings linked by an ethane bridge (Garrido, Borges 2013). Among them, *trans*-resveratrol is an important and leading stilbenoid compound occurring in some plants, and its biological activity for human-health benefits has been extensively studied for nearly 70 years (Aggarwal et al. 2004). In addition to resveratrol, its glycoside (piceid) has also shown beneficial effects on health – it can inhibit low-density lipoprotein oxidation, reduce platelet aggregation, and act as a tumour and metastatic carcinoma inhibitor (Fernández-Mar et al. 2012). The biological activities of the other isomers (*cis*-resveratrol, *trans*-piceid and *cis*-piceid) have not been thoroughly studied, but it seems that *cis*-resveratrol and both piceids may also have beneficial effects (Chong et al. 2009). Resveratrol has attracted much research interest because of its effects which have been demonstrated in all three stages of carcinogenesis (Bavaresco et al. 2012) and additionally, together with other stilbenoids, it possesess anti-inflammatory and anti-tumour properties and can also prevent cardiovascular disease (Li et et al. 2012).

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Agricultural biomass waste represents a largely ignored source of high-value phytochemicals which become value-added industrial products that could contribute to sustainability objectives (Rayne et al. 2008) as it is the case of cane pruning (Çetin et al. 2011). Moreover, summer trimming is performed to maintain desired canopy shape best suited for optimal grape production. During this process considerable amount of green mass (portions of shoots, leaves, tendrils) is removed. In grapes, *trans*-resveratrol is distributed mainly in a glycosylated form called piceid. *Trans*-Resveratrol and piceid were found in certain abundance in grape seeds, shoots and leaves (Lachman et al. 2004; Liu et al. 2013). The highest concentration of *trans*-resveratrol was reported in branches (specifically in the phloem tissue) and lower concentration in leaves (Wang et al. 2010). When comparing by-products, it was found that marc and rachis material contain higher amounts of polyphenols and impose higher antioxidant activities than lees (Alonso et al. 2002). It has been shown that berry rachises may contain even higher amounts of flavan-3-ols and flavonols than the berries at the stage of maturity (Doshi et al. 2006). Grape shoots are particularly rich in flavonoids and stilbenes, with *trans*-resveratrol and e-viniferin present in considerably high concentrations (Anastasiadi et al. 2012). Also leaves may contain high levels of *trans*-resveratrol and *cis*-resveratrol with differences between the position of leaves on the shoot i.e. insertion levels (Schoedl et al. 2012).

Different levels of stilbenoids have been reported in the cane of *V. vinifera* in different cultivars and different growing regions (Karacabey, Mazza 2008; Vergara et al. 2012). Environmental factors and type of post-pruning storage may induce stilbenoid biosynthesis (Gorena et al. 2014). The effect of grape genotype in promoting both the accumulation of resveratrol and piceid could also be affected by UV-C irradiation (Liu et al. 2010). Also, *Vitis amurensis* was found to be a rich source of resveratrol (Ji et al. 2014). In a recent study Barros et al. (2014) evaluated the grape shoots from Portuguese varieties as a rich source of polyphenolic compounds – flavanols, proanthocyanidins, flavonols, and hydroxycinnamic acids. The wine industry produces up to 30% (w/w) of grape pomace which might be used as a very valuable source of phytochemicals. The aim of the present study was to evaluate the levels of stilbenoids – *trans*-resveratrol and *cis*-resveratrol and *trans*-piceid and *cis*-piceid contained in the shoots, leaves and tendrils of six grapevine varieties grown in the Czech Republic, processed under different drying conditions. While limited studies have evaluated the levels of *trans*-resveratrol in grape shoots and leaves, to the best of our knowledge, no other work has extended this type of investigation to tendrils and to the effects of drying conditions on stilbene levels. For this reason, the amounts of stilbenes in the biomass of summer trimmings and the effects of two drying procedures on their preservation were evaluated.

MATERIAL AND METHODS

Plant material. Grape shoots, leaves and tendrils of six vine varieties (Hibernal, Pinot Gris, Pinot Noir, St. Laurent, Sauvignon Blanc, Zweigeltrebe) were collected after summer trimming from the vine-growing area Karlštejn (Bohemia region) in July–August 2013 (Table 1). In addition, samples of two varieties were also collected from two other vine-growing areas: Prague – Grébovka (Bohemia region) and Velké Bílovice (Moravia Region). To assess the effect of growing area on stilbene levels Pinot Gris and Pinot Noir were collected from all three localities. The trimmed biomass was separated manually into shoots, leaves and tendrils and subsequently dried at 35°C for 48 hours. A St. Laurent sample (Karlštejn) was subjected to two types of drying – at ambient temperature in the dark (22°C) and in the laboratory forced air oven. Agricultural practises at all growing locations were the same. Laboratory sample consisted of twenty canes.

Sample preparation. For extraction, 0.2 g of ground sample was weighed into 50-ml falcon tube and 10 ml of methanol:ethyl acetate (1:1) solution was added. Samples were left in the dark for 18 hours. Then, the sample was sonicated for 10 min. and centrifuged at 8,228 g (centrifuge 5810R, Eppendorf AG, Hamburg, Germany). The supernatant was transferred into 50 ml glass evaporation flask and the extraction repeated twice with 5 ml of methanol:ethyl acetate mixture. The pooled extract was evaporated to dryness in a vacuum evaporator R-200 (Büchi Labortechnik AG, Flawil, Switzerland) at 40°C and redissolved in 4 ml of 100% methanol, filtered through a 0.22 micron PVDF syringe filter

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Table 1. Grapevine varieties, localities and drying conditions used in the study

Vine variety	Part of vine plant	Date of pruning (2013)	Drying method	Vine growing location	Av. temp. (°C)	Av. S prec. (mm)	Av. SD (h)
St. Laurent	leaves	July 22	oven	Karlštejn (B)	15.0	97.00	–
St. Laurent	shoots	July 22	oven	Karlštejn (B)	15.0	97.00	–
St. Laurent	tendrils	July 22	oven	Karlštejn (B)	15.0	97.00	–
St. Laurent	leaves	July 22	amb., dark	Karlštejn (B)	15.0	97.00	–
St. Laurent	shoots	July 22	amb., dark	Karlštejn (B)	15.0	97.00	–
St. Laurent	tendrils	July 22	amb., dark	Karlštejn (B)	15.0	97.00	–
St. Laurent	pruned parts	July 20	oven	Karlštejn (B)	15.0	97.00	–
Hibernal	pruned parts	July 9	oven	Karlštejn (B)	15.0	97.00	–
Sauvignon Blanc	pruned parts	July 9	oven	Karlštejn (B)	15.0	97.00	–
Zweigeltrebe	pruned parts	July 10	oven	Karlštejn (B)	15.0	97.00	–
Pinot Gris	pruned parts	July 15	oven	Karlštejn (B)	15.0	97.00	–
Pinot Noir	pruned parts	August 6	oven	Karlštejn (B)	15.0	97.00	–
Pinot Gris	pruned parts	July 8	oven	Velké Bílovice (M)	15.9	53.73	220
Pinot Noir	pruned parts	July 8	oven	Velké Bílovice (M)	15.9	53.73	220
Pinot Gris	pruned parts	July 8	oven	Prague-Grébovka (B)	16.5	78.18	192
Pinot Noir	pruned parts	July 8	oven	Prague-Grébovka (B)	16.5	78.18	192

(B) – Bohemian region; (M) – Moravian region; Av. temp. – average temperature in April–July 2013; Av. S prec. – average sum of precipitation in April–July 2013, Av. SD – average sunshine duration in April–July 2013 (not available in Karlštejn); amb. – ambient

into amber HPLC vial and analysed. Samples were analysed in triplicates to assess varieties and localities; and in six replicates to assess drying and type of plant organ.

Determination of *cis-* and *trans-*resveratrol and *cis-* and *trans-*piceid in grape canopy. Analyte quantitation was carried out by HPLC (Ultimate 3000 RS, Thermo Fisher Scientific, Dionex, Sunnyvale, USA) equipped with a binary pump, autosampler and column heater. Pinnacle DB C18 column was used (50 × 2.1 mm, 1.9 μm; RESTEK, Bellefonte, Pennsylvania, USA) with gradient elution. Mobile phase consisted of acetonitrile (A) and 0.1% acetic acid (B) (V/V, HPLC super gradient). The following gradient was used: 0–0.2 min 5% A; 95% B (isocratic), 0.2–5 min 65% A, 35% B (linear gradient), 5–5.2 min 5% A, 95% B (linear gradient), 5.2–7 min 5% A, and 95% B (isocratic). Flow rate 0.35 ml/min; injection 1 μl, column temperature 30°C, sampler temperature 10°C, time of analysis 7 minutes. Detection was performed with a 3200 Q trap hybrid triple quadrupole – linear ion trap mass spectrometer (AB Sciex, Foster City, USA) with ESI (electro-spray ionisation). Data were evaluated with Analyst 1.4 Software. The LOD and LOQ values for individual analytes in ng/ml were for: *trans*-piceid 1 and 3, *cis*-piceid 1 and 3, *trans*-resveratrol 3 and 9, *cis*-resveratrol 1 and 4, respectively.

Statistical evaluation. Results for six grapevine varieties (Hibernal, Pinot Gris, Pinot Noir, St. Laurent, Sauvignon Blanc, Zweigeltrebe) from Karlštejn, and Pinot Gris and Pinot Noir from three vine-growing areas (namely Prague-Grébovka, Karlštejn and Velké Bílovice) were analysed by one-way and two-way analysis of variance; the Statistica ver. 12 at $P < 0.05$ was used. Tukey's HSD test was used *post hoc* to determine differences between varieties and growing areas. The data sets were tested for normal distribution and parametric tests (ANOVA) applied.

RESULTS AND DISCUSSION

Content of stilbenoids in different plant organs and effect of method of drying

It is evident that the highest level of *trans*-resveratrol was contained in grape shoots (90–91%) of St. Laurent variety from Karlštejn (Fig. 1, Table 2).

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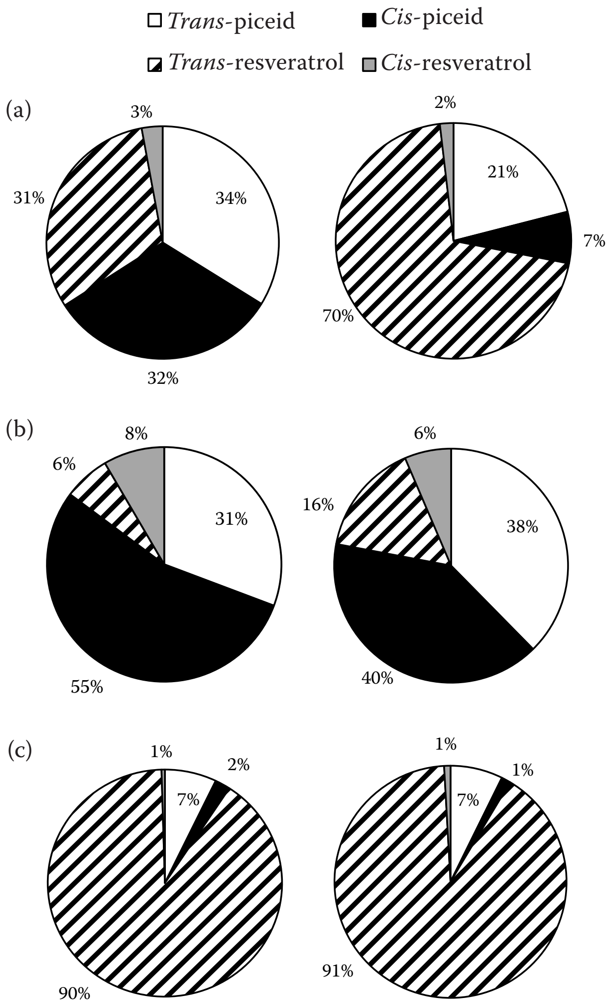

Fig. 1. Average proportions of major stilbenes in St. Laurent grapevine (a) tendrils, (b) leaves and (c) shoots dried in an oven (left) and at ambient temperature (right) in the dark, Karlštejn, Czech Republic

Also, *trans*-piceid was contained at a moderate level (7% of total stilbenoids). *Cis*-forms were present in only very small amounts (about 1%). Drying conditions did not affect the analytes except *cis*-piceid (Table 2). A similar situation in stilbene composition was observed for tendrils with the highest content of *trans*-resveratrol under drying at laboratory conditions (70% of total stilbenoid content) and *trans*-piceid (21%). When tendrils were dried in an oven, the ratio of *trans*-piceid, *trans*-resveratrol and *cis*-piceid were nearly equal (34%, 31%, and 32%, respectively). *Cis*-resveratrol was present in very small amounts (2–3%). A different situation occurred in leaves, where the dominant stilbene was *cis*-piceid (40% and 55%), followed by *trans*-piceid (38% and 31%). *Trans*-resveratrol (16% and 6%) and *cis*-resveratrol (6% and 8%) were present in lesser amounts. Higher concentrations were found for *trans*-resveratrol dried in a laboratory oven. Tukey's HSD test revealed that both methods of drying did not show statistically different levels of *trans*-piceid and *trans*- and *cis*-resveratrol.

Our findings are in agreement with the fact that the highest concentration of *cis*-piceid was found in all basipetal leaves whereas the *trans*-form seemed to occur randomly (Schoedl et al. 2012). The higher concentrations of *cis*-piceid in basipetal leaves may indicate earlier induction of stress response against UV irradiation in the elder leaves. *Trans*-resveratrol, *trans*-piceid and *cis*-piceid were found but *cis*-resveratrol was not detected in grape leaves in three grape cultivars, while of the total stilbenes, *cis*-piceid was dominant (Liu et al. 2013). Results indicated that grape stems comprise a rich source of bioactive stilbenes. In particular, *trans*-resveratrol was detected in considerable concentrations (Anastasiadi et al. 2012).

Variety differences of stilbene content

The highest level of *trans*-resveratrol among grape varieties was found in Pinot Noir shoots (15.90 ± 2.99 mg/kg dry matter (DM)), which differed from other varieties (Fig. 2). For *cis*-resveratrol no differences were found among varieties. High levels of *cis*-piceid were typical for Hibernal (17.16 ± 6.03 mg/kg DM) and Sauvignon Blanc (8.85 ± 2.99 mg/kg DM). Similarly, Hibernal and Sauvignon Blanc also contained higher levels of *trans*-piceid (9.39 ± 2.48 and 4.46 ± 0.42 mg/kg DM, respectively).

Different levels of stilbenoids were reported in grape canes. In particular, *trans*-resveratrol levels of 4.25 mg/kg DM were reported in Pinot Noir in Canada (Karacabey, Mazza 2008), which were similar to levels reported by Vergara et al. (2012) for the same cultivar in South Chile. Levels of *trans*-resveratrol of up to 6.53 mg/kg DM were reported in Gewürztraminer canes (Vergara et al. 2012). The *trans*-resveratrol concentrations in our samples compare favourably with this available dataset. However, higher levels of *trans*-resveratrol were reported in China by Zhang et al. (2011), who found concentrations between 570 and 1,751 mg/kg fresh matter (FM) in one-year-old canes. Püssa et al. (2006) reported between 1,100 and 3,200 mg/kg DM for hybrid cultivars from Estonia. Contrary,

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Table 2. Influence of drying methods on stilbene content in different parts of the plant of St. Laurent

	Trans-piceid			*Cis*-piceid	
	oven[a]	laboratory[a]		oven[a]	laboratory[b]
ten[3]	0.55 ± 0.04	0.53 ± 0.11	ten[2]	0.52 ± 0.04	0.18 ± 0.09
lea[1]	1.16 ± 0.03	1.22 ± 0.26	lea[1]	2.05 ± 0.07	1.31 ± 0.33
sho[2]	0.99 ± 0.17	0.75 ± 0.20	sho[2]	0.30 ± 0.08	0.13 ± 0.04
	Trans-resveratrol			*Cis*-resveratrol	
	oven[a]	laboratory[a]		oven[a]	laboratory[a]
ten[2]	0.51 ± 0.11	1.76 ± 0.51	ten[2]	0.05 ± 0.00	0.05 ± 0.03
lea[2]	0.24 ± 0.06	0.51 ± 0.33	lea[1]	0.32 ± 0.01	0.21 ± 0.05
sho[1]	12.5 ± 1.78	9.26 ± 1.53	sho[2]	0.06 ± 0.02	0.09 ± 0.05

data represent the average ± standard deviation (mg/kg DM, n = 6); ten – tendrils, lea – leaves, sho – shoots; different uppercase letters denote significant differences between the drying methods; different uppercase numbers denote significant differences between the plant parts. Statistical analysis was performed by two-way analysis of variance

trans-resveratrol was found in the canes between 9.5 and 39.4 μg/kg which confirmed that stilbene content largely depends on the grape cultivar, climate and extraction conditions (selection of extraction solvents) (Çetin et al. 2011). Grapes produce stilbenes in response to mold infections and physiological stresses such as UV light, heavy metals, etc. These types of treatments have been shown previously to increase stilbene levels up to several hundred-fold in grape skins (Gonzalez-Barrio et al. 2006) and leaves (Adrian et al. 1996). Rayne et al. (2008) also reported that *trans*-resveratrol of Pinot Noir cane extracts produced by different extraction solvents varied from 150 (propane-2-ol) to 3450 mg/kg DM (70:30 ethanol:water volume/volume (v/v)). Karacabey, Mazza (2008) reported *trans*-resveratrol contents varied between 1,300 and 4,100 mg/kg in cane extracts. Anastasiadi et al. (2012) found the average concentration of *trans*-resveratrol to be 149 mg/kg DM for red and 113 mg/kg DM for white varieties. Aaviksaar et al. (2003) determined the presence of *trans*-resveratrol in Estonian grape cultivars in concentrations between 100 and 4,700 mg/kg DM. In the survey of *trans*-resveratrol contents of grape cluster rachis from nine *Vitis vinifera* varieties, Melzoch et al. (2001) found levels ranging from 7 to 480 mg/kg DM. Postharvest stilbene contents of grape cane may possibly be further increased through the exposure to UV light, ozone, or other abiotic stresses.

Effect of growing grape locations on stilbene content

Two vine varieties (Pinot Gris and Pinot Noir) were investigated on growing locations differences (Karlštejn, Velké Bílovice and Prague-Grébovka). For Pinot Gris *trans*-resveratrol differed between all three locations; for Pinot Noir the Karlštejn location stood out (Fig. 3). Similar results were also found for the content of *trans*-piceid with different levels in Pinot Noir. Lower levels of *cis*-resveratrol were different in Pinot Gris, while lesser differences

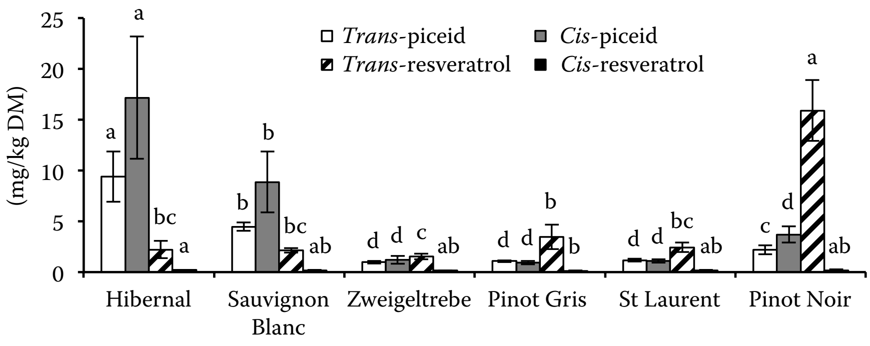

Fig. 2. Levels of major stilbenes, n = 3
columns marked with different letters were statistically different at P < 0.05 and vertical error bars represent standard deviation

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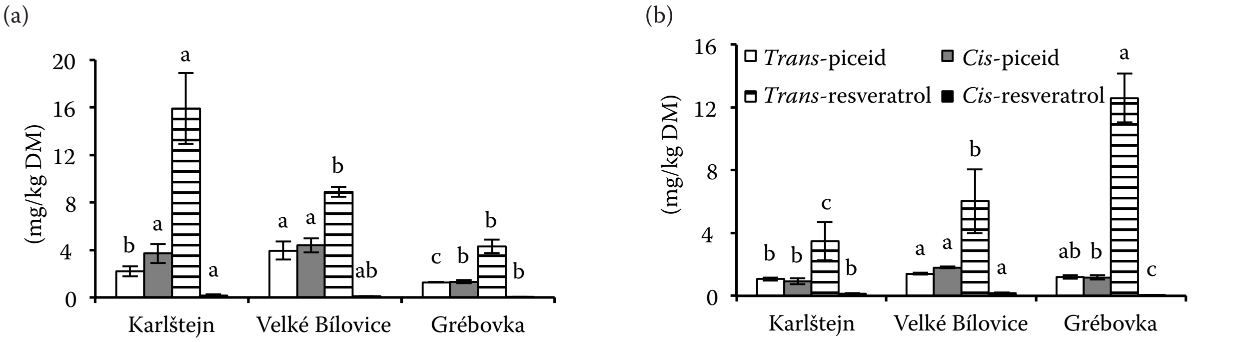

Fig. 3. Stilbenes content in (a) Pinot Noir and (b) Pinot Gris, $n = 3$
columns marked with different letters were statistically different at $P < 0.05$ and vertical error bars represent standard deviation

between localities in both varieties were characteristic for *cis*-piceid.

Interaction between genotype and grapevine location was evaluated by factorial two-way ANOVA analysis (Fig. 4). At assessing the three localities and two varieties it is possible to conclude that location and variety impacts significantly the amount of stilbenes which is nicely depicted in Fig. 4 which shows that each location is convenient for another variety. Localities Karlštejn and Prague-Grébovka demonstrated different effects on the major stilbene *trans*-resveratrol levels in Pinot Noir and Pinot Gris varieties (Fig. 3) and this affected total sum of stilbenes. The highest mean values were found in the case of *trans*-resveratrol which can be considered as a predominant stilbenoid compound.

In recent study a relationship between *trans*-resveratrol concentration in wines of cv. Riesling and vine-growing locality was observed by Kumšta et al. (2012). They found little influence of locality on the amounts of *trans*-piceid, *cis*-resveratrol and *cis*-piceid, while in the case of *trans*-resveratrol, the highly significant influence of the locality on its concentration was observed. The content of *trans*-resveratrol reflected the climatic conditions during the period of vegetation and fungal pressure which is higher in humid conditions (Melzoch et al. 2001). Abnormally high precipitations in the Karlštejn locality in May and June (175 and 162 mm) may have caused higher values of *trans*- and *cis*-resveratrol and *cis*-piceid in Pinot Noir which is a medium resistant variety. Also, a difference in *trans*-piceid between localities in Pinot Noir was found. Prague-Grébovka recorded high precipitations in May and June (108.9 and 151.1 mm). Our results demonstrated that the mutual interaction between genotype and locality is an important factor in the formation of stilbenes.

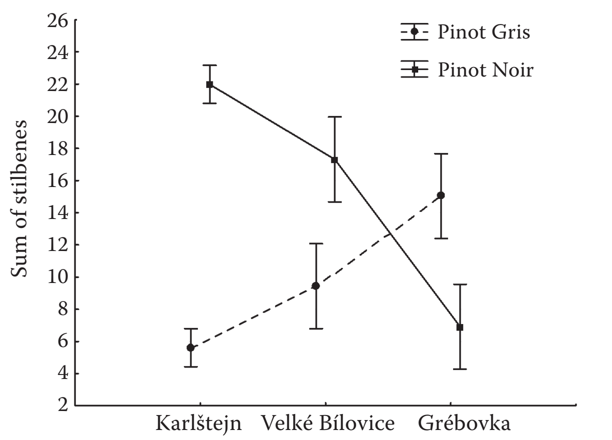

Fig. 4. Interaction between grapevine variety and locality (sum of stilbenes)
current effect: F (2, 36) = 76.248, $P = 0.0000$; vertical bars denote 0.95 confidence intervals

CONCLUSION

The highest levels of *trans*-resveratrol and *trans*-piceid were found in the shoots and tendrils, while *cis*-forms were present only in small amounts. There has been no observable effect recorded on the type of drying (except for *cis*-piceid). Leaves dried in the oven have shown higher levels of *trans*-forms of stilbenes compared with the leaves dried at ambient temperature. The highest level of *trans*-resveratrol was found in Pinot Noir. *Cis*- and *trans*-piceid showed the highest levels in Hibernal and Sauvignon Blanc. Growing locations had an effect on *trans*-resveratrol levels in Pinot Gris and Pinot Noir. Differences were also found between *trans*-resveratrol and vine-growing sub-regions. Our re-

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sults suggest that the trimmed clippings might be used as a valuable and inexpensive source of stilbenes. Clippings preserved by drying might be further processed to nutraceuticals or as an additive to the feed.

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