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ORIGINAL RESEARCH PAPER

INFLUENCE OF PATHOGENIC ATTACK ON VINE VARIETIES OF *CABERNET SAUVIGNON* ON THE CONTENT OF *TRANS*-RESVERATROL IN WINE

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Abstract: A study of the impact of pathogenic attack on vine varieties of *Cabernet Sauvignon* on the content of *trans*-resveratrol in wine was carried out. Some of the vines were attacked by two pathogens: *Botrytis cinerea* - a cause of "*gray mold*"; *Phyllosticta ampelicida* (asexual phase from *Guignardia bidwellii*) - the causer of "*black rot*". It was found that when *Botrytis cinerea* attacked the vines with high degree (93 %) and index (50.33) (variant V1), this reflected in lower levels of established *trans*-resveratrol in wine (3.23 mg·dm^{-3}), in compared to control pathogen-free V2 variant (3.74 mg·dm^{-3}). The same trend was observed in the attack of the other pathogen. *Phyllosticta ampelicida* (asexual phase from *Guignardia bidwellii*), even in low attack degree (10 %) (variant V4) and index (2.5), resulted in lower levels of *trans*-resveratrol in wine (2.34 mg·dm^{-3}), compared to control pathogen-free variant V3 (2.56 mg·dm^{-3}). The study found a lower content of *trans*-resveratrol in wine produced from grapes of organically grown vines compared to that of conventionally grown.

Keywords: *asexual phase from Guignardia bidwellii, Botrytis cinerea, black rot, gray mold, organic wine, Phyllosticta ampelicida, red wine, trans-resveratrol*

INTRODUCTION

Trans-resveratrol (*trans*-3,4,5-trihydroxy-stilbene) is a phenolic compound of the stilbenes group [1]. Now, it is well known that *Vitis vinifera* L. has a significant ability to synthesize it. The most important factors influencing the formation of this substance in the vine include: the genetic ability of the variety for active synthesis of resveratrol [2, 3], the impact of climate - significant differences between the daily and nightly temperatures [4]; the attack of the vine from different pathogens - *Botrytis cinerea* [5], *Plasmospora viticola* [6], *Rhizopus stolonifer* [7] and others. The largest amounts of *trans*-resveratrol are synthesized from the vine as a protective response to a pathogenic attack [2].

Resveratrol was found in wine for the first time in 1992 [8]. Since the stilbenes have a predominantly presence in grape skin, the amount of resveratrol in red wines is higher than white wines by technological reasons [9].

A large number of studies were concentrated on the health benefits of this compound - its high antioxidant activity [10], a preventive effect against cardiovascular diseases [11], anticancerogenic effect [12, 13].

The quantitative presence of *trans*-resveratrol in red wines ranges from 0.30 to 2.10 $mg \cdot dm^{-3}$ [1]. It is claimed that the influence of the vine genotype for high synthesis of resveratrol is most pronounced in the Pinot Noir variety - genetically determined to synthesize this substance in higher amounts [14].

Due to the increased research interest about this compound, there has been a large number of studies for its presence in wines and grapes of different varieties. It was found in red wines (0.352 – 1.99 $mg \cdot dm^{-3}$) produced from Greek varieties [15] in Romanian red wines (0.025 - 10.23 $mg \cdot dm^{-3}$) [16] in red wines from Portugal (2.64 $mg \cdot dm^{-3}$) [17], in Serbian red wines (0.11 - 1.69 $mg \cdot dm^{-3}$) [18], in Bulgarian wines [19] and others.

The aim of this study was to determine the impact of pathogenic attack on vine varieties of *Cabernet Sauvignon* on the content of *trans*-resveratrol in wine obtained from them.

MATERIALS AND METHODS

Sample variants and attack degree

Part of the experimental variants were attacked by two pathogens - *Phyllosticta ampelicida* (asexual phase from *Guignardia bidwellii*) [20] and *Botrytis cinerea* [21]. The first pathogen causes the disease named "*black rot*" on the vines. The disease induced by the second is known as "*gray mold*".

The development of the diseases (degree and index of attack) in the four variants of the vines of *Cabernet Sauvignon* varieties is presented in Table 1.

Table 1. *Development of black rot and gray mold on the vines of Cabernet Sauvignon variety*

Disease	**Black rot (causer – *Phyllosticta ampelicida* (asexual phase from *Guignardia bidwellii*)**		**Gray mold (causer – *Botrytis cinerea*)**	
Vine variety	Attack Degree [%]	Attack Index	Attack Degree [%]	Attack Index
Cabernet Sauvignon **V1**	0	0	93	50.33
Cabernet Sauvignon **V2**	0	0	0	0
Cabernet Sauvignon **V3**	0	0	0	0
Cabernet Sauvignon **V4**	10	2.5	0	0

The variants of the vines of which the red wines have been produced were as follows:
V1 - *Cabernet Sauvignon*, clone ILV 1/11 [22]; Degree of attack with *Botrytis cinerea* – 93 %; Attack Index - 50.33. The vine was not attacked by *Phyllosticta ampelicida* (asexual phase from *Guignardia bidwellii*);
V2 - *Cabernet Sauvignon*, conventionally grown; the vine was not attacked by the two pathogens;
V3 - *Cabernet Sauvignon*, conventionally grown; the vine was not attacked by the two pathogens;
V4 - *Cabernet Sauvignon*, organically grown; Degree of attack with *Phyllosticta ampelicida* (asexual phase from *Guignardia bidwellii*) – 10 %; Attack Index - 2.5; The vine was not attacked by *Botrytis cinerea*.
The grape harvest for variants V3 and V4 was done on 20 October 2015, whereas for variants V1 and V2 were carried out a week later (26 October 2015).
For determination of *Cabernet Sauvignon* response to the diseases "*gray mold*" (*Botrytis cinerea*) and "*black rot*" (*Phyllosticta ampelicida* - asexual phase from *Guignardia bidwellii*), an evaluation of 100 grapes (for each variant) was performed by seven rate scale for evaluation of diseases:

Rate 0 - no attack;
Rate 1 - up to 5 % of the cluster was affected by the disease;
Rate 2 - 6 to 15 % of the cluster was affected;
Rate 3 - 16 to 25 % of the cluster was affected;
Rate 4 - 26 to 50 % of the cluster was affected;
Rate 5 - 51 to 75 % of the cluster was affected;
Rate 6 - 76 to 100 % of the cluster was affected by the disease;
The attack index was calculated using Mc Kinney's formula [23].

Vinification

Experimental vineyards were grown at the Experimental Base of Institute of Viticulture and Enology (IVE). The varieties were located on an area of 0.2 ha.
The grapes (vintage 2015) were harvested and vinified at the Experimental Wine Cellar

of IVE. A classic scheme for the production of dry red wines [24] was applied – crushing and destemming, sulphitation (50 mg·kg^{-1} SO_2), inoculating with pure culture dry yeasts *Saccharomyces cerevisiae* Vitilevure CSM - 20 g·hL^{-1}, temperature of fermentation – 28 °C, separation from solids, further sulphitation, storage.

Determination of trans-resveratrol content in wines

High-performance liquid chromatography (HPLC) was used for determination of the *trans*-resveratrol content of the wines. The determination was carried out by a modified method of Anli *et al*. (2006) [25].

RESULTS AND DISCUSSION

The data about the content of *trans*-resveratrol in the wines from the four variants of *Cabernet Sauvignon* varieties attacked by various pathogenic forms are presented in Figure 1.

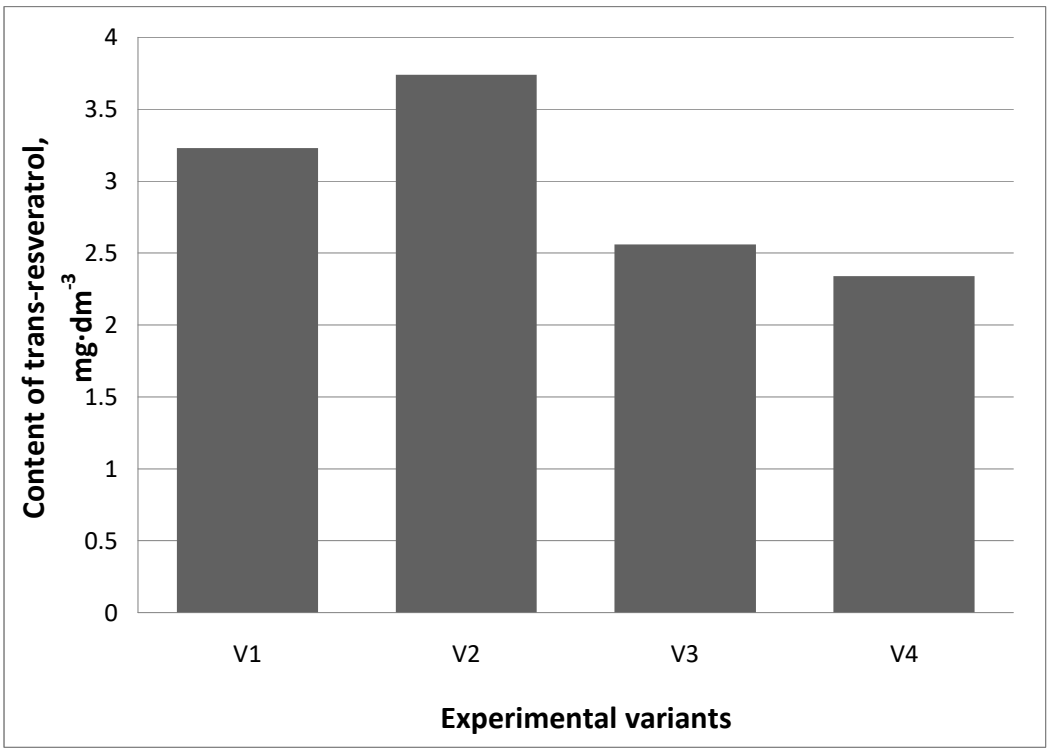

Figure 1. *Trans-resveratrol content in wines produced from grape vines attacked by pathogens*

The four experimental variants of the study provided information about the impact of the pathogens *Phyllosticta ampelicida* (asexual phase from *Guignardia bidwellii*) and *Botrytis cinerea* on the synthesis of *trans*-resveratrol in vines cultivated by different technologies - conventional (with all plant protection measures) and organic. The highest content of stilbene *trans*-resveratrol was found in the wine from variant V2 (3.74 mg·dm^{-3}). This variant was conventionally grown. There was not detected

pathogenic attack in it. Immediately after wine from this variant, according to the content of the stilbene, was the wine from variant V1, where *trans*-resveratrol was found in the amount of 3.23 mg·dm^{-3}. This variant was conventionally grown, too. However, there was a strong attack in it from one of the pathogens - *Botrytis cinerea*. The pathogen attacked the vines with a very high attack degree (93 %) with an attack index of 50.33.

From the compare of variants V1 and V2 (harvested at the same time), it was clear that *Botrytis cinerea* vine attack resulted in a lower level of stilbene *trans*-resveratrol found in the wine. Bavaresco *et al*. (2007) [26] concluded that the strong pressure of pathogenic fungi on the vine displaces the balance between phytoalexins produced by the vine (one of them is *trans*-resveratrol) and degraded (degraded from the pathogens) to degraded, i.e. the pathogens causes a reduction in phytoalexins level in the fruit of the vine. This explains the difference between the established content of *trans*-resveratrol in wines from not attacked and attacked vines. The content of *trans*-resveratrol in the wine produced from vines strongly attacked by *Botrytis cinerea* (V1) was with 0.51 mg·dm^{-3} lower.

The samples V3 and V4 reported the result and differences in the content of *trans*-resveratrol in wine produced from grape vines attacked by the other pathogen - *Phyllosticta ampelicida* (asexual phase from *Guignardia bidwellii*). The pathogen attack was carried out on variant V4. Compared to the *Botrytis*, it was much weaker, with an established attack degree of 10 % at index 2.5. However, the same trend was again observed - lower *trans*-resveratrol levels in the attacked variant V4 (2.34 mg·dm^{-3}), compared to non attacked V3 (2.56 mg·dm^{-3}). The grapes of the two variants were harvested at the same time. Again the pathogen attack was contributed to reducing the level of phytoalexin *trans*-resveratrol in the grapes, which reflected on its lower content in wine (0.22 mg·dm^{-3} less *trans*-resveratrol).

From the present study, a result can also be obtained for the differences in the synthesis of stilbene *trans*-resveratrol between conventional and organic grown vines. It was evident from the results obtained that the organic approach of grown (variant V4), even with low pathogenic attack, resulted in lower levels of *trans*-resveratrol in the grapes that reflected in the wine. The lowest content of *trans*-resveratrol compared to all other variants was found in variant V4.

The content of *trans*-resveratrol in wine as a quantitative indicator ranged from 2.56 to 3.74 mg·dm^{-3}. The quantitative levels observed corresponded to variations in *trans*-resveratrol content established in other studies [17, 16].

CONCLUSION

A conducted study on the impact of a pathogenic attack on vines of *Cabernet Sauvignon* varieties on the content of *trans*-resveratrol in wine revealed:

• *Botrytis cinerea* pathogenic attack (*gray mold*) resulted in a decrease of *trans*-resveratrol levels in wine produced from grapes of the attacked vines;

• Even a mild, weak attack of *Phyllosticta ampelicida* (asexual phase from *Guignardia bidwellii*) on the vines (*black rot*) led to the same effect as *Botrytis cinerea* - a reduction in *trans*-resveratrol levels in wine produced from the grapes of the attacked vines;

• The organic vine growing led to a probable lower *trans*-resveratrol synthesis, because in the wine obtained from the grapes of these vines, the lowest concentrations of stilbene were established, as compared to all other samples;
• The tested variants contained quantities of *trans*-resveratrol typical for red wines.

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