INFLUENCE OF STILBENOIDS ON THE PROPAGATION OF GRAY MOLD(*BOTRYTIS CINEREA)* ON GRAPE BERRIES UNDER LAB AND VINEYARD CONDITIONS

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ABSTRACT
The impact of vine stilbenoids on the propagation of gray mold (Botrytis cinerea) in grape berries under lab and vineyard conditions, was studied. As concerning the lab experiment, healthy berries of 3 red winegrape varieties were sampled, as follows: a) Alexandrouli, from a 12-year-old vineyard located in western Georgia (Racha-Lechkhumi) grown on Brown forest acid-Dystric cambisols type; b) Mujuretuli, from an 8-year-old vineyard cultivated in the same location on the same type of soil; c) Saperavi, from a 15-year-old vineyard grown on Eutric Cambisoil in Eastern Georgia (Gurjaani district). The experimental design included the following treatments: 1) berry pre-treatment with 1 mg/100mL, 3 mg/100mL, 5 mg/100 mL of a stilbenoid mixture, and then fungal infection; 2) berry pre-treatment with 1 mg/100ml, and 5 mg/100 mL of ε-viniferin, and then fungal infection; 3) berry pre-treatment with 1 mg/100mL, and 5 mg/100 mL of trans-resveratrol, and then fungal infection; 4) control berries with fungal infection but without pre-treatments. The pre-treatments 1), 2) and 3) also included freshly prepared or one-week-old stilbenoid mixture, ε-viniferin and trans-resveratrol solutions. As concerning the vineyard trial, healthy clusters of Saperavi (red winegrape variety) from a 15-year-old vineyard cultivated on Eutric Cambisols type in Eastern Georgia (Gurjaani district), were selected. The experimental design was as follows: 1) cluster pre-treatment with 5 mg/100 mL of stilbenoid mixture and then fungal infection; 2) cluster pre-treatment with Bordeaux mixture and then fungal infection; 3) control vines with fungal infection but without any pre-treatment. The stilbenoid mixture included a mix of trans-resveratrol and its derivatives. The degree of fungal infection at berry level was checked. Under lab conditions the pre-treatment with 5 mg/100 mL of stilbenoid mixture, ε-viniferin and trans-resveratrol completely inhibited Botrytis cinerea growth. Under vineyard conditions Bordeaux mixture showed a better fungal control than the stilbenoid mixture (97% versus 84%).

Keywords: *vine, Saperavi, stilbenoids, gray mold*

INTRODUCTION

Stilbenoids belong to a wide class of phenolic compounds. They include resveratrol and its derivatives (glucosides, dimers, trimers, tetramers, etc.) *cis-* and *trans-*isomeric forms[1-9]. Stilbenoids are characterized by several high biological activities and among them is important the phytoalexin activity for the plant, especially for the grapevine.The most important phytoalexin stilbenoids are: resveratrol[10,11], pterostilbene [12], piceid[13], viniferins [14]. Phytoalexins, under plant infection conditions, are actively synthesized and fight against disease-causing microorganisms (for example *Botrytis cinerea* and *Plasmopara viticola*). Beside the biotic factors, phytoalexins also respond to abiotic stresses such as UV rays and $AlCl_3$ [15].

Adrian et al. [16] studied the variability of stilbenoids in the berry skin of red (Pinot noir, Gamay) and white (Chardonnay) winegrape varieties infected by *Botrytis cinerea* and treated with UV rays. All samples infected with *B. cinerea* showed a decreased amount of resveratrol and an increased concentration after UV irradiation. Pterostilbene was found in low concentrations in infected berries of Chardonnay and Gamay. Pterostilbene was also observed in low concentrations in grape skins by other authors [17,18].According to Pezet and Pont [19] , pterostilbene plays an important role in the resistance of immature grapes against disease-causing microorganisms. Other authors studies the interaction between stilbenoids and *Botrytis cinerea* in gapevine [20-23].

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According to Bezhuashvili et al [24] stilbenoids have been identified in healthy and naturally diseased Georgian wine grape varieties - Rkatsiteli (white), Tsolikouri (white), Alexandrouli (red), Mujuretuli (red). *Trans*-resveratrol and *trans*-ε-viniferin were dominant for red varieties; moreover, *trans*-resveratrol was lower than *trans*-ε-viniferin in healthy grape skins, and the concentration of *trans*-resveratrol was significantly higher under gray mold infection than *trans*-e-viniferin; it decreased under disease conditions [24].In white wine grape varieties (Rkatsiteli and Tsolikouri), the main stress metabolite was *trans*-resveratrol, which increased significantly in gray mold disease conditions [25,26].The inhibitory effect of *trans*-resveratrol on *Botrytis cinerea* activity and consequently the spread of gray mold on grapes, has been established under laboratory conditions (in petri dishes) [27,28]. Stilbenoids had an inhibitory effect on the activity of the fungus- *Botrytis cinerea* pure culture in food areas placed in petri dishes and there was a negative correlation between the fungal propagation and the stilbenoids concentration [29].

According to Adrian et al. [30] a resveratrol concentration of 100 μg / ml completely inhibited the development of *B. cinerea* mycelium, while concentrations of pterostilbene at 20–40–60 μg/ml caused 50%, 80%, and 100% inhibition of *B.cinerea* mycelium development. According to Pezet and Pont [19] a concentration of pterostilbene of 18μg/ml caused a 50% inhibition of *B. cinerea* mycelium development while a concentration of 52 μg/ml resulted in an inhibition of 52%.

Evidences have been obtained on the capability of some highly pathogenic *B. cinerea* strains to circumvent the defence by detoxifying resveratrol through an oxidative process [22].

Other stilbenoids can be detoxified by enzymatic (laccase) activity of *B. cinerea*, resulting in the release of compounds like pterostilbene*trans*-dehydrodimer, pterostilbene*cis*-dehydrodimer, resveratrol *trans*- dehydrodimer [31]. All the physio-pathological aspects of stilbenoids are addressed in the review written by Jeandet et al [32]. Resveratrol synthesis can be also used as screening method in breeding programs for disease (gray mold and downy mildew) resistance [33]. Numerous other studies have confirmed a close association between phytoalexin accumulation and disease resistance [34-38].

The aim of the study was to determine the biological effect of vine stilbenoids on the propagation of gray mold on the berries of wine grapes under laboratory and vineyard conditions.

MATERIALS AND METHODS.

Lab experiments

Healthy berries of red winegrape varieties (*V. vinifera* L.) Alexandrouli, Mujuretuli and Saperavi were sampled at technological maturity (October 2020) in the following environments of Georgia: a) Alexandrouli berries from a 12-year-old vineyard located in Racha-Lechkhumi region (western Georgia) on brown forest acid-Dystric cambisols type; b) Mujuretuli berries from a 8-year-old vineyard located in Racha-Lechkhumi region (western Georgia) on brown forest acid-Dystric cambisols type; c) Saperavi berries from a 15-year-old vineyard located in Gurjaani district (eastern Georgia) on Eutric Cambisols type. The experimental design included the following treatments: 1) berry pre-treatment with 1 mg/100mL, 3 mg/100mL, 5 mg/100 mL of a stilbenoid mixture, and then fungal infection; 2) berry pre-treatment with 1 mg/100ml, and 5 mg/100 mL of ε-viniferin, and then fungal infection; 3) berry pre-treatment with 1 mg/100mL, and 5 mg/100 mL of *trans*-resveratrol, and then fungal infection; 4) control berries with fungal infection but without pre-treatments. The pre-treatments 1), 2) and 3) also included freshly prepared or one-week-old stilbenoids mixture, ε-viniferin and *trans*-resveratrol solutions.The pre-treatments were done by soaking 12 berries per variety in the previously described solutions (or just water in the case of the control), while fungal infection was done by spraying *Botrytis cinerea* conidial suspension over the berries placed on damp filter paper inside petri dishes. The fungal inoculum was prepared by recovering conidia from infected berries grown in the field. The stilbenoid mixture, which included a mix of *trans*-resveratrol and its derivatives, was extracted from grapevine tissues, as well as ε-viniferin and *trans*-resveratrol, by using ethyl acetate and fractions were separated in column with adsorbent "Sephadex G50".

Vineyard experiment

As concerning the vineyard trial, healthy clusters of Saperavi (red winegrape variety) from a 15-year-old vineyard (not sprayed by pesticides) cultivated on Eutric Cambisols type in Eastern Georgia (Gurjaani district), were selected between 10 and 30 August 2020. The experimental design was as follows: 1) cluster pre-treatment with 5 mg/100 mL of stilbenoid mixture and then fungal infection; 2) cluster pre-treatment with Bordeaux mixture and then fungal infection; 3) control vines with fungal infection but without any pre-treatment. The stilbenoid mixture, which included a mix of *trans*-resveratrol and its derivatives, was extracted from grapevine tissues. Three clusters of different sizes were selected per each treatment, according to the following table.

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Treatments	Cluster size	Treatment characteristics
Control	87 berries/cluster 127 berries/cluster 268 berries/cluster	Pre-treatment with water and then spraying with *Botrytis cinerea* conidial suspension
Pre-treatment 1	88 berries/cluster 135 berries/cluster 298 berries/cluster	Pre-treatment with stilbenoids water solution (5mg/100ml) and then spraying with *Botrytis cinerea* conidial suspension
Pre-treatment 2	85 berries/cluster 130 berries/cluster 275 berries/cluster	Pre-treatment with Bordeaux mixture and then spraying with *Botrytis cinerea* conidial suspension

Tested parameters in both experiments

The following parameters were tested: a) number of infected berries, depending on the pre-treatment; b) degree of the infection (%), depending on the pre-treatment; c) biological efficiency (%) of the pre-treatments according to the method of Berim and Sokolovskaya (39).

RESULTS AND DISCUSSION.

Table1. Lab trial. Impact of stilbenoids on the gray mold infectionof the Mujuretuli grape berries

##	Pre-treatments	# of berries in each petri dish	# of infected berries in each petri dish	Degree of infection (%)	Biological efficiency (%)
1.	P- 1mg/100ml	12	2/3 *	16.6/25.0 *	83.4/75.0 *
2.	P 3mg/100ml	12	½ *	8.3/16.6 *	91.7/83.4 *
3.	P 5mg/100ml	12	0/1 *	0/8.3 *	100/91.7 *
4.	V-1 5mg/100ml (1 week solution)	12	3	25.0	75.0
5.	V-2 1mg/100ml (fresh)	12	4	33.3	66.7
6.	V-3 5mg/100ml (fresh)	12	0	0	100
7.	R-1 1mg/100ml (fresh)	12	2	16.6	83.4
8.	R-2 5mg/100ml (fresh)	12	0	0	100
9.	R-3 5mg/100ml (1 week solution)	12	1	8.3	91.7
10.	Control	12	12	100	

- P – Stilbenoids mixture;
- V-*ε*-viniferin;
- R-*trans*-resveratrol;
- Fresh: Freshly made solution;
- 1 Week solution: Solution utilized after one week
- *: the first figure is related to fresh prepared stilbenoid mixture, while the second one is relared to 1 week old stilbenoid mixture

Table 2. Lab trial. Impact of stilbenoids on the gray mold infection of the Aleksandrouli grape berries.

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##	Pre-treatments	# of berries in each petri dish	# of infected berries in each petri dish	Degree of infection (%)	Biological efficiency (%)
1.	P 1 mg/100ml	12	2/3 *	16.6/25.0 *	83.4/75.0 *
2.	P 3 mg/100ml P 5 mg/100ml	12 12	½ * 0	8.3/16.6 * 0	91.7/83.4 * 100
3.	R-1 1mg/100ml, (1week)	12	4	33.3	66.7
4.	R-2 1mg/100ml, (fresh made)	12	2	16.6	83.4
5.	R-3 3mg/100ml, (fresh made)	12	1	8.3	91.7
6.	V-1 1mg/100ml (1week)	12	4	33.3	66.7
7.	V-2 3mg/100ml (1week) V-3 5mg/100ml (fresh made)	12 12	4 0	33.3 0	66.7 100
8.	Control	12	12	100	

- P – Stilbenoids mixture;
- V-ε-viniferin;
- R-*trans*-resveratrol;
- Fresh: Freshly made solution;
- 1 Week : Solution utilized after one week
- *: the first figure is related to fresh prepared stilbenoid mixture, while the second one is relared to 1 week old stilbenoid mixture

Table 3. Lab trial. Impact of stilbenoids on the grey mold infection of Saperavi grape berries

##	Pre-treatments	# of berries in each petri dish	# of infected berries in each petri dish	Degree of infection (%)	Biological efficiency (%)
1.	P 1 mg/100ml	12	1/3 *	8.3/25.0 *	91.7/75.0 *
2.	P- 3 mg/100ml P- 5 mg/100ml (fresh made)	12 12	½ * 0	8.3/16.6 * 0	91.7/83.4 * 100
3.	R-1 1mg/100ml, (1week)	12	3	25.0	75.0
4.	R-2 1mg/100ml, (fresh made)	12	2	16.6	83.4
5.	R-3 3mg/100ml, (fresh made)	12	1	8.3	91.7
6.	V-1 (1mg/100ml,1week)	12	3	25.0	75.0

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7.	V-2 (3mg/100ml,1 week) V-3 5mg/100ml (fresh made)	12 12	2 0	16.6 0	83.4 100
8.	Control	12	12	100	

- P – Stilbenoids mixture;
- V-ε-viniferin;
- R- *trans*-resveratrol;
- Fresh: Freshly made solution;
- 1 Week : Solution utilized after one week
- *: the first figure is related to fresh prepared stilbenoid mixture, while the second one is relared to 1 week old stilbenoid mixture

Table 4. Vineyard trial. Impact of stilbenoids mixture and Bordeaux mixture on gray mold infection of Saperavi clusters

Pre-treatments	# of berries/cluster	# of infected berries	Degree of infection (%)	Biological efficiency ,(%)
Control	85 127 268	81 120 253	95.3 94.5 94.7	4.7 5.5 5.6
Pre-treatmen 1	88 135 298	14 22 48	15.9 16.3 16.1	84.1 83.7 83.9
Pre-treatment 2	85 130 275	2 2 9	2.3 1.5 3.3	97.6 98.5 96.7

Pre-treatment 1: stilbenoid solution (5mg/100mL)

Pre-treatment 2: Bordeaux mixture

The results of the experiments in the laboratory indicate the inhibitory effect of the phytoalexin stilbenoids on the activity of gray mold. Inhibition activity is expressed by the intensity of fungal spread and developmen. The degree of infection on the berries of the tested varieties (Mujuretuli, Alexadrouli and Saperavi) was related to the concentration of the pre-treatment compounds (stilbenoid mixture, ε-viniferin and *trans*-resveratrol); the higher the stilbenoid concentration, the lower the degree of infection (Tables 1, 2, 3). At the same time, stilbenoid mixture, ε-viniferin and *trans*-resveratrol lost a significant activity within 1 week after preparing the solutions and had reduced inhibitory effect against *Botrytis cinerea*. In control berries (no pre-treatment with stilbenoids) of all the three grape varieties, the degree of infection was 100%, as expected. The pre-treatments with freshly prepared stilbenoid mixture, ε-viniferin and *trans*-resveratrol, at the concentration of 5 mg/100 mL showed 100% of biological efficacy in the case of Mujuretuli variety (Table 1). As concerning the other varieties (Alexandrouli and Saperavi) 100% of biological efficacy was observed only for freshly prepared stilbenoid mixture and ε-viniferin, at a concentration of 5 mg/100 mL, while a biological efficacy of about 90% was observed with *trans*-resveratrol at 5 mg/100 mL (Tables 2 and 3). The degree of gray mold infection on grapes of Saperavi was found to be different in laboratory and in vineyard conditions. This was the case for the control (untreated berries/clusters), being 100% the infection in the lab and about 95% the infection in the field (vineyard), and for the pre-treatment with stilbenoid mixture, being 100% the biological efficacy in the lab and about 84% the biological efficacy in the vineyard. The effectiveness of the stilbenoids mixture against gray mold under vineyard conditions was significantly lower than the efficiency of sulfur-containing Bordeaux mixture, which was about 97% (Table 4).

CONCLUSION

Stilbenoids had an inhibitory effect on the gray mold infection . A difference was found between the results obtained under lab and vineyard conditions. In particular, in the case of Saperavi, the biological efficiency of stilbenoids against spreading of disease under laboratory conditions far exceeds the efficiency observed under vineyard conditions. The results obtained are an important basis for determining the correlation of vine disease resistance with phytoalexin stilbenoids production. .

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