BIOLOGICAL CONTROL OF BOTRYTIS BUNCH ROT OF GRAPES IN THE REPUBLIC OF MACEDONIA

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Abstract
Bunch rot disease of grapes, caused by the necrotrophic fungus *Botrytis cinerea* is a chronic and serious problem in most of the vineyards in Republic of Macedonia. Its control is mostly achieved by application of synthetic fungicides. However, chemical control of *B. cinerea* is often difficult and incomplete, especially in vineyards where resistant strains have developed. A promising alternative strategy that could replace or be combined with fungicides are biofungicides. The main goal of the experiment was to observe the possibility for biological control of Botrytis bunch rot disease by the use of three novel biofungicides (Serenade Aso, Polyversum and Timorex gold). Experiment was conducted during the 2016 in two vineyard regions of Republic of Macedonia (Negotino and Kavadarci), on two grape varieties, Chardonnay and Cardinal. The destructive potential of this grape disease was confirmed in the untreated variant of the Chardonnay variety, where the intensity of infection was higher than 48%. Biofungicides Serenade Aso (a.m. *Bacillus subtilis* QST 713) and Timorex gold (extract of *Malaleuca alternifolia*) had similar efficacy, with average of 89% reduction of disease incidence in the region of Negotino and 91,66% in the region of Kavadarci. Biofungicide Polyversum (a.m. *Pythium oligandrum* M1), applied as preventive sprays achieved more than 85% reduction of the disease in both regions. The results showed that all tested biofungicides have a prospective use for control of bunch rot disease in grapes.

Keywords: biofungicides, Botrytis bunch rot, biocontrol.

Introduction

Botrytis cinerea Pers.:Fr. (*Botryotinia fuckeliana* (de Bary) Whetzel) is a polyphagous, necrotrophic fungal pathogen, which causes economically important pre- and postharvest diseases in more than 235 plant species (Williamson et al., 2007; Aboelghar and Wahab, 2013; Elad et al., 2015). Recently, *B. cinerea* was ranked second into the world Top 10 fungal plant pathogens list based on its scientific and economic importance (Dean et al., 2012). In grapevine, *B. cinerea* attacks leaves, developing shoots, inflorescences and berries, but symptoms become most evident at the stage of ripening when berries are most sensitive (Couderchet, 2003). During the last 60 years, chemical control was the most important mean of bunch rot disease control, with use of synthetic fungicides (Rosslenbroich and Stuebler, 2000; Leroux, 2002). However, rapid development of resistant strains to frequently applied fungicides (Latorre et al., 2002; Sergeeva et al., 2002; Leroux, 2004) and increased public concern about the human and environmental health (Janisiewicz and Korsten, 2002; Spadoro and Gullino, 2005), has opened the door for other, alternatives means of control of this pathogen, including plant defence stimulants and microorganisms to suppress disease epidemics (Elmer and Reglinski, 2006). Biological control agents has risen as a potential alternative to synthetic fungicides and offers economically sustainable and environmentally friendly control of many economically important pathogens, including *B. cinerea* (Diguta et al, 2016). They suppress the growth of plant pathogens through competition for space and nutrients, production of inhibitory metabolites, such as antibiotics and cell-well degrading enzymes, parasitism and induction of defense-related responses in host plants (Howell, 2003; Harman et al, 2004; Magnin-Robert et al., 2007; Trotel-Aziz et al., 2008; Sharma et al., 2009). Although many *in vitro* studies confirmed the potential use of antagonistic microorganisms in biological control of many economically important fungal pathogens, only a small number has exhibited field efficacy and even a smaller number has been developed into commercial products (Elmer and Reglinski, 2006). As a result, the main objective of this study was to assess the efficacy of three novel commercial biofungicides (Serenade Aso, Polyversum and Timorex gold) for control of *Botrytis cinerea* in grapevine.

Material and methods

The research was conducted during 2016 in two main vineyard regions in the Republic of Macedonia: Negotino (variety Chardonnay) and Kavadarci (variety Cardinal). The experiment consisted of four variants for each grape variety, in randomized block design with three replicates per treatment. Three of the variants were treated with biofungicides, while one was untreated and used as control (Table 1).

Table 1. Variants represented in Negotino (cv. Chardonnay) and Kavadarci (cv. Cardinal) region

Variant no.	Name of commercial biofungicide	Active ingredient	Content of a. i.	Producer	Dosage
1	SERENADE ASO	*Bacillus subtilis* QST 713 (spores)	5.13 x 10 CFU/g	BASF SE Germany	8 L/ha
2	POLYVERSUM	*Pythium oligandrum* M1 (oospores)	$1x10^6$/g	Biopreparaty spol., Czech Republic	0.25 kg/ha
3	TIMOREX GOLD	*Melaleuca alternifolia* (oil extract)	222.5 g/L	Biomor Israel Ltd	2 L/ha
4	CONTROL	Untreated			

During the vegetative period, based on the biology of the pathogen and phenological stage of the grapevine, three treatments were performed with the tested biofungicides (end of flowering - 80% caps fall, pre-bunch closure and veraison). Evaluation of the efficacy of all tested biofungicieds was performed 10 days after the last treatment. For that purpose, the Unterstenhöfer scale from 0 to 5 was used (Stojanovikj et al., 1971): Cluster: 0 = healthy cluster; 1 = up to 10% infected berries in the cluster; 2 = 10 – 25% infected berries in the cluster; 3 = 25 – 50% infected berries in the cluster; 4 = 50 – 75% infected berries in the cluster; 5 = 75 –100% infected berries in the cluster. The intensity of infection was calculated according to the formula of Townsend-Heuberger (1943), while the fungicide efficacy was evaluated by the formula of Abbott (1925). The statistical differences among biofungicide efficacy were assessed using Tukey's HSD test at probability level of P = 0.05.

Results and discussion

The obtained results regarding the intensity of infection and efficacy of the tested biofungicides are presented in Table 2 and Table 3. During the evaluation, Botrytis bunch rot infections were observed in all tested variants in the region of Negotino (Table 2). Very high intensity of infection (48.2%) was observed in the untreated (control) variant, which confirmed the destructive potential of the disease, especially in varieties with compact clusters, such as Chardonnay. This is in agreement with the hypothesis of Savage and Sall (1983) that the development of bunch rot is most pronounced in cultivars that develop compact fruit clusters and dense canopies (van Rooi and Holz, 2003). Moreover, between the untreated variant and all treated variants statistically significant difference was detected. Among the treated variants, the lowest intensity of infection was observed in the variant treated with the biofungicide Serenade ASO (5.1%). In the variant treated with the biofungicide Timorex Gold very similar intensity of infection was observed (5.3%), while the highest intensity of infection (6.8%) was observed in the variant treated with the biofungicide Polyversum. There was statistically significant difference between all variants treated with biofungicides (Serenade Aso and Polyversum and Timorex Gold) and the control (untreated variant). Regarding the efficacy, all tested biofungicides achieved great reduction of bunch rot incidence. Biofungicide Serenade Aso performed highest efficacy (89.41%), but almost equal efficacy (89%) was achieved by biofungicide Timorex gold. The lowest efficacy performed biofungicide Polyversum with ca 85% reduction of bunch rot incidence, significantly lower compared to Serenade Aso and Timorex Gold.

Table 2. Intensity of infection and efficacy of tested biofungicides in control of *Botrytis cinerea* on cultivar Chardonnay grown in the region of Negotino

No	Variant	Intensity of infection (%)	Efficacy of biofungicides (%)
1	SERENADE ASO	5.1a	89.41a
2	POLYVERSUM	6.8c	85.89b
3	TIMOREX GOLD	5.3b	89a
4	Control	48.2d	/

*The values marked with the different letter are significantly different

In the region of Kavadarci (Table 3), bunch rot infections were also observed in all tested variants during the evaluation, but the intensity of infection was considerably lower compared to region of Negotino. The intensity of infection in the untreated (control) variant was moderate to low (4.8), but between the untreated variant and all treated variants statistically significant difference was detected. In the variants treated with the biofungicides Serenade Aso and Timorex Gold equal intensity of infection (0.4%) was observed, while in the variant treated with the biofungicide Polyversum significantly higher intensity of infection (0.7%) compared to the other two biofungicides was observed. Concerning the efficacy of the tested biofungicides, even higher reduction of bunch rot incidence was observed in this region. The highest reduction of the gray mold incidence was achieved by the biofungicides Serenade Aso and Timorex gold, which performed equal efficacy of 91.66%. Biofungicide Polyversum performed the lowest efficacy, with 85.41% reduction of bunch rot incidence, significantly lower than Serenade Aso and Timorex Gold.

Table 3. Intensity of infection and efficacy of tested biofungicides in control of *Botrytis cinerea* on cultivar Cardinal grown in the region of Kavadarci

No	Variant	Intensity of infection (%)	Efficacy of biofungicides (%)
1	SERENADE ASO	0.4a	91.66a
2	POLYVERSUM	0.7b	85.41b
3	TIMOREX GOLD	0.4a	91.66a
4	Control	4.8c	/

*The values marked with the different letter are significantly different

The obtained results showed that all tested biofungicides were effective in suppression of bunch rot disease in grapes. This is in agreement with statement of Elmer and Reglinski (2006), that biocontrol treatments, whether operating by antibiosis, competition, and/or elevation of host resistance, can be highly effective against *B. cinerea* in grapes under controlled conditions and on occasions in vineyards. Moreover, levels of disease suppression can be equal those achieved with synthetic botryticides, particularly under low-to-moderate disease pressure conditions, as was the case in our study, but also in other studies. Thus, Esterio *et al*. (2000) reported that four applications of product Serenade (*B. subtilis* strain QST-713), which were compared with a traditional spray programme used to treat table grapes (cv. Thompson Seedless) resulted in postharvest disease control equivalent to a traditional botryticide programme.

Conclusions

Bunch rot disease, caused by *Botrytis cinerea* has great economic importance and in some cases can lead to complete loss of the grape production. Reducing these losses to a level that is acceptable still poses a great challenge for grape producers in the Republic of Macedonia. The results obtained in this study showed that all tested biofungicides (Serenade Aso, Timorex gold and Polyversum) performed great reduction of bunch rot incidence and have a prospective use for control of *Botrytis cinerea* in grapes. Moreover, combination of this biofungicides together with some preventive methods that control the development of the pathogen (less sensitive varieties, reduced fertilization, leaf removal for cluster ventilation and adequate protection against other pathogens such as powdery mildew and berry moth) can guarantee highly effective control of Botrytis bunch rot in grapes.

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