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SUSTAINABLE USE OF FUNGICIDES AND BIOCONTROL AGENTS FOR BOTRYTIS GRAY MOLD MANAGEMENT IN GRAPES

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Abstract

Gray mold disease or Botrytis rot, caused by Botrytis cinerea, it is well known for significant economic losses in vineyards worldwide, in both yield and quality. Management of gray mold it is traditionally based on fungicide treatments and cultural practices. Fungicide programmes specifically target critical periods during the growing season: late-bloom, bunch closure, veraison and preharvest. However, controlling Botrytis cinerea is still a challenge, because the pathogen is able to rapidly develop resistance. Also, more stringent regulations governing fungicide residues have severely restricted chemical control options in conventionally managed vineyards, particularly during the preharvest period. The objective of our study was to assess the potential of an integrated programme to reduce Botrytis rot, involving fungicides and biological control agents (BCAs). Two experimental bioproducts were tested: Trichopulvin Td85 25 PU (Trichoderma koningii Td85 strain) and Saccharopulvin 25 PU (Saccharomyces cerevisiae L30b yeast strain). The efficacy of the proposed integrated programme was assessed based on disease incidence and disease severity in field trials, conducted in Tohani vineyards. This integrated strategy that combines fungicides and bioproducts could offer the option to reduce the number of fungicide treatments, thus minimizing the chemical residues, and manage fungicide resistance development.

***Key words**: biocontrol agents, fungicides, sustainable viticulture.*

INTRODUCTION

Botrytis cinerea, the causal agent of gray mold or botrytis bunch rot in grapes, is responsible for significant economic damage in vineyards worldwide. Botrytis gray mold can develop in the vineyard after harvest, during long-distance transport, cold storage, and shelf-life. *Botrytis* can cause severe processing problems for winemakers also (loss of colour, enzymic oxidation, off-flavours, taints and reduced shelf life).

Fungicide programmes for botrytis bunch rot management are widely used, specifically targeting critical periods during the growing season: flowering, bunch closure, veraison and preharvest. This approach, however, is not regarded as sustainable: *B. cinerea* is well known as a high risk fungicide-resistant pathogen (Elmer and Raglinski, 2006). Also, there is an increasing restriction on botryticides available for protection during berry ripening because of some market specifications for low or no fungicide residues in wine (Hill, Beresford and Evans, 2010).

Biological control of plant pathogens by microorganisms has been considered a more natural and ecofriendly disease control measure. *Trichoderma* fungi are well known for their antagonism against plant pathogens. *Trichoderma harizanum*, *T. viride*, *T. virens*, *T. hamatum, T. roseum* and *T. koningii* are the most common fungal biological control agents (BCAs) that have been extensively researched and deployed throughout the world. Their activity is mainly due to faster metabolic rates, mycoparasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolites and induction of plant defence system (Verma *et al*., 2007).

Yeasts are a major component of the epiphytic microbial community on the surfaces of fruits and vegetables. A diverse range of yeasts (*Rhodotorula glutinis*, *Candida* spp., *Pichia membranifaciens*, *Kloeckerea apiculata*, *Saccharomyces* spp.) have shown efficacy against *B. cinerea*.
The objective of our study was to assess the potential of an integrated programme to reduce *Botrytis* rot, involving fungicides and biological control agents (BCAs).

MATERIALS AND METHODS

The trial was carried out in 2014 in Tohani Valley vineyard (Romania) on grapes of *Feteasca alba* variety, which is sensitive to grey mould disease (*Botrytis cinerea*). The trial was conducted using a completely randomized designed block. The tested variants (products, application rate and timing of aplication) are presented in table 1.

Table 1. Treatment variants tested against Botrytis cinerea infection in Feteasca alba grapes

V	Treatment	Rate(kg/ha)	Timing of application			
			A	B	C	D
V1	Control (untreated)	-	-	-	-	-
V2	Switch 62.5 WG	1.2	T1	T2	T3	T4
V3	Trichopulvin Td85 25 PU	2.0	T1	T2	T3	T4
V4	Switch 62.5 WG	1.2	T1	T2		
	Trichopulvin Td85 25 PU	2.0			T3	T4
V5	Switch 62.5 WG	1.2	T1	T2		
	Saccharopulvin 25 PU	2.0			T3	T4

Two commercial bioproducts produced by Research Institute of Plant Protection (RIPP) Bucharest-Romania were tested: Trichopulvin TD 85 (*Trichoderma pseudokoningii* Td85 strain) and Saccharopulvin (*Saccharomyces cerevisiae L30b* yeast strain) alone or integrated with Switch 62.5WG (cyprodinil 37.5% and fludioxonil 25%), produced by Syngenta. Four treatments were applied at the following stages: A (caps fall), B (bunch closure), C (beginning of color change) and D (three weeks before harvest). Botrytis bunch rot attack was assessed for each plot before harvest.
Disease incidence (frequency) was estimated as percentage of infected bunches on 100 bunches observed per plot). Disease severity was recorded using a grade scale, as the percentage of berries or tissue exhibiting botrytis infection symptoms for each bunch, on 100 bunches per plot. The average severity describes the amount of botrytis present in the vineyard and is sometimes referred as botrytis crop loss or percentage crop infection.
The efficacy of applied treatment was calculated based on disease severity compared to untreated control. The efficacy of fungicide was calculated according to Abbott's formula = (severity in untreated control culture - severity in treated culture) /severity in untreated control culture x 100. All data were subjected to statistical analysis based on ARM-8 software.

RESULTS AND DISCUSSIONS

Symptoms were assigned to botrytis bunch rot if there were brown or pink-brown (discoloured) berries, with a turgid or shrivelled aspect, in a manner characteristic of botrytis rot (figure 1) and if *B. cinerea* sporulation (grey mould) was visible on at least some berries or pedicels of the bunch.

Figure 1. Botrytis bunch rot symptoms

To assess the presence of botrytis bunch rot, disease incidence and severity were recorded (table 2). Disease severity is the variable of utmost interest to grape growers and wine makers, because it better reflects the impact of Botrytis bunch rot on yield and wine making than the variable called incidence (Hill, Beresford and Evans, 2010).
Almost a complete control of Botrytis bunch rot was achieved with four application of Switch 62.5 WG (cyprodinil and fludioxonil), at recommended rate, compared to untreated

control. In this variant, mean Botrytis incidence at harvest was 18.75%, with a severity of 3.5%.

Disease severity was significantly reduced (3.25%) when Switch 62.5 WG was applied in the first two treatments, followed by 2 other treatments with Trichopulvin 25 PU spray. Similarly, two applications of Switch 62.5 WG and two applications of Saccharopulvin 25 PU reduced the disease severity to 4.0% compared to untreated control (36.75%).

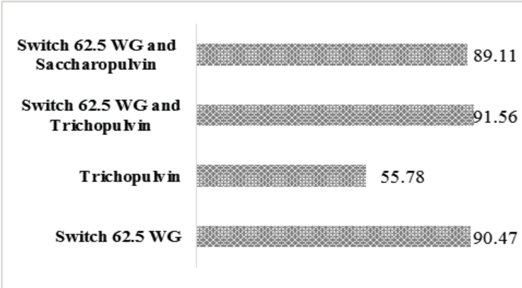
Botrytis bunch rot incidence was reduced in all treated variants, with values which slightly varies, between 15.0% (Trichopulvin 25 PUalone) and 18.25% (Switch 62.5 WG and Saccharopulvin 25 PU).

Table 2. Botrytis bunch rot incidence and severity in a Feteasca alba vine plantation

Var.	Treatment	incidence %	severity %
1	Untreated	76.00 a	36.75 a
2	Switch 62.5 WG	18.75 d	3.50 e
3	Trichopulvin Td85 25 PU	15.00 d	16.25 c
4	Switch 62.5 WG Trichopulvin Td85 25 PU	20.75 d	3.25 e
5	Switch 62.5 WG Saccharopulvin 25 PU	18.25 d	4.00 e
LSD (P=.05)		5.395	3.531
Standard Deviation		3.696	2.419
CV		12.83	18.14
Bartlett's X2		4.502	6.087
P(Bartlett's X2)		0.809	0.638
Replicate F		0.729	0.203
Replicate Prob(F)		0.5450	0.8936
Treatment F		106.242	82.495
Treatment Prob(F)		0.0001	0.0001

The efficacy of the tested programmes was assessed based on disease severity in field trials (figure 2). All treatments significantly reduced the overall severity of Botrytis bunch rot compared to untreated control. A spray programme with four Switch 62.5 WG applications (caps fall, bunch closure, beginning of colour change and 3 weeks before harvest) reduced disease severity (90.47% efficacy) with almost the same efficacy as a programme with only two early-season sprays (caps fall and bunch closure) and two late-season sprays (beginning of color change and three weeks before harvest) with Saccharopulvin 25 PU (89.11%).

Figure 2. Treatments efficacy (%) in botrytis bunch rot infection



The highest efficacy (91.56%) was registered for the programme with two early-season application of Switch 62.5 WG and two late-season application of Trichopulvin 25 PU. However, when applied alone, at four application, Trichopulvin 25PU was less effective compared to treated variants (55.78%). Biological control alone is often less effective compared with commercial fungicides or provide inconsistent control. To achieve a similar level of efficacy provided by conventional fungicides, the microbial antagonists must be integrated in the classical chemical programme, to protect berries, as late-season sprays.

CONCLUSIONS

Field trials conducted with four application of Trichopulvin 25 PU, an experimental bioproduct based on *Trichoderma pseudokoningii* provided only partial control of Botrytis bunch rot. This level of control is insufficient considering that *B. cinerea* is well known for significant economic losses in vineyards worldwide, in both yield and quality. A good level of efficacy and a significant reduction in disease severity it is possible when Trichopulvin 25 PU and Saccharopulvin 25 PU are applied at the beginning of colour change and three weeks before harvest, integrated with only two fungicide applications, at caps fall and bunch closure. This integrated strategy that combines fungicides and bioproducts could offer the option to reduce the number of fungicide treatments, thus minimizing the chemical residues and manage fungicide resistance of Botrytis.

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