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The evolution of the degree of attack by grey rot (*Botrytis cinerea*) on four wine grape cultivars under conventional and organic control treatments, in the Murfatlar viticultural center

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ABSTRACT

Between 2023 and 2024, in the vineyard of the Research and Development Station for Viticulture and Oenology Murfatlar, the susceptibility of four wine grape cultivars (*Vitis vinifera*) ('Columna', 'Pinot gris 13', 'Mamaia', and 'Fetească neagră') to grey mold (*Botrytis cinerea*) was tested under two types of disease control: organic and conventional. In both disease control systems, four *botrytis* treatments were applied each year, using the same commercial products with the same active substances per hectare. The treatments, both organic and conventional, were applied during the phenological stages when the grapevine is most vulnerable to this pathogen: after flowering, during bunch closure, at the beginning of veraison and during veraison. Thus, a trifactorial experiment was achieved: vintage year x disease control system x grape cultivar. In the two years of study, the two types of disease control were also differentiated by appropriate field and fertilization technologies. The degree of attack by grey rot (*Botrytis cinerea Pers.)* was determined on 100 grape clusters per variant at harvest. For the less affected cultivars, 'Columna' (2.48%) and 'Mamaia' (3.49%), the degree of attack (DA %) was statistically lower under all control systems and compared to the other cultivars. These cultivars showed greater native resistance to grey rot compared to 'Fetească neagră' (3.78%) and 'Pinot gris 13' (4.59%). These results are of interest for organic grey rot control practices, and consequently, the 'Columna' and 'Mamaia' cultivars can be recommended for organic cultivation.

Keywords: wine grape cultivars, conventional and organic control, grey rot, grapes, degree of attack (DA %)

INTRODUCTION

In the current conditions, controlling the main grapevine diseases - Downy mildew, Powdery mildew, and Grey rot - has become a particularly important issue. This is due to the increased virulence and evolution of phytopathogens caused by climate change, intensive grapevine cultivation technologies, and excessive use of toxic phytosanitary products. During the bioactive period, the vine, a perennial plant in monoculture, is vulnerable to a wide range of pathogens that cause various diseases on plant organs. To avoid environmental pollution, there is now a focus on the ecological adaptation of protection measures against grapevine diseases and pests. (Compant *et al.,* 2016)

Grey rot, caused by the fungus *Botrytis cinerea* (De Bary) Fck, attacks all grapevine organs but most frequently affects mature berries near harvest, causing significant yield losses and deteriorating wine quality.
To obtain healthy and stable yields, the protection program against *Botrytis cinerea* (De Bary) Fck includes four treatments applied during the phenological stages when grapevines are most vulnerable to this pathogen: after flowering, during bunch closure, at the beginning of veraison, and during veraison (20 days before grape harvest). (I. C. Alexandrescu *et al.,* 1994 Encyclopedia).
Given the risk of developing resistance to fungicides used to control *Botrytis cinerea* (De Bary) Fck (Fan *et al.,* 2017; Rupp *et al.,* 2017), as this pathogen has a highly pronounced adaptive mechanism to environmental conditions and changing virulence depending on abiotic factors (temperature, relative humidity, canopy moisture, etc.), and the persistence of these pesticides in the final product, it is necessary to justify the use of phytosanitary products in the grapevine protection program.
In this regard, biological control can be a safe alternative and an ecological approach for managing *B. cinerea* disease up to grape harvest, avoiding high doses of chemical substances (Li *et al.,* 2023; Pertot *et al.,* 2017).
All grapevine cultivars are susceptible to grey rot (*Botrytis cinerea* Pers.), with a high incidence during grape ripening when weather conditions are favorable: temperatures between 15 - 23°C, high relative humidity > 80%, generated by abundant precipitation, followed by large day-night temperature fluctuations.
Unlike conventional practices, organic agriculture involves controlling diseases and pests without using synthetic pesticides, herbicides, or fertilizers (Briar *et al.,* 2007) and promotes biodiversity, natural biological cycles, and product quality improvement.
This paper presents a comparison of the biological effectiveness of two grey rot control systems, conventional vs. organic. The level of biological effectiveness of the two systems was expressed by the degree of attack recorded on grapes of four wine grape cultivars grown under field conditions.

MATERIALS AND METHODS

Plant material and cultivation system: Four wine grape cultivars, two white and two red - 'Columna', 'Pinot gris 13', 'Mamaia', and 'Fetească neagră' - were tested during the period 2023-2024 in the vineyards of the Research and Development Station for Viticulture and Oenology Murfatlar, under two types of cultural practices: organic and conventional. Three of the four cultivars have medium resistance to gray rot, while 'Pinot gris 13' is more sensitive (Olteanu *et al.,* 2002).
The experimental plots were located in the plantation at S.C.D.V.V. Murfatlar, Romania, at an altitude of 27-50 m. The vineyard had distances of 2.2 x 1.1 m between rows and plants, and the orientation of the rows is North-South for all variants. The plants were grafted onto the same rootstock, *V. berlandieri* x *V. riparia*, Selection Oppenheim 4. The vine training system was a semi-trunk Guyot with a stem height of 75-80 cm and a bilateral cordon with two fruiting spurs. For each cultivar three experimental plots were organized with the following systems for controlling gray rot: untreated (control), conventional and organic. The treatment schemes specific for the two disease control systems remained identical in both years of the study. No treatment was applied in the control plot. Each experimental plot consisted of 300 plants. The experiment organization (year x disease control system x cultivar) is presented in Table 1.

Table 1. Design of the trifactorial experiment, Murfatlar, 2023-2024

Factor A – Year	Factor B - disease control system	Factor C- cultivar
Year 2023- a_1 Year 2024- a_2	- control- untreated – b_1 - conventional treatments- b_2 - organic treatments- b_3	'Columna'- c_1
		'Pinot gris 13' - c_2
		'Mamaia' – c_3
		'Fetească neagră' – c_4

The soil type is a typical calcareous chernozem, with a medium texture, having a humus content of 2-3%. The soil maintenance system under the vine and between rows was black furrow for all variants.

Four phytosanitary treatments were applied in the conventional and organic disease control variants. Conventional treatments consisted of using phytosanitary products with the following composition: Boscalid 50% (Cantus 1.0 kg/ha) after flowering, Fludioxonil 25% + Cyprodinil 37.5% (Switch 62.5 WG 1.0 kg/ha) during bunch compacting, Pyrimethanil 400 g/L (Pyrus 400 SC 1.5 L/ha) at the onset of veraison, and Fenhexamid 500 g/L (Teldor 500 SC 1 L/ha) during veraison.

Organic treatments consisted of spraying with 20% citrus seed extract (Zytron 1.5 L/ha) applied after flowering and during bunch compacting, and 80% Mimosa tenuiflora extract (Mimoten 2.5 L/ha) applied at the onset of veraison and in the veraison stage.

To determine the degree of attack by *Botrytis cinerea*, 100 grapes were harvested from each variant and using a severity scale with seven disease severity levels: 5, 15, 25, 35, 50, 75, and 100% (Caffi *et al.,* 2010), the frequency (F %) and the intensity (I %) of grey rot symptoms on the grapes were determined each year. F % referred to the number of grapes out of the 100 harvested that showed disease symptoms/total number of grapes evaluated, while I % referred to the percentage of the grape affected by rot/total surface area of the observed grape. AD % was calculated using the formula:

$$\text{AD } \% = \frac{F\ \% * I\ \%}{100}$$

Data on the degree of fungal attack were collected and analyzed using SPSS (Statistical Package for the Social Sciences). ANOVA (Analysis of Variance) was performed in SPSS to determine statistical differences between groups, assessing the significance of differences between means for each measured variable. After conducting ANOVA, the Duncan test was applied as a post-hoc test to identify specific differences between groups. The Duncan test allowed for the comparison of multiple means to determine which treatments (organic, conventional and control) were significantly different from each other in the context of the three variables of interest.

During the two years of study, all specific viticultural technological operations were carried out throughout the growing season, with treatments applied as needed using a 1000 LT tractor-mounted sprayer for grapevines. A series of data on meteorological conditions were recorded and processed, including mean, maximum, and minimum air temperatures, precipitation, relative air humidity, and sunshine duration.

RESULTS AND DISCUSSION

From a thermal point of view, during the two years, the average temperature during the growing season was recorded as 3.2-3.6°C above the normal average (18.3°C). In the Murfatlar wine-growing center, the normal accumulation of precipitation during the growing season is on average 245 mm. However, during the studied years, the precipitation was below the normal value in 2023 (110.2 mm), experiencing an excessive drought during the ripening period of the grapes (August - 3.6 mm and September 0.2

mm) and well above the normal average in 2024 (281.8 mm), with a significant contribution of rainfall between 28 - 31.08.2024 (62.0 mm) and in the first decade of September (62.4 mm), coinciding with the grape ripening phase.
Regarding the relative humidity, it is observed that in 2023, it was lower with reduced sunlight exposure compared to 2024. The data are presented in Table 2.

Table 2. Climatic data from the Murfatlar viticultural center during the growing season of 2023 and 2024

Month	Multiannual average	Year 2023						Year 2024					
		Monthly average (°C)	Absolute maximum (°C)	Absolute minimum (°C)	Rainfalls (mm)	Air Higroscopicity (%)	Sunshine (h)	Monthly average (°C)	Absolute maximum (°C)	Absolute minimum (°C)	Rainfalls (mm)	Air Higroscopicity (%)	Sunshine (h)
IV	10.5	10.8	20.3	-1.9	39.5	85.8	159.8	16.2	31.9	3.4	77.6	65.2	167.2
V	16.2	17.4	29.7	1.5	11.4	73.4	227.3	16.6	27.7	3.4	26.2	67.4	186.5
VI	20.4	22.8	33.6	7.9	28.4	64.8	227.5	26.1	37.7	12.5	11.2	53.6	230.8
VII	22.6	26.8	39.1	13.4	27.8	57.6	227.6	28.5	38.8	10.1	17.4	39.1	285.6
VIII	22.6	26.9	36.9	11.5	3.6	58.7	225.5	26.1	36.6	14.5	87.0	58.8	228.3
IX	17.6	22.2	31.4	8.6	0.2	59.5	244.1	21.9	27.5	16.6	62.4	72.4	224.3
Annual mean	18.3	21.5				66.6		22.6				59.4	
Annual sum					110.2		1311.8				281.8		1421.9

In the first part of the growing season, the degree of attack by the fungus *Botrytis cinerea* in all experimental plots was very low. However, with the onset of the grape ripening phenophase, both in 2023 and 2024, the incidence rate of the attack caused by *Botrytis cinerea* increased. In 2024, the frequency and intensity of the attack grew compared to 2023, favored and amplified by the excess precipitation recorded between August 28 – 31, 2024 (62.0 mm) and in the first decade of September (62.4 mm). At harvest, 100 grapes from each variant were evaluated, and observations and assessments were made using the disease severity diagram scale with seven levels of severity: 5, 15, 25, 35, 50, 75, and 100% (Caffi *et al.,* 2010).
As a result of the data analysis regarding the degree of infestation with gray rot over the two studied years, significant differences between the types of treatments are highlighted. Table 3 presents the mean values, standard deviations, and confidence intervals for each variable, along with the statistical significance of the differences between groups. Considering the degree of attack by *Botrytis cinerea* (AD %) for the tested cultivars, all three experimental factors had significant effects in the series of experiments.
Following the statistical analysis, the following significant data regarding the effect of control systems on the degree of fungal attack for the studied cultivars emerged: for the 'Columna' cultivar the mean degree of fungal attack in the control plot was 6.23 (standard deviation 5.83), whereas for the conventional control system, the mean significantly decreased to 0.20 (standard deviation 0.28), and for the organic control system, it was 1.03 (standard deviation 0.34). Similarly, the 'Mamaia' cultivar showed a notable reduction in the degree of attack, with a mean of 9.21 (standard deviation 9.81) in the control, 0.41 (standard deviation 0.42) in the conventional system, and 0.86 (standard deviation 0.74) in the organic system.
On the other hand, 'Fetească neagră' and 'Pinot gris 13' cultivars showed higher susceptibility to grey rot. 'Fetească neagră' cultivar recorded a mean of 9.50 (standard deviation 10.03) in the untreated (control) group, while 'Pinot gris 13' had a mean of 11.35

(standard deviation 12.71). These values indicate a more severe fungal attack compared to the 'Columna' and 'Mamaia' cultivars.
Statistics show that both the organic and conventional control systems had a significant effect on reducing the degree of fungal attack, especially for the 'Columna' and 'Mamaia' cultivars, which exhibit higher native resistance to grey rot compared to the other analyzed cultivars, 'Pinot gris 13' and 'Fetească neagră'.

Table 3. Descriptive statistics of fungal attack based on the applied treatment (organic, conventional, and control)

Descriptive Statistics					
Dependent Variable: Degree of fungal attack					
Cultivar	**Disease treatment system**	**Year**	**Mean**	**Std. Deviation**	**N**
1	2	3	4	5	6
'Columna'	Control	2023	2.1100	.	1
		2024	10.3500	.	1
		Total	6.2300	5.82656	2
	Conventional	2023	0.0000	.	1
		2024	0.4000	.	1
		Total	0.2000	0.28284	2
	Organic	2023	0.7800	.	1
		2024	1.2700	.	1
		Total	1.0250	0.34648	2
	Total	2023	0.9633	1.06688	3
		2024	4.0067	5.51068	3
		Total	2.4850	3.92185	6
'Fetească neagră'	Control	2023	2.4100	.	1
		2024	16.6000	.	1
		Total	9.5050	10.03385	2
	Conventional	2023	0.2500	.	1
		2024	0.8500	.	1
		Total	0.5500	0.42426	2
	Organic	2023	0.6500	.	1
		2024	1.9200	.	1
		Total	1.2850	0.89803	2
	Total	2023	1.1033	1.14914	3
		2024	6.4567	8.80066	3
		Total	3.7800	6.33296	6
'Mamaia'	Control	2023	2.2700	.	1
		2024	16.1500	.	1
		Total	9.2100	9.81464	2
	Conventional	2023	0.1100	.	1
		2024	0.7100	.	1
		Total	0.4100	0.42426	2
	Organic	2023	0.3300	.	1
		2024	1.3900	.	1
		Total	0.8600	0.74953	2
	Total	2023	0.9033	1.18867	3
		2024	6.0833	8.72462	3
		Total	3.4933	6.25000	6
'Pinot gris 13'	Control	2023	2.3600	.	1
		2024	20.3400	.	1
		Total	11.3500	12.71378	2
	Conventional	2023	0.1300	.	1

Descriptive Statistics					
Dependent Variable: Degree of fungal attack					
Cultivar	**Disease treatment system**	**Year**	**Mean**	**Std. Deviation**	**N**
1	2	3	4	5	6
		2024	1.5200	.	1
		Total	0.8250	0.98288	2
	Organic	2023	0.8900	.	1
		2024	2.3400	.	1
		Total	1.6150	1.02530	2
	Total	2023	1.1267	1.13368	3
		2024	8.0667	10.63692	3
		Total	4.5967	7.76021	6
Average disease system treatment	Control	2023	2.2875	0.13175	4
		2024	15.8600	4.12562	4
		Total	9.0738	7.74172	8
	Conventional	2023	0.1225	0.10243	4
		2024	0.8700	0.47237	4
		Total	0.4963	0.50968	8
	Organic	2023	0.6625	0.24240	4
		2024	1.7300	0.49511	4
		Total	1.1963	0.67515	8
Average Total		2023	1.0242	0.97324	12
		2024	6.1533	7.50311	12
		Total	3.5888	5.85154	24

To assess the impact of control systems on fungal attack, ANOVA tests were conducted for the degree of attack. The analyzed grey rot (*Botrytis cinerea* Pers.) control systems were: organic, conventional and control, with each group consisting of 24 variants. The ANOVA results are presented in Table 4.

Table 4. Results of the analysis of variance (ANOVA) for the degree of fungal attack based on the control system (organic, conventional and control)

Tests of Between-Subjects Effects						
Dependent Variable: Degree of fungal attack						
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**	**Partial Eta Squared**
Corrected Model	376.662[a]	5	75.332	3.300	0.027	0.478
Intercept	309.099	1	309.099	13.541	0.002	0.429
Cultivar	13.679	3	4.560	0.200	0.895	0.032
Treatment	362.983	2	181.491	7.951	0.003	0.469
Error	410.870	18	22.826			
Total	1096.631	24				
Corrected Total	787.532	23				
a. *R Squared = 0.478 (Adjusted R Squared = 0.333)*						

The ANOVA analysis highlighted significant effects of the control system on the degree of fungal attack ($F(2.18) = 7.951$, $p = 0.003$, Partial Eta Squared = 0.469). This result suggests that the type of control (organic, conventional, control) significantly influences the severity of the fungal attack, with an explanatory impact of nearly 47%.

Regarding the grapevine cultivars, the analysis did not find significant differences in the degree of fungal attack between experimental plots (F (3.18) = 0.200, p = 0.895, Partial Eta Squared = 0.032). This suggests that variability in fungal attack severity is not significantly influenced by the grapevine cultivar in the context of the applied control systems.
However, descriptive data indicate that the 'Columna' and 'Mamaia' cultivars show a lower degree of fungal attack compared to the other cultivars. 'Columna' and 'Mamaia' had significantly lower mean values for the degree of fungal attack, suggesting greater native resistance to grey rot compared to 'Fetească neagră' and 'Pinot gris 13' cultivars. These observations are supported by Duncan's post-hoc tests, which identified 'Columna' and 'Mamaia' as groups with significantly lower means compared to the other cultivars.
To explore specific differences between the organic, conventional, and control treatments regarding the degree, intensity, and frequency of fungal attack, Duncan's test was performed, which was applied after ANOVA to identify significant differences among the studied cultivars (Table 5).

Table 5. The comparison of cultivars regarding the degree of attack based on the control system (organic, conventional and control) using the Duncan test

Degree of fungal attack		
Duncan[a,b]		
Grapevine-cultivar	**N**	**Subset**
		1
'Columna'	6	2.4850
'Mamaia'	6	3.4933
'Fetească neagră'	6	3.7800
'Pinot gris 13'	6	4.5967
Sig.		0.492
Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square (Error) = 22.826.		
a. Uses Harmonic Mean Sample Size = 6.000.		
b. Alpha = 0.05.		

The Duncan test results for the degree of *Botrytis cinerea* attack show differences among the analyzed grapevine cultivars. The 'Columna' cultivar records the lowest degree of attack (2.485), indicating higher resistance to this pathogen. 'Mamaia', with a mean attack degree of 3.4933, falls into the same subset as 'Columna', suggesting a relatively high and comparable resistance. On the other hand, 'Fetească neagră' (3.7800) and 'Pinot gris 13' (4.5967) have higher mean values, indicating greater susceptibility to fungal attack, with 'Pinot gris 13' being the most sensitive.
Although the mean values suggest clear differences between cultivars regarding the resistance to *Botrytis cinerea*, the statistical significance of these differences (p = 0.492) indicates that they are not significant at the α = 0.05 level. Nevertheless, the results suggest that the 'Columna' and 'Mamaia' cultivars are more resistant to fungal attack compared to 'Fetească neagră' and 'Pinot gris 13'.

CONCLUSIONS

In the two years of the study, the climatic evolution during the growing season was distinctly different. The year 2023 was very dry (110.2 mm) compared to 2024, when

precipitation accumulations reached 281.8 mm, with 124.4 mm recorded during the grape ripening period.

Considering the degree of attack by *Botrytis cinerea* (AD %) for the tested cultivars, all three experimental factors had significant effects in the series of experiments. According to the statistical calculations, the type of control (organic, conventional) significantly influenced the severity of the fungal attack, with an explanatory impact of nearly 47%.

The 'Columna' and 'Mamaia' cultivars had significantly lower average fungal attack degrees under all control systems, each year, suggesting a higher native resistance to gray rot (*Botrytis cinerea*) compared to the 'Fetească neagră' and 'Pinot gris 13' cultivars.

This native resistance of the 'Columna' and 'Mamaia' cultivars offers an advantage in the selection of future cultivars for managing this disease and may be better suited to organic cultivation systems than other cultivars.

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