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THE INFLUENCE OF VEGETAL EXTRACTS AND NANOSTRUCTURED MIXTURES ON GRAPEVINE POLLEN GRAINS

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Abstract
Pesticides, plant bio-stimulants and chemical fertilizers used in intensified viticulture affects negatively the viability of the plants pollen causing relevant economic losses to viticulturists. Given these conditions, we aimed to identify the environmentally friendly phytosanitary treatments which does not negatively affect the evolution of pollen grains. The experiment took place in the greenhouse on 120 grapevine plants from the genotypes Feteasca alba 97 St., Feteasca regala 72 St., Feteasca neagra 6 St., Cabernet Sauvignon 131 St., 30 plants/genotype. These have been treated to combat the Plasmopara viticola and Uncinula necator pathogens with pesticides, biological products, plant extract and nanostructured mixture. The studied genotypes reacted differently regarding the evolution of the viability of pollen grains, the average pollen grains size and the total number of pollen grains. The best results have been registered at F. regala 72 St. (the viability was significantly improved at plants treated with plant extract and those with nanostructured mixture and the highest number of pollen grains was recorded at the plants treated with nanostructured mixture).

Keywords: Dryopteris filix-mas, fertility, inflorescence, viticulture.

1. INTRODUCTION

The grapevine is a plant with high ecological plasticity and is cultivated between 20-500 northern latitude and 20-400 southern latitude (Rotaru and Colibaba, 2011). In this species the fruit organs develop over two years, the current year's production being decided since the previous year (except for lateral shoots). Thus, to differentiate the fruit buds, the next steps are followed: floral induction, differentiation of inflorescence primordia and flower formation. The leaves are very important in the mechanism of floral induction, because the hormones synthesized in the leaf determine the beginning of the formation process of inflorescence primordia (Irimia, 2012).

The opening of the inflorescences and flowers takes place staggered: the first which bloom are the inflorescences at the base of the shoot and the last at the top; the first flowers which open are those from the middle of the inflorescence, followed by those from the base and, finally, those from the top. The flowering period it takes 6-12 days, depending on the variety, the favourable climatic conditions and the agrotechnical measures that are applied in the plantation. The maximum of

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flowering is considered when 75% of the flowers are open; the phenophase is considered complete when all the flowers have opened and are about to form berries (Irimia, 2012).
Weeds, pests and diseases can cause losses between 26 and 40% but they can reach up to 50 - 80%, depending on crop, cultivar and region (Oerke, 2006 cited by Bohme, 2018). Studies indicated that pesticides, plant bio-stimulants and chemical fertilizers used in intensified agriculture affects negatively the viability of the plants pollen reducing their fertility (Sabir, 2015). Accordingly, under certain conditions the low pollen viability may limit grapes production, causing relevant economic losses to the viticulturists (Tello et al., 2018).
The *Vitis vinifera* pollen grains are normally 3-zonocolporate, spheroidal to prolate, with very long, narrow, slit-like, slightly, but distinctly sunken ectoaperture (colpus). However, some research has shown the presence of pollen in a rounded shape, without furrows and germination pores (Jovanovic-Cvetkovic et al., 2016).

2. MATERIALS AND METHODS

The experiment took place in the greenhouse on 120 grapevine plants of the Feteasca alba 97 St., Feteasca regala 72 St., Feteasca neagra 6 St., Cabernet Sauvignon 131 St. genotypes, 30 plants/genotype each. These were treated to combat the *Plasmopara viticola* and *Uncinula necator* pathogens, in 2019, with: chemical and biological substances (V1 - Dithane 0,2%, Flint max 75 WG 0,16 kg/ha and Sublic + Nutryaction), plant extract of *Dryopteris filix-mas* (V2) and nanostructured mixture (V3). The extracts were applied to protect the environment by avoiding the use of pesticides. In 2020, pollen was harvested during flowering to observe if the applied treatments influenced the viability, the size of pollen grains and their total number. Thus, the pollen was harvested on 08.05.2020 from F. regala 72 St. and on 11.05.2020 from the other genotypes. The determinations were realised with the Luna II device (Figure 1) and the data processing was performed using the IBM SPSS Statistics Version 25 program.

a b

Figure 1. Luna II™ apparatus (a) and the cell counting slides (b)

3. RESULTS AND DISCUSSIONS

Statistical analysis performed in order to observe the effects of phytosanitary treatments on the evolution of the grapevine pollen showed that, pollen grains viability was significantly influenced only at F. regala 72 St. where the vegetal extract and the nanostructured mixture led to a significant increase in viability compared to the control. The average of pollen grains size was not influenced by the treatments to none of the variants. But, regarding the total pollen grains number from the 4

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genotypes studied, only pollen from C. Sauvignon 131 St. did not show significant differences between variants. However, at F. alba 97 St. the total number of pollen grains decreased significantly at V2 compared to the control, and at the F. neagra 6 St. their number decreased significantly in the variants treated with extracts and nanostructured mixture compared to the control. Probably, the lower values are due to the fact that the grapevine plants from variants 2 and 3 were in a more advanced stage of vegetation when were done these determinations, and the pollen had already shaken at these compared to the control (V1) (Table 1, 2, 3, 4).

Table 1. Evolution of the pollen grains at white wine varieties. The value represents Mean± Std. Deviation at P<0.05. The small letters represent the significance

Determination	Variant	Genotype	
		F. regala 72 St.	***F. alba 97 St.***
Viability of pollen grains (%)	V1	24.40±4.88 [a]	65.43±32.06 [a]
	V2	66.70±17.56 [b]	57.13±12.35 [a]
	V3	83.33±0.91 [c]	84.13±13.30 [a]
The average of pollen grains size (µm)	V1	27.40±0.85 [a]	21.00±9.36 [a]
	V2	24.90±2.31 [a]	25.57±8.38 [a]
	V3	29.33±0.12 [a]	29.57±0.50 [a]
Total pollen grains number (no)	V1	80.33±20.50 [a]	86.33±41.40 [a]
	V2	76.33±22.23 [a]	19.33±13.31 [b]
	V3	119.67±10.12 [b]	55.33±12.01 [a]

Table 2. Evolution of the pollen grains at red wine varieties. The value represents Mean± Std. Deviation at P<0.05. The small letters represent the significance

Determination	Variant	Genotype	
		F. neagra 6 St.	***C. Sauvignon 131 Șt***
Viability of pollen grains (%)	V1	46.47±7.50 [a]	87.37±13.58 [a]
	V2	65.70±25.00 [a]	79.93±10.14 [a]
	V3	42.90±26.06 [a]	79.27±1.70 [a]
The average of pollen grains size (µm)	V1	29.20±0.36 [a]	25.57±4.83 [a]
	V2	27.97±0.38 [a]	26.47±0.95 [a]
	V3	24.47±5.30 [a]	27.73±0.83 [a]
Total pollen grains number (no)	V1	87.00±10.54 [a]	64.00±55.65 [a]
	V2	34.67±20.65 [b]	50.33±16.29 [a]
	V3	27.33±11.93 [c]	28.33±12.50 [a]

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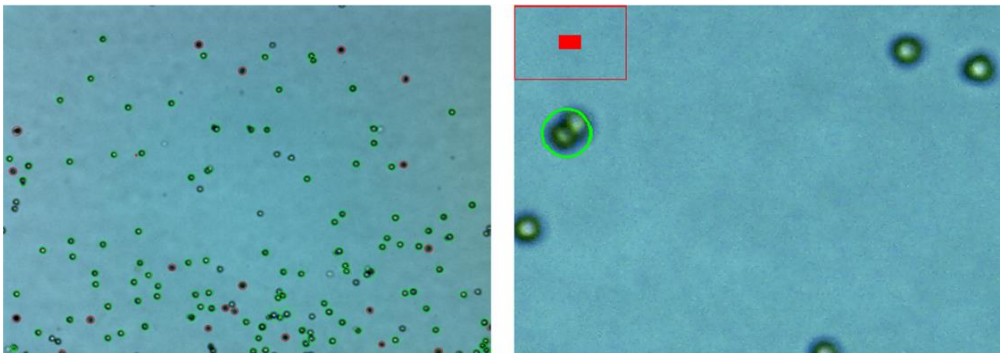

Figure 2 Pollen grains photographed with the Luna II™

The pollen grains number it is an important part of the reproductive strategies in plants and varies to a large extent between and within species. To count pollen is a laborious work using a counting chamber under a microscope. For this reason, some researcher uses for pollen counting a CASY cell counter (Kakui et. al., 2020a; Kakui et. al., 2020b) and LUNA-II™ automated cell counter.

4. CONCLUSIONS

The studied genotype reacted differently regarding the evolution of the pollen grains viability, average pollen grains size and total number of pollen grains after applying pesticides, biological products, plant extract and nanostructured mixture. Thus, at Feteasca regala 72 St. viability was significantly improved at plants from variant 2 and 3 compared to the control, and plants from variant 3 also recorded the highest number of pollen grains. The Feteasca alba 97 St. inflorescence registered a significantly decreased of the total number of pollen grains at variant 2 compared to the control. Also, a significant decrease of pollen grains number was recorded at Feteasca neagra 6 St. in the case of the variants 2 and 3 compared with the control, probably due to the fact that the plants were in a more advanced stage of vegetation and the pollen had shaken. At Cabernet Sauvignon 131 St. the evolution of the pollen did not register signified differences between the variants after applying the treatments.

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