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THE INFLUENCE OF TREATMENTS WITH AMINO-ACIDS AFTER HAIL FALL ON FETEASCĂ NEAGRĂ GRAPE HARVEST

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Abstract

Some fertilizers containing amino acids could help recover grapevine plants after hail stress and on the quality and quantity of harvest. After the hail damage on April 30th, 2019, three treatments with an amino-acids complex were applied to the grapevine canopy - at 20-25 cm, at 35-40 cm shoot length, and berries growth - on Fetească neagră grapevines at Serve Winery, Dealu Mare vineyard. At harvest, the quality and quantity of grape was tested for treated and untreated variant. The total anthocyanins for the treated variant were 211 mg/100 g FW and for the untreated variant - 198 mg/100 g FW. The content of total polyphenols total was 376 g/100 g FW for the treated variant, significantly higher than the untreated variant 320 g/100g FW. The content of flavonoids for the treated variant was of 147 g/100 g FW significantly lower than the untreated variant 130 g/100 g FW. The treatment with amino acids influenced the quantity and the quality of grape harvest affected by the hail, with significant results.

Key words: *Fetească neagră, amino-acids treatments, hail, quality, quantity.*

INTRODUCTION

Climate change poses an additional challenge to wine production at the level world. Historical records reveal significant signals of climate change, including an increase in global average surface temperature of about 0.6°C since 19th century (Trenberth et al., 2007). All these changes are very likely to have an impact significantly on the grapevine (van Leeuwen and Darriet, 2016). Actually, the vine (*Vitis vinifera* L.) is traditionally cultivated in geographical areas where the temperature average growing season is 12-22°C (Droulia and Charalampopoulos, 2021), and climate and climate are among the environmental factors that most influence the yield and quality of wine. Extreme weather events can influence significant yield losses over time how climate change can alter the vine phenological cycle, disease/pest patterns, ripening potential, wine characteristics and yield (Trenberth et al., 2007).

Hail can cause significant damage to the foliage and organs generative. Hail can reduce yield, shoot number and leaf tissue and partially or completely stop plant nutrition. As a result, a hail of over 50% damage suppresses growth and development with up to 20 days, while lighter hail destroys growth processes for about 10 to 14 days. At the same time, secondary buds, dormancy, side shoots from children, begin to develop more intensively. For this reason, apart from the low yield, production small, hail can lead to a poor quality harvest. From this point of view, it is important for the vine to be protected from pathogens and other stress factors after the impact of hail (Banită et al., 2020).

In terms of agriculture, this implies preserving the environment and public health by cooperating with nature. The "From Farm to Fork" strategy (European Commission, A Farm to Fork Strategy for a Fair, Healthy and Environmentally-Friendly Food System, 2020) is one of the initiatives of the "European Green Deal" and aims to promote a fair, healthy, and environmentally - friendly food system with equitable economic opportunities for all. This approach emphasizes the necessity of a sustainable food production chain by lowering the usage of agrochemical compounds (pesticides and fertilizers) while also guaranteeing the production of wholesome, premium, and reasonably priced food for customers (Artem et al., 2023).

The positive effects of the foliar application of amino acids and bio-stimulants based on amino acids on both the qualitative and quantitative characteristics of different cultivated plants have been reported (Sun et al., 2024).

Foliar application is a widespread practice in the agriculture business despite being highly energy - consuming. Enzymatic hydrolysis of both plant and animal protein hydrolysates produces amino acids that have been employed for foliar usage (Islam et al., 2022).

Abiotic factors are shown to control the synthesis and degradation of primary (sugars, amino acids etc.) and secondary (phenolic compounds) metabolites through the regulation of their biosynthetic pathways at different stages of berry development (Rienth et al., 2021).

The aim of this study was to investigate the influence of Delfan Plus treatment after hail fall on the profile of polyphenolic compounds in Feteasca Neagra grapes. In order to understand how the treatment with amino-acids might regulate the polyphenol metabolism, the appropriate spectrophotometry and high-performance liquid chromatography (HPLC) methods were used to monitor for Fetească neagră grapes after harvest.

MATERIALS AND METHODS

The research was carried out during 2019 on Fetească neagră *Vitis vinifera* L. cultivar - at Serve winery, Ceptura wine growing center, Dealu Mare vineyard. The vines were planted in 2006, with distance of 2.2 m between rows and 0.9 m between vines/row, with a density of 5050 vines/ha. The vines were trained as double Guyot with 24 buds/vine. On April 30th 2019, the hail that felt in Ceptura wine growing center and damaged 90% of shoots. The shoots were 10-15 cm height, uniformly growth and in good phytosanitary shape. In 30 minutes felt an amount of 25 mm/m^2 of rain. On May 2nd, at 2 days after the hail felt, a contact fungicide (Funguran OH, 2 l/ha) was applied. The damaged shoots were cut down to stimulate the growth of dormant, secondary and coronary buds.

Two experimental variants were set on: Control variant (CV) and treated variant (TV) with amino-acids (Delfan Plus) were applied at 20-25 cm shoots length, at 35-40 cm shoots length and at berries growth (treated variant). Also, during vegetative period, nine phytosanitary treatments using contact and systemic pesticides were applied.

At harvest, on September 18, 2019, the grape samples were collected. The analyses consisted on sugar content, total acidity, pH, total polyphenols, anthocyanins and flavonoids.

The quantification of total phenolic concentration in the wine samples was conducted utilizing the Folin-Ciocalteu (FC) method, a widely accepted protocol in analytical chemistry (Girelli et al., 2015). The experimental procedure involved combining 0.1 mL of the wine sample with 5 mL of deionized water, 0.5 mL of Folin-Ciocalteu reagent, and 2 mL of a 20 percent (w/v) Na_2CO_3 solution. Subsequently, the volume was adjusted to 10.00 mL with deionized water. After a specified incubation period of 30 minutes at 40°C, the absorbance of the resulting solution was measured at 760 nm, relative to a blank containing 0.1 mL of water in lieu of the wine sample.

The determination of the total concentration of anthocyanins in wine was facilitated through the distinctive absorption band exhibited by this class of compounds at approximately

520 mn. Given that the color intensity of anthocyanic pigments reaches its zenith at pH 1 and becomes colorless at pH 4.5, a meticulous procedure was employed for sample preparation. Specifically, aliquots of each wine sample were judiciously diluted with a 10 mL solution, composed of 1.49 grams of KCl dissolved in 100 milliliters of deionized water, combined with 67 milliliters of 0.2 molar HCl, and adjusted to pH 1.0. Additionally, a 10 mL aliquot was mixed with a pH 4.5 buffer, consisting of 1.64 grams of sodium acetate dissolved in 100 milliliters of deionized water and adjusted with hydrochloric acid. Spectrophotometric analysis was conducted at 700 mn for degradation product correction and at 520 nanometers (the peak of the visible spectra) for each wine sample, as elucidated by Bora et al. (2018a) and Bora et al. (2018b). It is noteworthy that anthocyanins manifest an absorption band within the 490-550 nanometers range, distinct from the ultraviolet bands characterizing other phenolic compounds. This comprehensive methodology ensures the accurate determination of anthocyanin concentrations in the grape samples.

The quantification of total flavonoids was conducted employing a colorimetric method as described by Kim et al. (2003). The initial step involved the dilution of the extract to a final volume of 5 ml with distilled water. Subsequently, 300 µl of 5% $NaNO_2$ was added to the solution, and the mixture was allowed to stand for 5 minutes. Following this, 300 µl of 10% $AlCl_3$ was introduced, and after an additional 6 minutes, 2 ml of 1N NaOH was incorporated. The resulting solution was thoroughly homogenized, and the absorbance was measured at 510 nm against a blank containing water. This methodological approach, outlined in Kim et al. (2003), ensures the accurate determination of the total flavonoid concentration in the wine samples, providing valuable insights into the compositional aspects of these compounds within the analyzed specimens.

Statistical analysis

The statistical interpretation of the results was performed using the Tukey test, using the SPSS, version 24 (SPPS Inc. Chicago, IL, USA). The statistical processing of the results was primarily made to calculate the following statistical parameters: arithmetic average, standard deviation, average error, using the SPSS version 24 (SPPS Inc. Chicago, IL, USA).

RESULTS AND DISCUSSIONS

Even if the vines were pruned with 24 buds/vine, the hail fall on April 30th, damaged 90% of shoots. In this situation, the number of grapes on vines was low – 3.3 for Control variant and 4.3 for DelfanPlus Treated vines. The chemical analyses of the red grape samples produced in 2019 harvest are present in Table 1. The parameters are reported for the two variants Control and Delfan Plus Treated. The results show that the grape harvest recorded a normal to high sugar content, ranging, in accordance to the experimental variants, from 271 g/l sugar for Delfan Plus Treated Variant to 265 g/l sugar for Control variant, differences being insignificant. The Control variant with reduced number of grapes/vine – 3.3, recorded lower values for the sugar in grapes compared to the Delfan Treated variant, and this could explain the role of this product in increasing the quality of harvest.

Table 1. Grape characteristic - harvest 2019

Variant	No. of grapes/ vine	Sugar content (g/l)	Total acidity (g/l sulphuric acid)	pH
Control Variant	3.3 a	265 a	5.7 b	3.4 a
Treated Variant	4.3 b	271 a	5.9 a	3.5 a

Average values ± standard errors (n=3). The letters in the brackets show the statistical difference among results for $p<0.05$. For the same compound, a common letter for the variants shows no significant difference among them according to Tukey test.

Regarding the total acidity, this parameter was influenced by the treatment, between the two variants being a significant difference, with a 5.9 g/l sulphuric acid for Delfan Plus Treated Variant and a 5.7 g/l sulphuric acid for Control Variant (Table 1).

Table 2. Effect of Delfan Plus Treatment on the concentration of anthocyanins in Feteasca neagra grapes at harvest

Anthocyanin types	Delfan PlusTreated variant	Control variant	Sign
Delphinidin-3-O-glucoside	26.83±7.89 B	20.74±5.61 A	***
Cyanidin-3-O-glucoside	13.43±3.32 B	11.45±3.45 A	**
Petunidin-3-O-glucoside	24.62±6.89 B	22.67±5.88 A	**
Peonidin-3-O-glucoside	38.85±6.99 B	36.45±7.76 A	**
Malvidin-3-O-glucoside	95.23±10.03 A	95.31±12.05 A	Ns
Malvidin-3-O-pcumaroilglucoside	12.16±3.57 A	11.67±7.96 A	ns

Average values ± standard errors (n=3). The letters in the brackets show the statistical difference among results for p<0.05. For the same compound, a common letter for the variants shows no significant difference among them according to Tukey test.

Anthocyanins are orchestrated within the phenylpropanoid pathway beneath the complex control of all basic chemicals and various administrative qualities at the transcriptional level. Therefore, the most important anthocyanins in red grapes are the 3-O-monoglucosides of cyanidin, peonidin, delphinidin, petunidin, and malvidin (He et al., 2010). Cyanidin in grapes were chemically changed over not as it were to catechins but too to cyanidin-3-glucoside and peonidin-3-glucoside by C3 glucosylation responses catalysed in specific by the action of anthocyanidin/anthocyanin glycosyltransferases. Based on the information gotten after HPLC investigation of anthocyanins, it can be concluded that treatment with Delfan Plus upgraded the quality expression related with anthocyanin biosynthesis, especially within the biosynthesis of cyanidin-3-glucoside. In the case of cyanidin-3-glucoside and peonidin-3-glucoside, an increase of almost 15%, and 7%, respectively. Moreover, in this situation, the existence of directly relationship between the Delfan Plus treatment on the vines and the intensification of anthocyanin biosynthesis in these grapes was confirmed (Table 2).

The HPLC results also show differences between the delphinidin-3-glucoside concentration in the treated grapes and the control grapes were significant. An increase in the concentration level of 23% in the Fetească neagra grapes treated with Delfan Plus was observed, which proves that the application of this products on grapes leads to an enhancement of the enzyme activity of UDP-D-glucose flavonoid 3-O-glycosyltransferase, according to Artem et al. (2023), an increase of about 8% in the petunidin 3-glucoside concentration was observed in the Delfan Plus treated grapes. This could be explained by the statetment that delphinidin 3-O-glucoside underwent two successive methylations, mediated by O-methyltransferases and with S-adenosylmethionine as a methyl donor, at the oxygen in the 30 - and 50 -positions, giving successively petunidin 3-O-glucoside and the malvidin 3-O-glucoside (Artem et al., 2023). For malvidin-3-glucoside, the concentrations were similar in both the control and treated grapes (Table 2). According to He et al., 2012, the most abundant monomeric anthocyanin in red grapes is malvidin-3-O-glucoside. In our study, the major monomeric anthocyanin was malvidin-3-O-glucoside, with values of 95.23 mg/100 g FW (Delfan Plus treated grapes) and 95.31 mg/100 g FW (Control grapes), the differences being insignificant. The anthocyanins, the red pigments of grape, influence the colour shade and intensity through their concentration and their proportion, each anthocyanin having colours which depend on their chemical structure and external factors, such as the pH. Thus, any change in the anthocyanin profile leads to colour change. Some study, showed that anthocyanins like delphinidin, peonidin, petunidin accumulated better in warmer years. Anthocyanins is the class of polyphenols which is the most influenced by the grape yield. In our study, even if the Delfan Plus treated variant had a higher number of grapes – 4.3 grapes/vine, the concentration of total anthocyanins were higher - 221 mg/100 g FW, then Control variant – 3.3 grapes/vine – 198 mg/100 g FW, differences being significant.

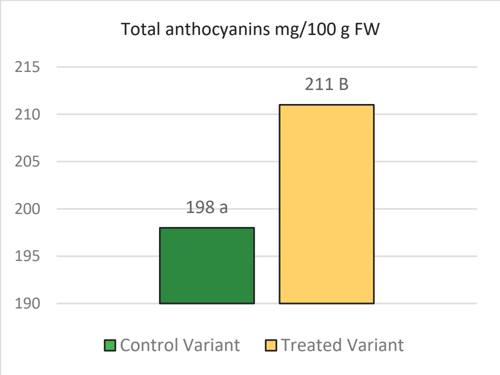

Figure 1. Total anthocyanin content in experimental variants. All the analysis were performed in triplicate. Different letters indicate the existence of significant differences (α≤ 0.05)

The most important flavonoids in wine are anthocyanidins, flavanols (also known as catechins or flavan-3-ols), and flavonols (including quercetin and myricetin). Red wine is a major source of flavonoids, and it is known to reduce cardiovascular events when consumed in moderation (Fernandes et al., 2017) and an appropriate intake of those compounds may be beneficial in preventing, and eventually managing, allergic diseases.

Our results indicate a significant difference of the flavonoids content between the two experimental variants, with values of 130 mg/100 g FW for control grapes and 147 mg/100 g FW for Delfan Plus treated variant (Figure 2). In grape there are two main types of phenolic compounds non-flavonoids, and flavonoids, respectively. The relative amounts and distribution of these compounds depend on many different factors such as grape variety, vineyard location, climate, soil type, agricultural practices, harvest time, production process and wine brewing process (Colleta et al., 2014).

Our results indicate a significant difference of the polyphenols content between the two experimental variants, with values of 230 mg/100 g FW for control grapes and 347 mg/100 g FW for Delfan Plus treated variant (Figure 3), the difference being significant.

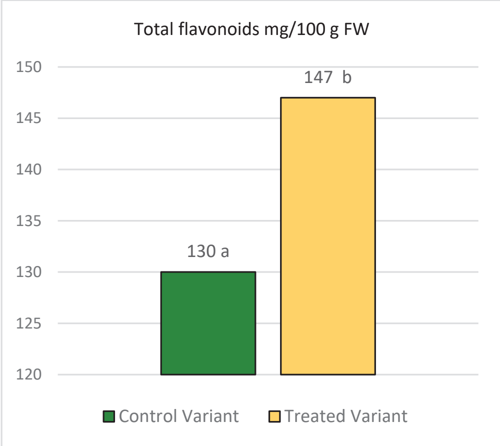

Figure 2. Total flavonoids (mg GAE/L) content in experimental variants. All the analysis were performed in triplicate. Different letters indicate the existence of significant differences (α≤ 0.05)

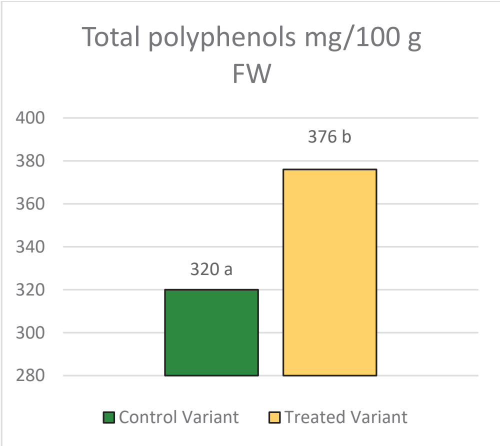

Figure 3. Total polyphenols (mg/100 g FW) content in experimental variants. All the analysis were performed in triplicate. Different letters indicate the existence of significant differences (α≤ 0.05)

CONCLUSIONS

For the first time, at SERVE winery treatments with Delfan Plus was used in treatment grapevines affected by hail. Furthermore, Delfan Plus – a biostimulator containing amino-acids was found to stimulate the grape quality.

It can be concluded that significantly higher concentrations of polyphenols in Fetească neagră red grapes can be achieved by applying Delfan Plus in the preliminary stage of berry development. However, further studies are needed to observe the evolution of the profile of polyphenolic compounds during the applying of biostimulants containing amino-acids to determine the appropriate concentration of chemical compounds required as a quality grape harvest.

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