

HERBICIDE AND FOLIAR FERTILIZER EFFECTS ON CABERNET SAUVIGNON WINE VOLATILES

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Abstract: The influence of combined treatment with herbicides and foliar fertilizers on the volatile composition (by GC-FID) of four harvests (2021 – 2024) red wines of the Cabernet Sauvignon variety from the region of Central Northern Bulgaria was studied. The strongest influence (in two of the harvests – 2022 and 2023) on the accumulation of high final levels of total volatile compounds and the highest total accumulation of esters in the wines was exerted by the single treatment with herbicides (variant V2). It was found that the treatments with herbicides and foliar fertilizers alone or in combination led to the synthesis of higher levels of total higher alcohols in the experimental variants, compared to the untreated control. This indicated for an influence on improved amino acid metabolism in the grapevine plant, and from there the synthesis (by metabolic degradation of amino acids by yeast) of higher levels higher alcohols in the young wines. The main higher alcohols were 3-methyl-1-butanol and 2-methyl-1-butanol. Ethyl acetate and isopropyl acetate dominated the ester fraction, and there were no clear trends in terms of terpenes.

Keywords: *aroma profile, amino acids, esters, grapes, higher alcohols, terpenes*

INTRODUCTION

The application of herbicide treatments is a widely used practice in conventional viticulture. The aim is to provide control over weed vegetation and to reduce its competitive impact on the vine nutrition [1 – 3]. In the scientific literature, there are not many studies that analyze the impact of herbicides on the wine quality, and in particular focused on its volatile profile. Matsuda *et al.* [4] monitored the influence of herbicide residues (from treatment with glyphosate, glyphosinate and pendimethalin) on some volatile components in red wines from Cabernet Sauvignon variety and found that the treatment significantly reduced the amounts of ethyl acetate (reduction from 65.00 mg·L⁻¹ in the control variant to 58.00 – 63.00 mg·L⁻¹ in the treated variants), isoamyl alcohol (reduction from 140.00 mg·L⁻¹ to 125.00 – 130.00 mg·L⁻¹) and linalool (decreasing from 40.00 µg·L⁻¹ to 25.00 – 30.00 µg·L⁻¹). On the other hand, the team [4] found an increase in the concentration of hexanol, which increased from 12.00 mg·L⁻¹ in the untreated control to 20.00 mg·L⁻¹ in the pendimethalin-treated experimental variant. Hartwell *et al.* [5] found that the application of herbicides (glyphosate, ammonium glyphosate and pendimethalin) to Cabernet Sauvignon resulted in a reduction in the content of the esters ethyl acetate (decreasing from 35.00 mg·L⁻¹ in the untreated control to 28.00 mg·L⁻¹ in the herbicide-treated wines), ethyl hexanoate (reduced from 2.1 mg·L⁻¹ to 1.5 mg·L⁻¹), decreased the concentration of the higher alcohols isoamyl (from 110.00 mg·L⁻¹ to 95.00 mg·L⁻¹), hexanol (from 12.00 mg·L⁻¹ to 9.00 mg·L⁻¹), and a reduction in the monoterpene linalool (from 0.9 mg·L⁻¹ to 0.6 mg·L⁻¹) was also observed. Jensen *et al.* [6] also found that herbicide treatment (glyphosate, 2,4-D and paraquat) of red grapevine varieties Cabernet Sauvignon, Merlot and Syrah led to a change in the volatile profile of their wines, reducing the content of ethyl acetate and isoamyl acetate and increasing the content of 1-hexanol.

The application of foliar fertilizers is an agro-chemical practice that aims to improve the physiological development and fruiting of the vine, as well as to increase the qualitative composition of the grapes obtained from it [7 – 9]. El-Sayed *et al.* [10] examined the effect of foliar application of amino acids in combination with potassium sulfate on the physiology, yield and quality of grapevines (*Vitis vinifera*). The combination shown a significant improvement in the grape's quality indicators, which suggests a synergism between macronutrients and amino acids in foliar feeding. The biostimulants, in which amino acids and macronutrients act together, fall within a broader concept of biostimulation, which has been shown to affect physiological and biochemical processes in vines and potentially metabolites related to the aroma profile [11]. Analyzes show that foliar application of biostimulants and trace elements improves the physiological resistance of vines to various abiotic stress factors, such as drought, high temperatures and salinity. These treatments lead to improved water economy, increased photosynthetic activity and enhanced antioxidant status, which can support vine growth and development under adverse conditions [12]. Combinations of amino acids with macronutrients support the physiological resistance of vines under abiotic stress conditions. This includes enhanced antioxidant capacity and accumulation of osmoprotectants under water deficit, as well as improved membrane stability under cold stress. Such effects are particularly relevant for sustainable management of Cabernet Sauvignon under climatic challenges [13].

A number of studies investigated the effects of applying foliar fertilization on the volatile profile of wines. Ren *et al.* [14] investigated the effect of foliar fertilization of Cabernet Sauvignon vines with Mn (solutions with concentrations of 0.300, 1200.00 and 2400.00 mg·L⁻¹) on the volatile profile of wines from the treated variety and found 8 volatile compounds as key to the aroma (floral-fruity) of the Mn-treated variants with a dominant presence of ethyl acetate, phenylethyl acetate and phenylethanol. Miliordos *et al.* [15] investigated the effect of applying traditional nitrogen fertilization with ammonium sulfate and a variant of nitrogen fertilization + 3,4 - Dimethylpyrazole phosphate (DMPP) applied to the Greek white variety Savvatiano, finding in wine that the combined variant of nitrogen fertilization leads to an increase in the intensity of the aroma - floral aroma and tropical fruit. Lacroux *et al.* [16] applied foliar nitrogen and sulfur to Sauvignon Blanc vines and found that their combined application resulted in increased levels of volatile thiols and glutathione in wines of the treated variety.

The aim of the present study was to define the influence of the combined treatment with herbicides and foliar fertilizers on the volatile composition of wines from four harvests of the Cabernet Sauvignon variety from the Central Northern Bulgaria region.

MATERIALS AND METHODS

Plantation and soil

The fruiting plantation with the Cabernet Sauvignon variety, clone ILV 1/11 [17] was located on the territory of the Experimental Base of Institute of Viticulture and Enology (IVE – Pleven). It was created in 2014. The soil type on which the plantation was grown, in terms of mechanical composition, was heavy sandy-clay chernozem, formed on a clay loess, with favorable water-physical properties and very good aeration [18]. Soil samples taken with a hand probe during the period of this study according to the methodology of Yonev [19] showed that the average content of total mineral nitrogen (NH₄⁺ + NO₃⁻) in the profile from 0 to 90 cm was 15.4 mg·kg⁻¹, and the concentrations of mobile phosphorus and potassium were 19.4 and 24.8 mg·100g⁻¹, respectively. The most enriched with them was the uppermost, humus horizon (0 – 30 cm) – mg·kg⁻¹ (N), 28.25 mg·100g⁻¹ (P₂O₅) and 34.4 mg·100g⁻¹ (K₂O). The average amount of active carbonates in it was 8.25 % and increased significantly with increasing of the depth. The soil reaction (pH aqueous solution) varied in a narrow range – from 7.68 (0 – 30 cm) to 8.13 (60 – 90 cm).

Determination of the total mineral nitrogen (NH₄⁺ + NO₃⁻) content

The mineral nitrogen content in the soil samples was determined by the distillation-titrimetric method in an automatic Kjeldahl distillation apparatus (UDK 129). The method is based on the extraction of mineral nitrogen forms from the soil with a solution of potassium chloride (KCl), followed by alkalization with MgO and reduction of nitrate nitrogen with Devard's alloy, in which the mineral nitrogen forms are converted to ammonia. The determination was carried out according to a laboratory protocol based on the recommended procedures for the determination of mineral nitrogen in soils described in FAO Soils Bulletin 38/2 [26].

For the analysis, 20 ml of 7.5 % KCl solution, 0.2 g of magnesium oxide (MgO) and 0.2 g of Devard's alloy (Cu 49 – 51 %, Al 44 – 46 %, Zn 4 – 6 %) were added to 4 g of air-dried and sieved soil. The resulting suspension was subjected to distillation in a Kjeldahl apparatus. The released ammonia was collected in a receiving flask containing 5 ml of a 4 % solution of boric acid (H_3BO_3) and a mixed indicator of methyl red and bromocresol green, dissolved in ethanol. After the distillation, the resulting solution was titrated with a 0.01 N solution of H_2SO_4 until the indicator changes color.

The concentration of mineral nitrogen was calculated according to a calculation scheme approved for the laboratory, in which the recorded titration volume was multiplied by a factor of 35, and the results were expressed as $\text{mg N}\cdot\text{kg}^{-1}$ soil.

Determination of mobile phosphorus content

Mobile phosphorus in soil samples was determined by the acetate-lactate method (modification of Ivanov), described by Tomov *et al.* [27]. To prepare the soil extract, 2 g of air-dried, sieved soil was extracted with 50 ml of acetate-lactate extraction solution for 1 h with shaking, after which the suspension was filtered. For the photolorimetric determination, Murphy and Riley's reagent (reagent A) [28] was added to 5 ml of the obtained extract, followed by reagent B (ascorbic acid), after which the volume was brought to 50 ml with distilled water. After the blue coloration developed, the optical density was measured at a wavelength of 630 nm with a SPEKOL 11 spectrophotometer. The concentration was determined by calibration with standard solutions and the results were expressed as $\text{mg P}_2\text{O}_5$. The content of mobile potassium in the soil samples was determined according to an internal laboratory protocol, by extraction with 2N hydrochloric acid solution and subsequent measurement by flame photometry. For the analysis, 5 g of air-dried and sieved soil were extracted with 50 ml of 2N HCl by shaking for 2 h. After settling of the suspension, the extract was filtered, and the obtained filtrates were analyzed with a flame photometer by pre-calibration with standard KCl solutions. Calibration was performed with standard solutions with concentrations corresponding to 10, 20, 30, 40 and 50 $\text{mg K}_2\text{O}\cdot\text{L}^{-1}$, prepared in 2N HCl. The potassium content was calculated against the calibration curve and the results were presented as $\text{mg K}_2\text{O}\cdot 100\text{g}^{-1}$ soil according to the method of Tomov *et al.* [27].

Determination of mobile potassium content

The content of mobile potassium in the soil samples was determined according to an internal laboratory protocol, by extraction with 2N hydrochloric acid solution and subsequent measurement by flame photometry. For the analysis, 5 g of air-dried and sieved soil were extracted with 50 ml of 2N HCl by shaking for 2 h. After settling of the suspension, the extract was filtered, and the obtained filtrates were analyzed with a flame photometer by pre-calibration with standard KCl solutions. Calibration was performed with standard solutions with concentrations corresponding to 10, 20, 30, 40 and 50 $\text{mg K}_2\text{O}\cdot\text{L}^{-1}$, prepared in 2N HCl. The potassium content was calculated against the calibration curve and the results were presented as $\text{mg K}_2\text{O}\cdot 100\text{ g}^{-1}$ soil.

The vines were grafted onto the Berlandieri x Riparia selection Oppenheim 4 rootstock, at distances of 1.20 m (in-row) and 1.80 m (between rows). The rows were oriented in a northwest-southeast direction. The exposure was with a slight slope to the southwest. The formation had one stem and a double-sided cordon at a height of 80 cm from the ground. The support structure consisted of one wire carrying the cordons and two pairs for

piercing the shoots, respectively 40 and 80 cm above it. The loading of the vines was consistent with the planting scheme and the varietal characteristics. It was carried out with buds - 24 winter eyes. The variants included 3 repetitions of 5 experimental vines.

Foliar fertilizers treatments

During each of the study years, the combination of foliar fertilizers was applied four times. The treatments were tailored to the following phenophases: 1) before flowering; 2) after flowering; 3) when the grape berries swelled to the size of a pea (3-4 mm); 4) when the grapes turned gray (beginning of the ripening process), but not later than August 1-2. To avoid high daily temperatures, the treatments of the respective experimental variants were made in the early hours of the day. Two preparations were applied:

- Green Aktiv AMINO - an active liquid concentrated microbiological product containing 18 types of vital L-amino acids, enriched with trace elements, vitamins, gibberellins and auxins, produced by hydrolysis of natural marine products. It is produced through a process in which natural marine products were hydrolyzed and transformed into a 100 % absorbable source of plant nutrients. It can be combined with foliar fertilizers. Maximum dose for foliar feeding - 300 mL·da⁻¹.
- MaxGrow PK - universal liquid water-soluble complex fertilizer, which could be applied for foliar and root feeding, increasing the resistance of plants to low temperatures and drought. Contained the following nutrients in chelated form: macronutrients - total nitrogen: 3 % (N), phosphorus: 20 % (P₂O₅), potassium: 22 % (K₂O); micronutrients – iron: 0.05 % (Fe), manganese: 0.025 % (Mn), copper: 0.01 % (Cu), zinc: 0.01 % (Zn), boron: 0.025 % (B) and molybdenum: 0.002 % (Mo). This fertilizer can be applied equally well by the known methods of foliar and root feeding, by fertigation (with irrigation water). It is suitable for use in horticulture, floriculture, viticulture, fruit growing and for technical crops. Stimulates growth, increases yields and improves the quality of production.

The fertilizer mixture was: Green Aktiv AMINO, 200 mL·da⁻¹ + Max Grow PK, 200 mL·da⁻¹. It was applied four times during each growing season, at intervals of 10 – 14 days, in phases: before flowering, after flowering, when the grape berries swell to the size of a pea and in the initial phase of ripening (in red varieties, graying).

Herbicides treatments

In the variants treated with herbicides, a tank mixture of Targa super 5 EK (50 g·L⁻¹ quizalofop-p-ethyl) and Pledge 50 VP (500 g·kg⁻¹ flumioxazin) was applied.

Quizalofop-p-ethyl had a selective systemic foliar action and was known as an effective herbicide with a wheat weed control effect - it is absorbed by their leaves and moves throughout the plant to the growth points, disrupting lipid synthesis and inhibiting the growth of roots and rhizomes [20]. Flumioxazine is a selective systemic herbicide, partially contact, with soil and foliar application against annual dicotyledonous and some monocotyledonous species and inhibits protoporphyrinogen oxidase, blocking chlorophyll biosynthesis [21].

Their use in a combined working solution (tank mix) showed a synergistic effect between quizalofop-p-ethyl and flumioxazine, expressed in increased efficacy against weeds and increased durability of action [22].

Targa super 5 EK (dose $0.300 \text{ L} \cdot \text{da}^{-1}$) and Pledge 50 VP (dose $0.040 \text{ kg} \cdot \text{da}^{-1}$) were applied to the inter-row strip with a backpack sprayer, at a working solution consumption of $400 \text{ L} \cdot \text{ha}^{-1}$ and nozzle pressure $P_{\text{max}} = 300 \text{ kPa}$. The treatments were carried out on 13.04. and 03.06.2021; 06.04. and 17.06.2022; 23.05.2023 and 20.05.2024. The technological control was dug up 3 times during the growing season. The inter-row distances were processed mechanized 4 times during the year.

The active substances flumioxazine and quizalofop-p-ethyl are currently authorised for use in the EU with the relevant regulations (Commission Implementing Regulation (EU) 2022/43 of 13 January 2022; Commission Implementing Regulation (EU) 2023/1757 of 11 September 2023). The manufacturer recommends a maximum dose of $0.040 \text{ kg} \cdot \text{da}^{-1}$ for flumioxazine and $0.200 \text{ L} \cdot \text{da}^{-1}$ for quizalofop-p-ethyl [29].

Vinification and experimental variants

Four harvests (2021 – 2024) were studied. The grape harvest was carried out at technological maturity of the variety and optimal content of sugars and titratable acids. The grapes, an amount of 30 kg for each experimental variant, were processed and vinified in the Experimental Wine Cellar of IVE – Pleven according to the classical scheme for red wine production, under microvinification conditions: red grapes → crushing → destemming → sulphitation ($50 \text{ mg} \cdot \text{kg}^{-1} \text{ SO}_2$) → inoculating with a pure culture of dry wine yeast *Saccharomyces cerevisiae* ($10 \text{ g} \cdot \text{hL}^{-1}$) → fermentation temperature $25\text{--}28^\circ\text{C}$ → separation from solids → malolactic fermentation → post-sulphitation ($50 \text{ mg} \cdot \text{kg}^{-1} \text{ SO}_2$) → storage [23]. Post sulphitation was made with the aim to suppress bacterial activity and protect the wine from oxidation and microbiological spoilage.

Four experimental variants were formed:

- V1 - Control - untreated (N0P0K0);
- V2 - Treatment only with herbicides - tank mixture of Targa super 5 EK ($0.300 \text{ L} \cdot \text{da}^{-1}$) and Pledge 50 VP ($40 \text{ g} \cdot \text{da}^{-1}$);
- V3 - Combined treatment with the indicated herbicide mixture and foliar fertilization with GreenAktiv AMINO, 200 mL/dka + MaxGrow PK, $200 \text{ mL} \cdot \text{da}^{-1}$ (N3P20K22+ME);
- V4 - Single treatment with the same combination of foliar fertilizers.

Gas chromatographic analysis

The content of the main volatile compounds was determined based on a stock standard solution prepared in accordance with IS [24]. The method describes the preparation of a standard solution of one congener, but the preparation step was followed to prepare a solution of more compounds. The standard solution in the present study included the following compounds (Merck, Darmstadt, Germany; compounds purity $> 99.0 \%$): acetaldehyde, ethyl acetate, methanol, 2-propanol, isopropyl acetate, 1-propanol, 2-butanol, propyl acetate, 1-butanol, isobutyl acetate, ethyl butyrate, 2-butyl acetate, 2-methyl-1-butanol, 3-methyl-1-butanol, 4-methyl-2-pentanol, 1-pentanol, pentyl acetate, 1-hexanol, ethyl hexanoate, hexyl acetate, 1-heptanol, linalool oxide, dimethyl succinate, phenyl acetate, linalool, ethyl caprylate, 2-phenylethanol, α -terpineol, nerol, β -citronellol, geraniol, ethyl decanoate. The prepared standard solution containing all compounds was

injected in an amount of 2 μL into a gas chromatograph Varian 3900 (Varian Analytical Instruments, Walnut Creek, California, USA) with a capillary column VF max MS (30 m, 0.25 mm ID, DF= 0.25 μm), equipped with flame ionization detector (FID). The carrier gas was helium. Hydrogen to support combustion was supplied to the chromatograph via a hydrogen bottle. The injection was manual, using a microsyringe. The gas chromatographic determination parameters were: injector temperature – 220 $^{\circ}\text{C}$, detector temperature – 250 $^{\circ}\text{C}$, initial oven temperature – 35 $^{\circ}\text{C}$ with 1 min retention, rise to 55 $^{\circ}\text{C}$ with a step of 2 $^{\circ}\text{C}$ per min for 11 min, rise to 230 $^{\circ}\text{C}$ with a step of 15 $^{\circ}\text{C}$ per min for 3 min. Total chromatography time – 25.67 min. After the retention times of the compounds in the standard solution were determined, the identification and quantification of the volatile compounds in the wines were conducted. The volatile composition was determined based on the injection of wine distillates. Samples were injected in an amount of 2 μL into a gas chromatograph and identification and quantification of volatile compounds was performed.

RESULTS AND DISCUSSION

The data on the identified volatile compounds by variants for the four studied harvests are presented in Tables 1 and 2.

Looking at the established total volatile content (by harvest), the highest levels were identified in the 2021 harvest. In almost all studied variants, more volatile compounds were accumulated in this harvest (with exception for V2) compared to the other three harvests. The highest total amount of volatile compounds (1687.84 $\text{mg}\cdot\text{L}^{-1}$) in the 2021 harvest was found in the control variant V1. Second, with a not very large difference, was variant V4 (1541.32 $\text{mg}\cdot\text{L}^{-1}$), where only foliar fertilization was applied.

Variants V2 and V3 did not show major differences between them, although in the second a slightly higher volatile content was found (1123.29 $\text{mg}\cdot\text{L}^{-1}$), compared to the first (1000.19 $\text{mg}\cdot\text{L}^{-1}$). In the next studied harvest (2022), the trend was changed, with the highest accumulation of volatile compounds (1555.51 $\text{mg}\cdot\text{L}^{-1}$) found in variant V2, where only herbicide treatment was applied. Variant V3 showed slightly higher total levels of volatile compounds (857.66 $\text{mg}\cdot\text{L}^{-1}$), compared to V4 (739.84 $\text{mg}\cdot\text{L}^{-1}$), and the lowest volatile content was identified in the untreated control (528.89 $\text{mg}\cdot\text{L}^{-1}$). In this harvest, it could be concluded that all three treatments had an impact on the increasing of the wine total volatile fraction, which was the most significant for the variant of herbicide treatment alone.

The trend for the highest levels found in this variant was maintained for the next studied harvest too – 2023. In it, V2 showed an accumulation of 1422.43 $\text{mg}\cdot\text{L}^{-1}$. It was followed by variant V4 (734.89 $\text{mg}\cdot\text{L}^{-1}$) and V3 (592.61 $\text{mg}\cdot\text{L}^{-1}$), and the lowest levels of volatile compounds (507.17 $\text{mg}\cdot\text{L}^{-1}$) were established in the untreated control. Considering the fourth studied harvest (2024), the dominant in terms of total volatile content (589.35 $\text{mg}\cdot\text{L}^{-1}$) was the control variant V1. The concentration of the detected total volatile compounds varied between the experimental variants in a narrow range – from 212.87 $\text{mg}\cdot\text{L}^{-1}$ (V4) to 389.12 $\text{mg}\cdot\text{L}^{-1}$ (V3).

Table 1. Results of gas chromatographic analysis of red wines from Cabernet Sauvignon variety, variants V1 – V4, harvests 2021 and 2022 (*ND – not detected)

Identified compounds, mg·L ⁻¹	Wines							
	Cabernet sauvignon							
	V1		V2		V3		V4	
	2021	2022	2021	2022	2021	2022	2021	2022
Ethyl alcohol, vol. %	12.22	13.38	12.06	12.82	12.40	12.47	11.60	12.22
Acetaldehyde	113.33	37.36	20.22	327.61	15.31	352.84	28.03	360.06
Methanol	140.52	13.05	150.66	28.07	53.14	21.64	80.89	12.55
2-methyl-1-butanol	110.94	17.76	124.62	33.18	463.81	42.53	96.36	27.13
3-methyl-1-butanol	214.13	78.66	173.63	92.51	435.17	160.91	348.56	108.99
4-methyl-2-pentanol	ND	ND	ND	ND	ND	ND	ND	1.32
1-propanol	ND	2.38	ND	6.95	ND	3.06	ND	4.17
2-propanol	27.02	0.47	ND	2.46	9.52	1.52	ND	ND
1-butanol	ND	7.91	71.86	315.93	ND	33.89	ND	23.05
2-butanol	26.80	7.87	195.12	158.90	108.17	28.16	364.40	11.49
1-pentanol	ND	11.43	ND	9.37	ND	ND	ND	8.05
1-hexanol	ND	21.80	ND	ND	ND	ND	ND	22.18
1-heptanol	ND	ND	ND	ND	ND	ND	ND	ND
2-phenylethanol	40.46	54.26	ND	ND	ND	ND	ND	ND
2-methyl-1-propanol	197.90	ND	ND	ND	ND	ND	ND	ND
Total higher alcohols	617.25	202.54	565.23	619.30	1016.67	270.07	809.32	206.38
Ethyl acetate	112.61	28.43	75.34	41.97	11.08	58.37	66.35	51.81
Isobutyl acetate	323.73	ND	ND	ND	ND	ND	ND	ND
Propyl acetate	37.15	ND	ND	ND	ND	10.22	ND	4.01
Isopropyl acetate	184.16	1.39	135.84	5.32	20.70	4.48	46.91	1.01
Isopentyl acetate	ND	ND	34.13	ND	ND	ND	42.10	ND
Pentyl acetate	ND	33.78	ND	ND	ND	ND	418.91	ND
Ethyl caprylate	27.82	ND	18.77	6.06	6.08	80.99	ND	ND
Hexyl acetate	57.14	105.96	ND	479.16	ND	ND	ND	ND
Ethyl hexanoate	73.91	19.80	ND	ND	ND	58.51	ND	70.65
Phenyl acetate	ND	86.27	ND	ND	ND	ND	ND	ND
Ethyl butyrate	ND	ND	ND	15.72	ND	ND	48.67	15.31
Ethyl decanoate	ND	ND	ND	32.00	ND	ND	ND	ND
Total esters	816.52	275.63	264.08	580.23	37.86	212.57	622.94	160.72
Linalool oxide	ND	0.31	ND	ND	ND	0.54	ND	ND
α – terpineol	ND	ND	ND	ND	0.21	ND	0.14	ND
Nerol	0.22	ND	ND	0.30	ND	ND		
Geraniol	ND	ND	ND	ND	0.10	ND	ND	0.13
Total terpenes	0.22	0.31	0.00	0.30	0.31	0.54	0.14	0.13
TOTAL CONTENT	1687.84	528.89	1000.19	1555.51	1123.29	857.66	1541.32	739.84

Table 2. Results of gas chromatographic analysis of red wines from Cabernet Sauvignon variety, variants V1 - V4, harvests 2023 and 2024 (*ND – not detected)

Identified compounds, mg·L ⁻¹	Wines							
	Cabernet sauvignon							
	V1		V2		V3		V4	
	2023	2024	2023	2024	2023	2024	2023	2024
Ethyl alcohol, vol.%	13.00	16.36	13.40	16.51	13.00	16.15	13.46	15.57
Acetaldehyde	84.61	177.26	231.34	54.51	130.76	86.68	118.87	38.53
Methanol	7.32	7.43	56.47	6.73	114.77	5.24	5.50	6.11
2-methyl-1-butanol	14.44	10.13	20.20	2.67	34.84	4.10	26.68	2.83
3-methyl-1-butanol	61.27	54.74	100.26	12.32	146.24	40.69	113.60	16.05
4-methyl-2-pentanol	ND	ND	ND	ND	ND	ND	ND	ND
1-propanol	ND	ND	ND	ND	6.36	ND	ND	ND
2-propanol	ND	6.57	0.94	5.36	2.92	5.48	1.88	2.43
1-butanol	65.00	ND	155.26	4.27	94.79	24.01	81.15	ND
2-butanol	ND	ND	ND	ND	ND	ND	ND	ND
1-pentanol	0.84	ND	ND	ND	ND	ND	ND	ND
1-hexanol	4.80	1.27	ND	ND	ND	ND	ND	ND
1-heptanol	ND	6.34	ND	ND	ND	ND	ND	3.33
<i>Total higher alcohols</i>	146.35	79.05	276.66	24.62	285.15	74.28	223.31	24.64
Ethyl acetate	267.77	308.13	203.30	153.55	38.94	186.27	383.52	93.49
Propyl acetate	ND	ND	ND	ND	ND	ND	ND	ND
Isopropyl acetate	0.47	ND	2.31	5.26	7.64	ND	3.69	ND
Diethyl succinate	ND	ND	ND	ND	ND	4.20	ND	ND
Butyl acetate	ND	ND	ND	ND	0.43	ND	ND	ND
Isopentyl acetate	ND	ND	ND	ND	ND	ND	ND	ND
Pentyl acetate	ND	5.52	ND	ND	ND	ND	ND	ND
Ethyl caprylate	ND	11.84	40.57	ND	14.92	ND	ND	9.97
Hexyl acetate	ND	ND	185.43	ND	ND	ND	ND	40.00
Ethyl hexanoate	ND	ND	ND	ND	ND	ND	ND	ND
Phenyl acetate	ND	ND	380.39	ND	ND	ND	ND	ND
Ethyl butyrate	ND	ND	ND	7.41	ND	0.81	ND	ND
Ethyl decanoate	ND	ND	45.80	ND	ND	31.64	ND	ND
<i>Total esters</i>	268.24	325.49	857.80	166.22	61.93	222.92	387.21	143.46
Linalool oxide	0.65	ND	ND	ND	ND	ND	ND	0.13
α – terpineol	ND	0.12	ND	ND	ND	ND	ND	ND
Geraniol	ND	ND	0.16	ND	ND	ND	ND	ND
<i>Total terpenes</i>	0.65	0.12	0.16	0.00	0.00	0.00	0.00	0.13
TOTAL CONTENT	507.17	589.35	1422.43	252.08	592.61	389.12	734.89	212.87

Looking at the total established content of higher alcohols in the 2021 harvest, they were identified in the highest amount (1016.67 mg·L⁻¹) in the wine of the variant with combined treatment with herbicides and foliar fertilizers (V3). Immediately after it was

situated the wine of variant V4 (809.32 mg·L⁻¹), where a separate treatment with foliar fertilizers was applied. The lowest levels were identified in variant V2 (565.23 mg·L⁻¹), and the untreated control showed a total amount of higher alcohols – 617.25 mg·L⁻¹. These results outlined a trend of the strongest influence on the increased accumulation of higher alcohols when combined treatment with herbicides and foliar fertilizers was applied.

In the next studied harvest (2022), the highest amount of higher alcohols (619.30 mg·L⁻¹) was found in the wine of the variant that was treated only with herbicides (V2). Immediately after it were situated variants V3 (270.07 mg·L⁻¹) and V4 (206.38 mg·L⁻¹), and the lowest content of higher alcohols in this studied harvest showed the untreated control V1 (202.54 mg·L⁻¹). The highest accumulation of higher alcohols in the Cabernet Sauvignon wines of the 2023 harvest was found in the variant with combined treatment of herbicides and foliar fertilizers V3 (285.15 mg·L⁻¹). Variant V2 ranked second (276.66 mg·L⁻¹) with a small difference from the previous variant. In variant V4, total higher alcohols of 223.31 mg·L⁻¹ were found, and the lowest levels were again identified in the untreated control V1 (146.35 mg·L⁻¹). The wines from the last studied harvest (2024) showed the lowest levels of total higher alcohols, and the trend of their distribution was different. Practically the lowest accumulation of higher alcohols was found in variants V2 (24.62 mg·L⁻¹) and V4 (24.64 mg·L⁻¹), with their levels being almost absolutely similar. The other two variants V1 and V3 showed very close concentrations of total higher alcohols with a slight predominance of the control (79.05 mg·L⁻¹), compared to the variant of combined treatment with herbicides and foliar fertilizers (74.28 mg·L⁻¹).

In terms of total ester accumulation for the 2021 harvest, the highest levels (816.52 mg·L⁻¹) were found in the wine of the control variant V1. It was followed by variant V4 (622.94 mg·L⁻¹), where only foliar fertilization was applied. Variant V2 showed an ester concentration of 264.08 mg·L⁻¹, and the lowest levels of this volatile fraction were recorded in the wine of variant V3 (37.86 mg·L⁻¹). In the next studied harvest (2022), variant V2 (580.23 mg·L⁻¹), where only herbicide treatment was applied, was dominant in terms of ester concentration. In second place was the untreated control V1 (275.63 mg·L⁻¹). Variant V3 showed ester levels of 212.57 mg·L⁻¹, and the lowest ester accumulation for this harvest was found in variant V4 (143.46 mg·L⁻¹), where only foliar fertilization was applied. The trend for the highest ester accumulation in the wine of the variant with herbicide treatment alone V2 (857.80 mg·L⁻¹) was maintained for the next studied harvest (2023). Immediately after it was the established concentration of the variant where foliar fertilizer treatment alone was applied – V4 (387.21 mg·L⁻¹). Variant V1 showed lower levels (166.22 mg·L⁻¹) of esters, and the lowest (significantly) were in the wine of variant V3 (61.93 mg·L⁻¹) with combined treatment with herbicides and foliar fertilizers. The highest ester content (325.49 mg·L⁻¹) in the last studied harvest (2024) was found in the control variant V1. In the remaining three experimental variants dominated the wine in which a combined treatment with herbicides and foliar fertilizers was applied – V3 (222.92 mg·L⁻¹), and the remaining two variants showed very close concentrations with a slight dominance of V2 (166.22 mg·L⁻¹) compared to V4 (143.46 mg·L⁻¹).

The terpene composition of the analyzed wines was not very diverse. The highest levels of total terpenes for the first studied harvest (2021) were found in the wine of variant V3 (0.31 mg·L⁻¹), in which a combined treatment with herbicides and foliar fertilizers was

applied. It was followed by the control variant V1 ($0.22 \text{ mg}\cdot\text{L}^{-1}$). The third in terms of concentration of terpenes was the variant of treatment only with foliar fertilizers – V4 ($0.14 \text{ mg}\cdot\text{L}^{-1}$), while in variant V2 terpenes were not identified. In the next studied harvest (2022), the trend for the highest accumulation of terpenes was maintained for variant V3 ($0.54 \text{ mg}\cdot\text{L}^{-1}$). Here, as in the previous harvest, the untreated control V1 ($0.31 \text{ mg}\cdot\text{L}^{-1}$) ranked second. Its difference with variant V2 was minimal, since a total terpene concentration of $0.30 \text{ mg}\cdot\text{L}^{-1}$ was established in it. Variant V4 showed the lowest levels ($0.13 \text{ mg}\cdot\text{L}^{-1}$). In the third studied harvest (2023), terpenes were identified only in the control variant V1 and the herbicide treatment variant V2, with the levels in the control being significantly higher ($0.65 \text{ mg}\cdot\text{L}^{-1}$) compared to V2 ($0.16 \text{ mg}\cdot\text{L}^{-1}$). In variants V3 and V4, terpenes were not identified. In the last analyzed harvest (2024), terpenes were identified only in the control variant V1 ($0.12 \text{ mg}\cdot\text{L}^{-1}$) and in the variant of applying only foliar fertilization V4 ($0.13 \text{ mg}\cdot\text{L}^{-1}$). It was noteworthy that the terpene concentrations in the two variants were almost similar. In the remaining two variants, terpenes were not identified.

A single aldehyde – acetaldehyde – was identified in the wines. At concentrations up to about $110.00 \text{ mg}\cdot\text{L}^{-1}$, this compound has a positive effect with the appearance of fruity nuances in the aroma, and above this concentration its influence is negative with the appearance of an oxidized tone [25]. In the wines from the 2021 harvest, its levels were the highest in the control variant V1 ($113.33 \text{ mg}\cdot\text{L}^{-1}$) – its concentration was slightly above the threshold for a positive effect. In the remaining three variants, it was in normal amounts, which varied in a very narrow range – from $15.31 \text{ mg}\cdot\text{L}^{-1}$ (variant V3) to $28.03 \text{ mg}\cdot\text{L}^{-1}$ (variant V4). In the next examined harvest, it was in normal concentration only in the control variant V1 ($37.36 \text{ mg}\cdot\text{L}^{-1}$). In the other three experimental variants, it was found in high levels, which distinctly exceeded its threshold for a positive aromatic effect. In them, it ranged from $327.61 \text{ mg}\cdot\text{L}^{-1}$ (variant V2) to $360.06 \text{ mg}\cdot\text{L}^{-1}$ (variant V4). Its levels were an indicator of an oxidation process, which could lead to the formation of oxidized notes in the wine aroma. This trend was also observed in the next studied harvest (2023), where the most optimal levels of acetaldehyde were also found in the control variant V1 ($84.61 \text{ mg}\cdot\text{L}^{-1}$). In the three experimental variants, its amounts were higher, ranging from $118.87 \text{ mg}\cdot\text{L}^{-1}$ (V4) to $231.34 \text{ mg}\cdot\text{L}^{-1}$ (V2), which again outlined a trend for a possible manifestation of oxidized notes in the aroma. In the last studied harvest (2024), the trend was reversed, with the highest levels of acetaldehyde ($177.26 \text{ mg}\cdot\text{L}^{-1}$) being found in the untreated control. In the three experimental variants, its amounts ranged from $38.53 \text{ mg}\cdot\text{L}^{-1}$ (2024) to $86.68 \text{ mg}\cdot\text{L}^{-1}$ (2024) – levels that are associated with a positive effect on the wine's aromatic quality.

3-methyl-1-butanol and 2-methyl-1-butanol were the main higher alcohols identified in all the wines studied. The first was found in higher concentrations. In the wines of the 2021 harvest, it was found in the highest concentration ($435.17 \text{ mg}\cdot\text{L}^{-1}$) in the variant of combined treatment with herbicides and foliar fertilizers V3. The next in concentration presence ($348.56 \text{ mg}\cdot\text{L}^{-1}$) was the wine, where treatment with foliar fertilizers only was applied – V4. The control variant V1 showed levels of 3-methyl-1-butanol – $214.13 \text{ mg}\cdot\text{L}^{-1}$, and the lowest amounts were found in variant V2 ($173.63 \text{ mg}\cdot\text{L}^{-1}$). In the next studied harvest (2022), the levels of this higher alcohol were lower, but they were again the highest in the wine of variant V3 ($160.91 \text{ mg}\cdot\text{L}^{-1}$). It was followed by variant V4 ($108.99 \text{ mg}\cdot\text{L}^{-1}$) – a trend similar to that established in the previous harvest. In this harvest, the herbicide treatment variant V2 accumulated amounts of 3-methyl-1-butanol

in the wine of 92.51 mg·L⁻¹, and the lowest levels of this higher alcohol were found in the control variant V1 (78.66 mg·L⁻¹). The trend was maintained in the third harvest (2023), where again the highest levels of 3-methyl-1-butanol (146.24 mg·L⁻¹) were found in the Cabernet Sauvignon wine with combined treatment with herbicides and foliar fertilizers – V3. It was again followed by variant V4 (113.60 mg·L⁻¹) and V2 (100.26 mg·L⁻¹), and the lowest levels were identified in the control variant V1 (61.27 mg·L⁻¹). The levels of this higher alcohol were the lowest in the last studied harvest (2024). However, the trend was different in it. The highest levels were found in the control V1 (54.74 mg·L⁻¹). In second place in terms of concentration of the higher alcohol was the variant of combined treatment with herbicides and foliar fertilizers – V3 (40.69 mg·L⁻¹). Variant V4 showed a concentration of 16.05 mg·L⁻¹, and the lowest levels of 3-methyl-1-butanol in this harvest were found in the wine of variant V2 (12.32 mg·L⁻¹).

It could be concluded that 3-methyl-1-butanol exhibited a tendency of quantitative decrease in the studied variants with each subsequent harvest. At the same time, the highest levels in three of the studied harvests were identified in the variant of combined treatment with herbicides and foliar fertilizers (V3). This indicated an improved metabolism of the grapevine plant, probably leading to the synthesis of higher amounts of amino acids in the grapes, which are the precursor of higher alcohols (yeast metabolic products) in the wine.

2-methyl-1-butanol in the 2021 harvest was found in the highest concentration (463.81 mg·L⁻¹) in the variant of combined treatment with herbicides and foliar fertilizers – V3. It was followed by variant V2 (124.62 mg·L⁻¹). The control variant V1 showed amounts of 2-methyl-1-butanol of 110.94 mg·L⁻¹, and the lowest (96.36 mg·L⁻¹) were the levels found in the variant of single treatment with foliar fertilizers – V4. Its levels decreased in the next studied harvest (2022). However, the trend of the highest amount (42.53 mg·L⁻¹) in the variant of combined treatment with herbicides and foliar fertilizers (V3) was again maintained. It was followed by variant V2 (33.18 mg·L⁻¹) – a trend similar to that found in the previous harvest. In the wine of the variant of single treatment with foliar fertilizers (V3), a quantity of 2-methyl-1-butanol of 27.13 mg·L⁻¹ was found, and the lowest levels of this higher alcohol were identified in the untreated control V1 (17.76 mg·L⁻¹). The trend of decreasing levels of 2-methyl-1-butanol in the next studied harvest (2023) continued. Analogously to the previous harvests, here too, the highest levels of this higher alcohol (34.84 mg·L⁻¹) were found in the wine of the variant of combined treatment with herbicides and foliar fertilizers – V3. Here too, it was followed by the variants V2 (33.18 mg·L⁻¹) and V4 (26.68 mg·L⁻¹), and the lowest levels were established in the untreated control V1 (14.44 mg·L⁻¹). The trend of decreasing in the levels of 2-methyl-1-butanol continued in the last studied harvest (2024). The lowest levels of this higher alcohol were found in it. In this specific harvest (2024), they were the highest in the control variant V1 (10.13 mg·L⁻¹). It was followed by variant V3 (4.10 mg·L⁻¹), and the lowest levels were in variant V4 (2.83 mg·L⁻¹) and V2 (2.67 mg·L⁻¹).

This higher alcohol, as with 3-methyl-1-butanol, showed a decrease in quantities, recorded over the four harvests, while at the same time the highest levels were found in the variants of combined treatment with herbicides and foliar fertilizers (in three of the studied harvests).

The remaining higher alcohols were identified in separate variants. 4-methyl-2-pentanol was found only in variant V4 in the 2022 harvest, but in a low concentration – 1.32 mg·L⁻¹. 1-propanol was identified in the 2022 harvest of the control variant V1 (2.38

mg·L⁻¹), in the 2022 harvest of variant V2 (6.95 mg·L⁻¹), in the 2022 and 2023 harvests of variant V3, where it occupied concentrations of 3.06 mg·L⁻¹ and 6.36 mg·L⁻¹, respectively, it was also identified in the 2022 harvest of variant V4 (4.17 mg·L⁻¹). As was clear, the concentration of 1-propanol in all experimental variants, where it was found, was higher than the control. 2-propanol was detected in three of the researched harvests (2021, 2022 and 2024) of the control variant V1, where it ranged from 0.47 mg·L⁻¹ to 27.02 mg·L⁻¹. In the variant with applied herbicide treatment V2, it was present again in three of the harvests (2022, 2023 and 2024) and ranged from 0.94 mg·L⁻¹ to 5.36 mg·L⁻¹. In the third variant V3, where combined treatment with herbicides and foliar fertilizers was applied, 2-propanol was identified in all four studied harvests with a variation from 1.52 mg·L⁻¹ (2022) to 9.52 mg·L⁻¹ (2021). In the last studied variant V4, this higher alcohol was identified only in the wines of two harvests – 2023 (1.88 mg·L⁻¹) and 2024 (2.43 mg·L⁻¹). 1-butanol was found in the wines of two of the studied harvests of the control – 2022 (7.91 mg·L⁻¹) and 2023 (65.00 mg·L⁻¹). In variant V2, where herbicide treatment was applied alone, this higher alcohol was present in all four studied harvests, where it ranged from 4.27 mg·L⁻¹ (2024) to 315.93 mg·L⁻¹ (2022). In the wines of the variant of combined treatment with herbicides and foliar fertilizers, 1-butanol was present in three of the studied harvests (2022, 2023 and 2024), where it ranged from 24.01 mg·L⁻¹ to 94.79 mg·L⁻¹. In the last studied variant V4, 1-butanol was present in the wines of only two harvests – 2022 (23.05 mg·L⁻¹) and 2023 (81.15 mg·L⁻¹). 2-butanol was identified in two of the harvests of the control variant V1 – 2021 (26.80 mg·L⁻¹) and 2022 (7.87 mg·L⁻¹). In the same two harvests, but in significantly higher quantities, it was also found after treatment with herbicides – variant V1 in quantities of 195.12 mg·L⁻¹ (2021) and 158.90 mg·L⁻¹ (2022), respectively. In the wines of the third studied variant V3, 2-butanol was also present in only two of the studied harvests – 2021 (108.17 mg·L⁻¹) and 2022 (28.16 mg·L⁻¹). In the same two harvests, this higher alcohol was also found in the last studied variant – V4, where it occupied amounts of 364.40 mg·L⁻¹ (2021) and 11.49 mg·L⁻¹ (2022), respectively. 1-pentanol was found in two of the harvests of the control variant – 2022 (11.43 mg·L⁻¹) and 2023 (0.84 mg·L⁻¹), in one of the harvests of variant V2 – 2022 (9.37 mg·L⁻¹) and in one of the harvests of variant V4 – 2022 (8.05 mg·L⁻¹). 1-hexanol was found in three of the harvests of the control variant (2022-2024), where it ranged from 1.27 mg·L⁻¹ to 21.80 mg·L⁻¹ and only in one of the harvests of variant V4 – 2022 (22.18 mg·L⁻¹). 1-heptanol was detected only in the 2024 harvest of the control variant V1 (6.34 mg·L⁻¹) and in the 2024 harvest of the variant V4 (3.33 mg·L⁻¹). The aromatic alcohol 2-phenylethanol was identified only in the wines of two harvests of the control variant V1 – 2021 (40.46 mg·L⁻¹) and 2022 (54.26 mg·L⁻¹), and 2-methyl-1-propanol was detected only in the 2021 harvest of the control variant V1 (197.90 mg·L⁻¹).

The main representatives of the ester fraction were ethyl acetate and isopropyl acetate. Ethyl acetate was practically identified in all studied wines. The characteristic of this ester is that in concentrations up to about 200.00 mg·L⁻¹ it positively affects the wine aromatic quality, giving fruity nuances, while above these concentrations its influence is negative with the formation of acetic-acid notes in the aroma and taste [25]. In the wines of the 2021 harvest, the ester was found with the highest concentration (112.61 mg·L⁻¹) in the control sample. In the experimental variants it varied from 11.08 mg·L⁻¹ (V3) to 75.34 mg·L⁻¹ (V2). All levels of the ester for this harvest were within the norm in which it exerted a positive effect on the wine aromatic quality. In the next studied harvest (2022),

the wine of the untreated control (V1) showed the lowest levels of ethyl acetate ($28.43 \text{ mg}\cdot\text{L}^{-1}$). In the three experimental variants, its concentration ranged in a very narrow range – from $41.97 \text{ mg}\cdot\text{L}^{-1}$ in the wine of the variant in which only herbicide treatment was applied (V2) to $58.37 \text{ mg}\cdot\text{L}^{-1}$ in variant V3 with a combined treatment with herbicides and foliar fertilizers. In three of the variants from the third harvest (2023) the ester was identified in slightly higher quantities – the wine of the control variant V1 from this harvest showed a quantity of $267.77 \text{ mg}\cdot\text{L}^{-1}$ – a content that was above the threshold for a positive influence. The same was the case with variant V4, where the highest accumulation of ethyl acetate was found for this harvest – $383.52 \text{ mg}\cdot\text{L}^{-1}$ – levels that were significantly above the threshold value with the possibility of negatively affecting the aromatic and taste wine quality. Variant V2 also showed high levels of ethyl acetate ($203.30 \text{ mg}\cdot\text{L}^{-1}$), but they were right on the threshold. The most optimal was the content of the ester in the wine of variant V3, where it was present in an amount of $38.94 \text{ mg}\cdot\text{L}^{-1}$. In the last studied harvest (2024), the ester was found in the highest concentration in the wine of the control sample V1 ($308.13 \text{ mg}\cdot\text{L}^{-1}$) – an amount that exceeded the threshold of its positive influence. In the remaining experimental variants, its levels were normal and varied from $93.49 \text{ mg}\cdot\text{L}^{-1}$ in the variant of treatment only with foliar fertilizers to $186.27 \text{ mg}\cdot\text{L}^{-1}$ in the wine of the variant with combined treatment with herbicides and foliar fertilizers V3.

The other ester, which was predominant in most of the analyzed wines, was isopropyl acetate. In the control variant V1, it was absent only in the wine of the 2024 harvest. In the other studied harvests, it varied in amounts from $0.47 \text{ mg}\cdot\text{L}^{-1}$ (2023) to $184.16 \text{ mg}\cdot\text{L}^{-1}$ (2021). In the next studied variant V2, in which only herbicide treatment was applied, isopropyl acetate varied between harvests from $2.31 \text{ mg}\cdot\text{L}^{-1}$ (2023) to $135.84 \text{ mg}\cdot\text{L}^{-1}$ (2021). In the third studied variant V3 (combinedly treated with herbicides and foliar fertilizers), the ester was not detected only in the 2024 harvest. In the other three harvests, it varied from $4.48 \text{ mg}\cdot\text{L}^{-1}$ (2022) to $20.70 \text{ mg}\cdot\text{L}^{-1}$ (2021). In the last studied variant V4 (with only foliar fertilization applied), the ester was again not available in the fourth harvest (2024). In the remaining three, it varied from $1.01 \text{ mg}\cdot\text{L}^{-1}$ (2022) to $46.91 \text{ mg}\cdot\text{L}^{-1}$ (2021). It was noteworthy that its levels were the highest in all variants in the first studied harvest (2021), which was probably due to specific climatic features of the year. The remaining esters were identified in separate variants. Isobutyl acetate was identified only in the wine of the control variant V1 from the 2021 harvest in an amount of $323.73 \text{ mg}\cdot\text{L}^{-1}$. Propyl acetate was also found in the control in the 2021 harvest ($184.16 \text{ g}\cdot\text{L}^{-1}$), as well as in the variant of combined treatment with herbicides and foliar fertilizers V3 – harvest 2022 ($10.22 \text{ mg}\cdot\text{L}^{-1}$), as well as in the same harvest of the next experimental variant V4 ($4.01 \text{ mg}\cdot\text{L}^{-1}$). Diethyl succinate was identified only in the wine of the 2024 harvest ($4.20 \text{ mg}\cdot\text{L}^{-1}$) of variant V3. Butyl acetate was found in the 2023 harvest of variant V3 ($0.43 \text{ mg}\cdot\text{L}^{-1}$) and in the 2022 harvest of variant V4 ($17.93 \text{ mg}\cdot\text{L}^{-1}$). Isopentyl acetate was identified in the 2021 harvest in two of the experimental variants – V2 ($34.13 \text{ mg}\cdot\text{L}^{-1}$) and V4 ($42.10 \text{ mg}\cdot\text{L}^{-1}$). Pentyl acetate was present in two of the harvests of the untreated control – 2022 ($33.78 \text{ mg}\cdot\text{L}^{-1}$) and 2024 ($5.52 \text{ mg}\cdot\text{L}^{-1}$), and also in the 2021 harvest of variant V4, where it was found in significantly higher amounts ($418.91 \text{ mg}\cdot\text{L}^{-1}$). Ethyl caprylate was detected in two of the harvests of the control variant V1 – 2021 ($27.82 \text{ mg}\cdot\text{L}^{-1}$) and 2024 ($11.84 \text{ mg}\cdot\text{L}^{-1}$), in three of the harvests of variant V2 (2021 – 2023), where it ranged from $6.06 \text{ mg}\cdot\text{L}^{-1}$ to $40.57 \text{ mg}\cdot\text{L}^{-1}$, in the same three harvests of the next studied variant (V3), where it ranged

from $6.08 \text{ mg}\cdot\text{L}^{-1}$ (2021) to $80.99 \text{ mg}\cdot\text{L}^{-1}$ (2022) and in one of the harvests of variant V4 – 2024 ($9.27 \text{ mg}\cdot\text{L}^{-1}$). Hexyl acetate was identified in harvests 2021 ($57.14 \text{ mg}\cdot\text{L}^{-1}$) and 2022 ($105.96 \text{ mg}\cdot\text{L}^{-1}$) of the control variant V1, as well as in harvests 2022 ($479.16 \text{ mg}\cdot\text{L}^{-1}$) and 2023 ($185.43 \text{ mg}\cdot\text{L}^{-1}$) of the variant with herbicide treatment V2, as well as in harvest 2024 of variant V4 ($40.00 \text{ mg}\cdot\text{L}^{-1}$), where only foliar fertilizer treatment was applied. Ethyl hexanoate was identified in harvests 2021 ($73.91 \text{ mg}\cdot\text{L}^{-1}$) and 2022 ($19.80 \text{ mg}\cdot\text{L}^{-1}$) of the untreated control variant V1, as well as in harvest 2023 ($380.39 \text{ mg}\cdot\text{L}^{-1}$) of the variant in which treatment with herbicides only was applied – V2. Ethyl butyrate was found in two of the harvests of variant V2 – 2022 ($15.72 \text{ mg}\cdot\text{L}^{-1}$) and 2024 ($7.41 \text{ mg}\cdot\text{L}^{-1}$), in one of the harvests of variant V3 – 2024 ($0.81 \text{ mg}\cdot\text{L}^{-1}$) and in two of the harvests of variant V4 – 2021 ($48.67 \text{ mg}\cdot\text{L}^{-1}$) and 2022 ($15.31 \text{ mg}\cdot\text{L}^{-1}$). Ethyl decanoate was identified in two of the harvests of variant V2 – 2022 ($32.00 \text{ mg}\cdot\text{L}^{-1}$) and 2023 ($45.80 \text{ mg}\cdot\text{L}^{-1}$), as well as in one of the harvests of variant V3 – 2024 ($31.64 \text{ mg}\cdot\text{L}^{-1}$).

Different terpene representatives were distributed in different variants. Linalool oxide was identified in harvests 2022 ($0.31 \text{ mg}\cdot\text{L}^{-1}$) and 2023 ($0.65 \text{ mg}\cdot\text{L}^{-1}$) of the control untreated variant V1, as well as in harvests 2022 of variant V3 ($0.54 \text{ mg}\cdot\text{L}^{-1}$) and 2024 of variant V4 ($0.13 \text{ mg}\cdot\text{L}^{-1}$). α -terpineol was identified in harvest 2024 of the untreated control ($0.12 \text{ mg}\cdot\text{L}^{-1}$), as well as in harvest 2021 of variants V3 ($0.21 \text{ mg}\cdot\text{L}^{-1}$) and V4 ($0.14 \text{ mg}\cdot\text{L}^{-1}$). Nerol was identified only in harvest 2021 of the control ($0.22 \text{ mg}\cdot\text{L}^{-1}$) and harvest 2022 of variant V2 ($0.30 \text{ mg}\cdot\text{L}^{-1}$). Geraniol was identified in only one of the harvests of the experimental variants, respectively 2023 of variant V2 ($0.16 \text{ mg}\cdot\text{L}^{-1}$), 2021 of variant V3 ($0.10 \text{ mg}\cdot\text{L}^{-1}$) and 2022 of variant V4 ($0.13 \text{ mg}\cdot\text{L}^{-1}$).

Methanol was also identified. It is normally present in red wines [30] in permissible concentrations up to $300.00 \text{ mg}\cdot\text{L}^{-1}$ and it is produced on the basis of hydrolysis of fruit pectin under the action of the pectolytic enzyme complex of grapes during fermentation [25]. In the wines of the control variant V1 it was found with a variation between harvests from $7.32 \text{ mg}\cdot\text{L}^{-1}$ (2023) to $140.52 \text{ mg}\cdot\text{L}^{-1}$ (2021). In the wines of the variant with applied only herbicide treatment, the methanol content between harvests varied from $6.73 \text{ mg}\cdot\text{L}^{-1}$ (2024) to $150.66 \text{ mg}\cdot\text{L}^{-1}$ (2021). In the variant of combined treatment with herbicides and foliar fertilizers V3, a variation in methanol content was found from $5.24 \text{ mg}\cdot\text{L}^{-1}$ (2024) to $114.77 \text{ mg}\cdot\text{L}^{-1}$ (2023), and in the last experimental variant V4, where treatment with foliar fertilizers only was applied, its levels were the lowest, varying between harvests from $5.50 \text{ mg}\cdot\text{L}^{-1}$ (2023) to $80.89 \text{ mg}\cdot\text{L}^{-1}$ (2021).

All established methanol levels were within the norm.

CONCLUSIONS

The following conclusions could be made from the study:

- The variant of herbicide treatment V2 affected the accumulation of the highest final total amounts of volatile compounds in the studied wines from two harvests – 2022 and 2023.
- The treatments affected the total concentration presence of higher alcohols, reflecting in higher levels of total higher alcohols in the wines, where treatments of the grapevine with herbicides and foliar fertilizers were applied. This result indirectly indicated a direct impact on improved metabolism of amino acids in the

vine plant, which are precursors (transformed by the yeast) of wines higher alcohols.

- Increased total ester accumulation was found in the wine of the variant with applied herbicide treatment – V2, but in two of the studied harvests – 2022 and 2023.
- The total content of terpenes was not high and no trend was observed outlining the influence of the treatments on this indicator.
- Main higher alcohols identified were 3-methyl-1-butanol and 2-methyl-1-butanol. The first was found in higher quantities, and it also showed a tendency of quantitative decrease in the studied variants with each subsequent harvest. The highest levels of 3-methyl-1-butanol and 2-methyl-1-butanol were established in three of the studied harvests in the variant of combined treatment with herbicides and foliar fertilizers (V3). This indicated an improved metabolism of the vine plant, probably leading to the synthesis of higher amounts of amino acids in the grapes, which are the precursor of higher alcohols (metabolic products of yeasts that break down amino acids) in the wine.
- Main esters were ethyl acetate and isopropyl acetate. Ethyl acetate levels were at an optimum, determining its positive effect on wine aroma.
- There was no trend directly outlining the influence of treatments on the amounts of individual terpenes.

The overall perspective of the results obtained indicated that treatment with herbicides alone led to a significant quantitative increase in the total content of volatile compounds in the analyzed wines in two of the harvests - 2022 (1555.51 mg·L⁻¹) and 2023 (1422.43 mg·L⁻¹). This is a significant final amount of volatile compounds, the reason being that the herbicide destroyed the weeds, creating more favorable conditions for enhanced metabolism of the vine plant, due to reduced competition for nutrient substrate. In the three experimental variants (V2, V3 and V4), in which treatments with herbicides, combined treatment with herbicides and foliar fertilizers and treatment with foliar fertilizers alone were applied, a trend of accumulation of higher final amounts of higher alcohols was observed in a significant part of the harvests, which was probably due to improved amino acid metabolism of the grape plant - secretion of more amino acids in the grapes (precursors of higher alcohols in the wine).

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