AGRICULTURAL UNIVERSITY – PLOVDIV

FACULTY OF VITICULTURE AND HORTICULTURE

DEPARTMENT OF VITICULTURE AND HORTICULTURE

ABSTRACT

of a dissertation submitted for the award of the educational and scientific degree "Doctor" (PhD)

Scientific field: "Viticulture"

by

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Title:

"Application of organic products and their impact on the growth and reproductive performance of Merlot variety grown under non-irrigated conditions"

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The dissertation comprises 147 pages and contains 29 tables and 54 figures. The cited literature includes 275 references.

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1. INTRODUCTION

Viticulture has existed in the Bulgarian lands for more than 3,000 years, with its earliest development along the Maritsa River valley. Its principal branch is wine-grape production, which is of considerable importance to the national economy. This sector is traditional in many of the country's rural regions. Since 2006, a persistent two-way trend has been observed: new vineyards have been established while, in parallel, existing vineyards have been uprooted or abandoned, and the rate of new planting has been insufficient to compensate for the loss of harvested vineyard area. This trend has also been driven by the increasing frequency of unpredictable climatic events during the growing season, which grape and wine producers must address.

Maintaining the most economically efficient balance between vegetative growth and the reproductive capacity of the plants is one of the major challenges in modern viticulture. Cultivars exhibiting excessively vigorous vegetative growth tend to produce smaller and less typical grape crops, with direct implications for wine quality. The wine market and consumers are increasingly attentive to organoleptic characteristics, thereby raising producers' requirements regarding the quality of grapes used for winemaking.

Numerous approaches are available for managing vine vigour and the quality of reproductive performance. Grapevine is a drought-tolerant plant with a deep root system and can perform well on relatively poor soils that are of limited suitability for other agricultural crops. At the same time, grapes are energy-rich fruit with a complex chemical composition. Grapevine is among the most ancient plants cultivated by humankind and has retained its general botanical characteristics almost unchanged to the present day.

Merlot is one of the most widely cultivated red wine grape cultivars in the world's principal viticultural countries, including Bulgaria. According to data from the Ministry of Agriculture, in 2020 Merlot wine-grape vineyards in Bulgaria covered 9,345 ha, accounting for 28.32% of the total area planted with red wine cultivars and 15.53% of the total area planted with wine-grape cultivars. Wines produced from Merlot grapes mature relatively rapidly and, when young, are more harmonious owing to their lower acidity. They are refined and comparatively soft. Because of its valuable agrobiological and technological attributes, Merlot is expected to retain a leading position in Bulgaria (MAF, 2020).

As a result of climate change and the insufficiently functional underground and surface irrigation-channel network, wine-grape vineyards in Bulgaria are increasingly exposed to water deficit and drought stress. These adverse effects compromise grape quality and consequently lead to wines that lack typical varietal characteristics. Given the influence of microclimatic and soil conditions on the physiological and biochemical characteristics of Merlot, there is a need to develop and test new organic products that can act as antitranspirants and mitigate vine water stress, particularly under non-irrigated conditions. Severe water stress can be alleviated by irrigation, which enables control of plant water availability and the maintenance of an optimal water status required to achieve a specific level of grape and wine quality.

2. AIM OF THE STUDY

To determine the potential of organic products and their effects on the vegetative growth and reproductive performance of Merlot grapevines grown under non-irrigated conditions, with particular reference to overcoming stress during dormancy and the growing season.

The following objectives were set to achieve this aim:

- To investigate the climatic and soil conditions in the land area of the town of Kuklen and their effects on grape yield and quality;

- To assess the metabolic activity of soil bacterial communities;
- To investigate differences in phenological development;
- To identify differences in the indicators characterising potential bud fruitfulness;
- To identify differences in the indicators characterising actual vine fruitfulness;
- To determine the intensity of photosynthetic activity in the leaf canopy;
- To determine the dynamics of grape ripening;
- To quantify changes in grape yield and quality;
- To characterise grape vinification through comprehensive physicochemical analysis;
- To conduct an organoleptic assessment of the experimental wines in order to identify differences in their sensory profiles.

3. MATERIALS AND METHODS

The study was conducted on the Merlot cultivar grafted onto Berlandieri × Riparia SO4 rootstock. The vines were planted in 2011 at the Educational, Experimental and Extension Base of the Department of Viticulture and horticulture, Agricultural University – Plovdiv, located near the village of Brestnik, Rhodopi Municipality, and the town of Kuklen. Merlot was selected because of its widespread cultivation in the Republic of Bulgaria, its importance to the wine industry, and the growth suppression observed in vineyards cultivated under non-irrigated conditions.

The vineyard was in full fruiting. Planting distances were 3.0 m between rows and 1.00 m between vines within the row (3,330 vines/ha). The vines were trained to a medium-height trunk using a bilateral cordon system with the corresponding trellis structure. In all treatments, vine bud load was established by spur pruning to two buds per spur, with six spurs per vine and a total of 12 buds per vine.

The inter-row spaces were grassed, while the soil surface within the vine rows was maintained weed free through herbicide application.

3.1. Ampelographic characteristics of the Merlot cultivar used in the study

Name – Merlot; Merlot noir in France. According to one interpretation, the cultivar name derives from the French word "merle", meaning blackbird, in reference to the very dark colour of the bunches, which resembles the plumage of the bird.

Origin and distribution – An old French wine-grape cultivar grown mainly in the Saint-Émilion and Pomerol appellations of the Bordeaux region. It was subsequently introduced into Moldova, Northern Italy, the USA, Chile and many other countries. In Bulgaria, it is grown mainly in the Southern Viticultural Region, where it expresses its biological potential most fully.

Botanical description – The leaf blade is large, five-lobed and reticulate-wrinkled; the upper surface is dark green with sparse cobwebby pubescence, while the lower surface is bristly. The upper lateral sinuses are deep and closed, with an ovate opening and occasionally a tooth at the base. The lower lateral sinuses are moderately deep and closed, with an ovate opening. The petiolar sinus is open, deep and broad, with an arched base. Flowers are hermaphroditic. Bunches are medium-sized, winged (with one or two wings), and moderately compact to loose. Berries are very small, spherical and juicy, with a medium-thick blue-black skin (Babrikov et al., 2000).

Agrobiological characteristics – Merlot is a mid-season cultivar, ripening around mid-September. Under good cultural practices and an appropriate crop load, vine vigour is high and shoots mature well. The cultivar is highly fruitful: under trunk-trained conditions, 80–82% of shoots are fruitful and the fruitfulness coefficient ranges from 1.30 to 1.60. It is prone to coulure, particularly under unfavourable conditions during flowering, and is relatively sensitive to low winter temperatures (Babrikov et al., 2000).

Technological characteristics – Merlot is a red grape cultivar used for the production of varietal and blended wines. Together with Cabernet Sauvignon, Cabernet Franc, Malbec and Petit Verdot, it is among the principal cultivars used in Bordeaux wines and is one of the most widely planted cultivars in the region. These cultivars together form the well-known Bordeaux blend. Beyond Bordeaux, Merlot is one of the world's best-known red cultivars and one of the best-known grape cultivars overall; in 2018 it ranked second only to Cabernet Sauvignon in planted area, with 657,300 acres worldwide. It was initially used primarily in blends with Cabernet Sauvignon; however, following its introduction into the United States, its soft palate and low tannin levels encouraged the production of 100% Merlot wines. It has since become a favoured cultivar in New York and Washington State. Although Merlot is grown almost worldwide, two broad wine styles have emerged. The "international style", preferred in many New World regions, is based on later harvesting to produce riper, darker, violet-hued wines with a full body, high alcohol and tannin levels, and intense blackberry aromas. The other, the so-called "Bordeaux style", is based on earlier harvesting to retain acidity and produce medium-bodied wines with moderate alcohol, fresh raspberry and strawberry fruit characters, and potentially herbaceous notes (vinopoly.com/encyclopedia-grape-varieties-merlot-bg, 2022).

3.2. Description of the organic product

"Humat Rost" is a fully organic plant-nutrition product derived from peat. It is a concentrated liquid with an intense dark-brown colour. This relatively new product, manufactured in Bulgaria, belongs to a group of organic materials obtained from peat-deposit extracts. The principal sources of energy in soil are plant residues, animal excreta and water-soluble soil organic compounds, from which "Humat Rost" is also derived. The product is mineralised (decomposed) by soil microorganisms, releasing energy that contributes to the stimulation of plant growth.

3.3. Experimental design

The experiment comprised seven treatments. In the foliar-application treatments, "Humat Rost" was applied at the following phenological stages: first inflorescence visible, before flowering, after full bloom, during berry growth, and at veraison, using the doses specified below:

V0 – untreated control
V1 – foliar treatment at 200 mL/da
V2 – foliar treatment at 300 mL/da
V3 – foliar treatment at 400 mL/da
V4 – foliar treatment at 500 mL/da

In the two soil-incorporation treatments, "Humat Rost" was applied as a solid residue at two rates at the beginning of the growing season.

V5 – soil/root-zone application at the beginning of the growing season at 15 kg/da
V6 – soil/root-zone application at the beginning of the growing season at 25 kg/da

Each treatment comprised 60 vines (four replicates of 15 vines).

3.4. Study parameters

Soil and climatic characteristics of the site at the Educational, Experimental and Extension Base of the Agricultural University near the village of Brestnik

Climatic characteristics of the region:

- mean daily air temperature (°C); mean monthly maximum and minimum air temperatures (°C); and absolute maximum and minimum air temperatures (°C);
- duration of the growing season (days);
- precipitation regime (mm).

Climatic data for the study period were obtained from the meteorological station at the Educational and Experimental Viticulture Base in Brestnik.

Characteristics of the soil cover:

Geology, geomorphology and relief; particle-size distribution determined by the gravimetric method described in the Soil Science Practical Manual (Trendafilov et al., 2017).

Soil chemical composition:

- humus content;
- soil reaction (pH), determined potentiometrically;
- water-soluble salt content, determined conductometrically;
- active lime and total carbonate contents, determined titrimetrically;
- contents of the major plant nutrients:
- nitrogen – KCl extraction followed by determination using a Kjeldahl apparatus;
- phosphorus – colorimetric determination in an Egner–Riehm extract;
- potassium – determination by flame photometry.

The parameters were determined using methods generally accepted in Bulgaria and described in the Agrochemistry Practical Manual (Tomov et al., 2009) and the Soil Science Practical Manual (Trendafilov et al., 2017). Soil analyses were performed twice, before and after treatment application.

3.5. Determination of metabolic activity and calculation of key functional indices as indicators of the status of soil microbial communities using Biolog EcoPlates

The metabolic activity of the microbial communities was assessed from their utilisation of different substrates. Biolog EcoPlate™ microplates (Biolog, Hayward, CA, USA) contain 96 wells arranged in three replicate sets of 31 carbon substrates and three control wells without an added substrate. Substrate utilisation was indicated by reduction of a tetrazolium dye. For plate inoculation, 1 g of soil, previously cleared of plant residues and sieved through a 1-mm mesh, was used. Appropriate dilutions were prepared in sterile water after repeated vortex mixing for 30 s and sedimentation of soil particles for 5 min. Each well was inoculated with 150 µL of a 10^{-3} dilution. The plates were incubated at 25 ± 2 °C for seven days. Optical density was measured spectrophotometrically every 24 h until the end of the incubation period using the automated BIOLOG MicroStation™ reader at two wavelengths, 590 and 750 nm. The optical-density values were used to: (1) calculate average well colour development (AWCD), and (2) calculate functional indices as indicators of the structure of the resulting microbial communities.

The substrates were grouped into six categories—amines, amino acids, carbohydrates, carboxylic acids, phenolic compounds and polymers—following Xu et al. (2015).

Data processing and graph preparation were performed in Microsoft Excel. Means, standard deviations and statistical analyses were calculated using IBM SPSS Statistics, version 26. Statistically significant differences were assessed by one-way analysis of variance

(ANOVA), with the measured parameter as the response variable and treatment as the factor, followed by Tukey's test at $p < 0.05$.

3.6. Phenological observations during the grapevine growing season by developmental stage:

- sap flow (bleeding);
- budburst to flowering;
- flowering;
- berry growth;
- berry softening/veraison to technological maturity;
- leaf fall.

These parameters were assessed using the generally accepted methods described in the Viticulture Practical Manual (Roychev et al., 2014).

3.7. Parameters characterising potential bud fruitfulness:

- potential fruitfulness coefficient;
- inflorescence dimensions—length and width (μm);
- dead buds (%).

These parameters were determined according to the method of Babrikov, Braykov and Pandeliev (1977) in 40 buds (four replicates of 10 buds) collected during dormancy from nodes 1, 3, 5, 7, 9 and 11 of one-year-old canes arising from two-year-old wood.

3.8. Parameters characterising actual vine fruitfulness:

- burst buds (%);
- fruitful shoots (%);
- fruitfulness coefficient.

These parameters were assessed using the generally accepted methods described in Ampelography (Roychev, 2012).

3.9. Studies on grapevine photosynthetic activity

- leaf gas-exchange rate measured with an LCA-4 portable photosynthesis system (Fig. 3);
- chlorophyll content determined with a CCM-300 portable chlorophyll-content meter.

These parameters were measured before and after flowering during the growing season under comparable conditions, using shoots of similar size, crop load, orientation and light exposure (Berova et al., 2007; Kerin et al., 2011).

3.10. Dynamics of shoot growth and maturation, assessed by weekly measurements of 10 shoots per treatment

3.11. Studies on grape yield and quality

- Mean bunch mass, g;
- Grape yield per vine, kg;
- Grape yield per decare, kg;
- Sugar content determined by hydrometry, %;
- Yeast-assimilable nitrogen in grape must, mg/dm^3;
- Titratable acidity determined by titration with 0.1 N NaOH, g/dm^3.

Sugar content and titratable acidity were monitored throughout ripening in order to determine the date of technological ripeness. The parameters were analysed using the generally accepted methods described in the Practical Manual of Ampelography (Roychev, 2014) and the Practical Manual of Winemaking (Bambalov, 2009).

Experimental wines were produced from the grapes of all treatments in accordance with the technological scheme for red-wine production (Fig. 4), and the following physicochemical parameters were determined:

Physicochemical parameters:

- Relative density, g/dm^3;
- Alcohol content, % vol.;
- Sugars, g/dm^3;
- Total dry extract, g/dm^3;
- Sugar-free extract, g/dm^3;
- Titratable acidity, g/dm^3;
- Volatile acidity, g/dm^3;
- Free sulphur dioxide, mg/dm^3;
- Total sulphur dioxide, mg/dm^3;
- Total phenolic compounds, mg/dm^3;
- Anthocyanins, mg/dm^3;
- Colour intensity;
- Colour hue;
- Percentage contribution of yellow;
- Percentage contribution of red;
- Percentage contribution of blue;
- Sensory analysis;
- Quantitative determination of total protein by the Bradford method (Richard, O.A., 2002).
- Isoelectric separation and determination of protein fractions by SDS-PAGE electrophoresis.

The analyses were performed in the laboratory of the Training and Experimental Winery at the Agricultural University – Plovdiv, using the methods described in the Practical Manual of Winemaking (Bambalov, 2009), and in the laboratory of the Department of Wine and Beer Technology at the University of Food Technologies, Plovdiv.

The overall wine score was based on the results of the sensory analysis—the arithmetic mean scores assigned by an expert tasting panel—using the 100-point scale.

3.12. Reserve status of one-year-old canes with respect to nitrogen (total nitrogen), phosphorus, potassium, calcium, magnesium, starch and reducing sugars

These parameters were determined once in dry matter from canes collected by random sampling during dormancy (November) from all experimental treatments.

3.13. Statistical analysis

Data processing and graph preparation were performed in Microsoft Excel. Means, standard deviations and statistical analyses were calculated using SPSS (IBM, version 26). Before analysis of variance, homogeneity of variances was tested using Levene's test. Results are presented as mean ± standard deviation, and statistically different groups are denoted by different letters. Data were collected over three consecutive years, with each year treated as an independent replicate in order to assess interannual variability.

Statistically significant differences were identified by analysis of variance (ANOVA), followed by the LSD multiple-comparison test at $p < 0.05$. For the Biolog assay, differences among treatments were assessed by one-way ANOVA, with the measured parameter as the response variable and treatment as the factor, followed by Tukey's multiple-comparison test at $p < 0.05$. One biological sample per treatment was analysed in each year; the replicates were technical replicates at EcoPlate level.

4. RESULTS AND DISCUSSION

The results are presented sequentially according to the principal components of the dissertation research: soil and climatic characteristics, metabolic activity of soil microbial communities, physiological status of the vines, dynamics of shoot growth and maturation, biochemical composition of the canes, fruitfulness, grape yield and quality, and the physicochemical and sensory profiles of the experimental wines. The discussion focuses on the practical evaluation of foliar and root-zone applications of Humat Rost to Merlot grapevines grown under non-irrigated conditions.

5. CLIMATIC CHARACTERISTICS

5.1. Mean monthly air temperature

Analysis of C3S/ECMWF data showed that the global mean air temperature in November 2023 was 0.85 °C above the mean for the new climatological reference period (1991–2020) and 1.09 °C above that for the previous reference period.

At the regional scale, 2023 was characterised by marked differences between below-normal temperatures in northern Europe and above-normal temperatures in the south. In Bulgaria, August and November 2023 were warmer than in 2020–2022. Data from the automatic weather station at the Training, Experimental and Demonstration Centre in Brestnik indicated a temperature anomaly broadly consistent with the national mean, approximately 1.0–1.5 °C above normal.

The mean monthly air temperature averaged over 2023 was 14.96 °C, and the annual temperature range was 20.99 °C. The latter was calculated as the difference between the warmest month, July (27.24 °C), and the coldest month, January (6.25 °C).

In 2023, growth processes in the root zone began considerably earlier because of higher spring temperatures, thereby shortening the period available for dormant pruning. This tendency persisted in the second year, while temperature anomalies varied throughout 2024. February 2024 was exceptionally warm in Bulgaria and exhibited one of the largest positive monthly departures from the 1991–2020 norm. The mean monthly anomaly for the country exceeded 5 °C (Matev, 2024). Although the anomaly was substantially smaller in southern Bulgaria, it remained pronounced, reaching approximately +2.97 °C in the study area.

The tendency established in February persisted throughout the active growth period until August 2024. In the second year, mean monthly air temperatures continued to increase, reaching an annual mean of 15.53 °C, while the annual temperature range was 23.96 °C. This range was 2.97 °C greater and confirmed the warming tendency during 2024, both within the study period and over the preceding decade. Such rapid change also altered the timing of growth processes in the below-ground and above-ground parts of the vine.

In 2025, the mean air temperature over the annual cycle was 14.4 °C and the annual temperature range was 25.6 °C. The third year therefore maintained the tendency towards wider temperature ranges in the study area. Lower temperatures in February prolonged the period from the onset of sap flow to budburst by 3–10 days, depending on treatment. Flowering in June was completed over a shorter period than in the previous two years, at a temperature of 24.7 °C, whereas veraison began 4–8 days later.

5.2. Mean monthly maximum air temperature

The mean monthly maximum air temperature averaged over 2023 was 28.97 °C, and the annual range was 19.09 °C. Winter air temperatures in 2023 were higher than those recorded in the 2020–2022 calendar years. The lowest mean monthly maximum, 21.49 °C, was recorded in December, whereas the absolute maximum of 40.58 °C occurred in August. High air temperatures are important for achieving phenolic maturity in grapes; however, they must be accompanied by adequate plant-available water reserves, particularly in the rhizosphere soil horizon, as well as sufficient atmospheric humidity.

The 2023 observations demonstrated the occurrence of marked temperature anomalies. Although 2023 was not the warmest calendar year on record, the annual anomaly reached 1.5 °C. The data presented in Figure 6 support the view that it represented a turning point in the course of climatic change. At the regional level, the highest winter monthly maximum was recorded in March (26.25 °C), and the difference between the first and second experimental years was 2.94 °C. This favoured more dynamic vegetative development in 2024. During the second experimental year, the weather station recorded values close to the highest ever measured at the Training, Experimental and Demonstration Centre in Brestnik. The lowest monthly maximum was 17.94 °C in January, while the mean monthly maximum averaged over 2024 was 29.5 °C. From March to August, values were higher in 2024 than in 2023, which is assumed to have contributed to the earlier harvest. The resulting upward trend amounted to 0.66 °C. In February 2025, the mean monthly maximum declined to 17.9 °C; the differences relative to the preceding two years reached 4.99 °C, while the difference between 2024 and 2025 was 1.95 °C. Owing to the cooler beginning of the year, the mean monthly maximum averaged over 2025 was 27.6 °C. Consequently, technological maturity in 2025 occurred 9 days later than in 2024 and 19 days later than in 2023. According to Hunter and Visser (1989), shoot growth depends on the efficient interception and use of solar energy not only by individual leaves but also by the vine canopy as a whole. This supports the proposition that solar radiation is a principal driver of grapevine growth.

5.3. Mean monthly minimum air temperature

Mean monthly minimum air temperature varied within a relatively narrow range: 3.31 °C in 2023, 4.15 °C in 2024 and 3.2 °C in 2025, the lower value in 2025 being attributable to the colder winter–spring period. The lowest monthly minimum was recorded in February (−9.16 °C), and the highest in July (12.55 °C). A shift in the timing of the lowest temperature was again observed: in 2024 it occurred in January (−7.95 °C), whereas the February value was −4.98 °C and was higher than in the other two years. In 2025, prolonged and frequent temperature inversions produced minima of −1.6 °C in March and −0.7 °C in April, and the spring was long and cool. No critically low temperatures in the range of −15 to −18 °C were recorded in any of the three years; such temperatures could have caused freezing injury to the primary and some replacement buds within the compound winter buds, thereby reducing reproductive potential. The data in Table 6 indicate that the minimum-temperature pattern in 2025 resembled that of 2023, particularly in March and April. This pattern was atypical because the vines had already left endodormancy and entered ecodormancy, which normally begins in early February. This period is particularly important for achieving a crop with the desired characteristics because root water-uptake capacity begins to recover.

Grape growers should therefore promote adequate carbohydrate reserves in the xylem and phloem. This would enhance vine resilience to late spring cold events and support both vegetative and reproductive potential during the growing season.

5.4. Biologically active temperatures and conditions governing the onset of the principal grapevine phenophases

Normal growth processes, which involve changes in starch metabolism within the vine xylem, require the accumulation of an adequate sum of active temperatures. This heat accumulation supports the resumption of active growth in both below-ground and above-ground vine organs. Under the climatic conditions of the study area, the sum of active temperatures should be at least 2,200 °C. It amounted to 3,634 °C in 2023 and increased to 4,136.37 °C in 2024. The upward tendency continued in 2025. The growing season lasted 214 days in 2023 and 222 days in 2024; the longest period was recorded in 2025, comprising 263 days with air temperatures above 10 °C. This represented a new maximum, exceeding the 2023 value by 581.65 °C. These observations are consistent with data from the Copernicus Climate Change Service (C3S). In 2025, the increase in the sum of active temperatures remained high, amounting to 558.16 °C, and was accompanied by the prolonged 263-day growing season.

Marked meteorological variability was observed during the three experimental years. January 2023 had the smallest monthly anomaly, whereas the opposite pattern was recorded in 2024; the variability reappeared in 2025, although to a lesser degree. The climate has undergone profound and highly dynamic changes, which are reflected in the observed conditions. Their causes include numerous global factors: natural physical processes, such as atmospheric circulation favouring above-normal temperatures and low precipitation, together with the absence of the cooling effect of snow cover in the temperate zone, as well as substantial anthropogenic influences.

Treatment-specific group patterns were observed in the phenological study. High temperatures shortened the duration of individual phenophases. In 2023, and particularly in 2024, the growing season was shorter because elevated temperatures affected vine physiology, whereas growth in 2025 was more regular and prolonged. The maturation of both vegetative growth and berries was accelerated by the lack of rainfall and enhanced water loss through the berry skins during the experimental period. An earlier harvest was consequently required.

5.5. Precipitation

Grapevine growth and development are determined not only by temperature but also by soil-water availability derived from precipitation during the autumn–winter and winter–spring periods. Spring 2023 was characterised by negative anomalies in mean seasonal temperature and relatively large departures in precipitation. The season was comparatively cool, with rainfall close to the long-term norm. Across much of the Upper Thracian Plain, spring precipitation totals exceeded the climatic norm.

Approximately one-third of the annual precipitation norm for the Southern Viticultural and Wine-Producing Region was received during April, May and June 2023. Total precipitation at the Training, Experimental and Demonstration Centre in Brestnik was 350.4 mm in 2023, with the greatest monthly amount recorded in June (79.8 mm). Precipitation recorded in the experimental area in 2024 was close to the climatic norm, but monthly totals varied widely, from 0 mm in October to 159.4 mm in December. Departures from the norm were negative in most months, with the largest deficits in February (−88.8%), June (−94.4%) and October (−100%). Preliminary estimates indicated an annual total of 441.6 mm in 2024, equivalent to approximately 85.74% of the expected rainfall. In contrast to the preceding two years, rainfall in 2025 was distributed more evenly between spring and autumn, and the annual total reached 679.2 mm. This was one reason for the longer growing season and the later leaf fall, which occurred from 30 November to 1 December. Annual precipitation in 2025 exceeded the climatic norm of 515 mm for Plovdiv Province.

The relatively dry conditions in February 2023 delayed the onset of sap flow in the experimental vines. Greater rainfall in January and February 2025 created suitable conditions for an earlier onset of sap flow, further promoted by higher mean daily air temperatures. Temperature is the principal factor initiating winter-bud development, but only at the outset; progression from one phenophase to the next cannot occur without sufficient water in the root zone. Rainfall during April and the first half of May in 2023, 2024 and 2025 was highly beneficial in preparing the vines for flowering. Both excessive rainfall and its absence, however, may adversely affect flowering. In 2023, temperatures during this period were below those required for uniform flowering and, together with rainfall, promoted coulure and millerandage. Flowering consequently lasted longer than the usual 20 days. A related response occurred in 2024, when insufficient rainfall impeded normal progression through the fourth phase of the growing cycle and contributed to the formation of smaller berries within the clusters.

High summer temperatures and the absence of rainfall in 2024 caused growth to cease earlier, although some treatments produced a greater amount of vegetative biomass.

During the 2025 experimental year, several rainfall periods were concentrated in January–March, April–May and October–November. Spring rainfall provided favourable conditions for growth, but its effect coincided with lower temperatures, which disrupted flowering. Summer precipitation was substantially lower in all three years: 125.2 mm in 2023, 61.8 mm in 2024 and only 43 mm in 2025. The vines were therefore assumed to have experienced water deficit and drought stress; in some treatments this was manifested as sunburn and even premature leaf abscission at the basal nodes of the shoots.

Nevertheless, the greater spring rainfall in 2025 resulted in a slightly later harvest, during the last ten days of August, approximately 9 days later than in the previous year. More than 208.8 mm of precipitation was recorded in October and November. This rainfall favoured the prolonged growing season and the accumulation of assimilates in both one-year-old and perennial vine organs.

6. DETERMINATION OF METABOLIC ACTIVITY AND CALCULATION OF KEY FUNCTIONAL INDICES AS INDICATORS OF THE STATUS OF SOIL MICROBIAL COMMUNITIES USING BIOLOG ECOPLATES

6.1. Inoculation of Biolog EcoPlates™

The metabolic activity of microbial communities was assessed from their utilisation of different carbon substrates. BIOLOG EcoPlates™ (Biolog, Hayward, CA, USA) contain 96 wells arranged as three replicate sets of 31 substrates and three control wells without added substrate. Reduction of a tetrazolium indicator dye was used as an indicator of substrate utilisation. For plate inoculation, 10 g of soil was cleared of plant residues and sieved through a 1.5-mm mesh. Appropriate dilutions were prepared in sterile physiological saline (0.85% NaCl) after the samples had been shaken for 30 min at 185 rpm and the soil particles allowed to settle for 10 min. Each well was inoculated with 150 µL of a 10^{-3} dilution. The plates were incubated at 25 ± 2 °C for seven days. Optical density was measured spectrophotometrically every 24 h until the end of incubation using a BIOLOG MicroStation™ automated reader at two wavelengths, 590 and 750 nm. The optical-density values were used to: (1) calculate average well colour development (AWCD); and (2) calculate functional indices as indicators of the structure of the microbial communities formed.

The substrates were assigned to six categories—amines, amino acids, carbohydrates, carboxylic acids, phenolic compounds and polymers—according to Xu et al. (2015).

6.2. Calculation of average well colour development (AWCD)

Average well colour development (AWCD) was calculated according to Sofo and Ricciuti (2019), using readings at 590 and 750 nm. Each set of 31 substrates on a plate was treated as a separate replicate. The initial calculation of changes in optical density during plate incubation comprised three steps. First, the value at 750 nm was subtracted from that at 590 nm. Second, the resulting value was corrected against the control well containing the initial suspension at the dilution used. Third, C_i was obtained by subtracting the value recorded at the initial reading (24 h in the present study) from each control-corrected value at every subsequent incubation time, thereby removing the optical-density contribution of the initial suspension in accordance with Urakawa et al. (2013). Negative values after correction were set to zero, and AWCD at each measurement time was calculated using the equation of Chen et al. (2020):

6.3. Results and discussion

Total metabolic activity (AWCD) of the microbial communities differed among the treatments. Following the initial lag phase after plate inoculation, characteristic of the first hours of EcoPlate incubation, metabolic activity increased throughout the incubation period. The lag phase depends both on the number of microorganisms in the initial suspension and on their rate of adaptation to the supplied substrates, and may vary substantially with sample origin. For example, Ge et al. (2018), studying microbial communities isolated from stored rice, reported a 24-h lag phase, whereas studies of soil samples enriched with sludge have observed a 60-h lag phase. In the present study, V0 exhibited lower microbial metabolic activity from 72 h until the end of incubation. The experimental treatments showed similar activity up to 96 h, after which V5 exhibited higher activity. At 168 h, a clear difference among treatments was observed. Optical density was highest in V5 (0.519 ± 0.087), followed by V6 (0.455 ± 0.121), and lowest in the control (0.337 ± 0.118). In the absence of inhibitory factors suppressing bacterial growth and metabolism, the AWCD curve generally has a characteristic sigmoidal shape (Campbell et al., 1997; Lima et al., 2015). A similar pattern was observed in the present study.

A distinct difference between V0 and the experimental treatments was observed for microbial utilisation of amino acids. Metabolic activity in V5 and V6 was similar up to 96 h, after which V5 showed higher activity. At the end of the study period, the lowest optical density was recorded in V0 (0.327 ± 0.148). This mean was almost twofold lower than that of V5, which reached 0.646 ± 0.110. The value for the other treated variant, V6, was slightly lower, at 0.558 ± 0.127.

In contrast to total metabolic activity and the other five substrate groups, V6 consistently exhibited the highest rate of amine utilisation in all samples analysed during the three experimental years. This response may reflect selection for a particular group of microorganisms as a result of the treatment dose or a specific response of the soil microbiota. After the initial 24-h lag phase observed in all treatments, optical density in V6 increased relatively uniformly by 0.120 units between 24 and 96 h. After 120 h, the rate of increase slowed to 0.058 units. At the end of the study period, the highest value was recorded in V6 (0.525 ± 0.172), followed by V5 (0.372 ± 0.103) and V0 (0.269 ± 0.103).

For carboxylic-acid utilisation, a clear difference in metabolic activity among treatments emerged after 72 h. At the end of the study period, the highest value was recorded in V5 (0.542 ± 0.090), followed by V6 (0.440 ± 0.092), while V0 had the lowest value (0.330 ± 0.136).

For carbohydrate utilisation, V6 initially exceeded the other treatments, with a mean value of 0.432 ± 0.170; by the end of the study period, however, its values were lower than those of V5, which exhibited a more uniform and sustained increase in optical density. At 168 h, the numerical differences among treatments were small, averaging 0.072 units. The highest mean was recorded in V5 (0.524 ± 0.106), followed by V6 and V0, with respective means of 0.471 and 0.416.

The increase in optical density associated with polymer utilisation followed a pattern similar to that observed for the other substrate groups and was characterised by a clear difference among treatments. The largest difference, 0.208 units, occurred between V5 and V0, whose respective end-point means were 0.519 and 0.312, with standard errors of 0.073 and 0.089. The mean for V6 was 0.409 ± 0.090.

Phenolic compounds were utilised to a much smaller extent than the other substrate groups. In most cases, the measured optical density resulted from utilisation of only one of the two substrates in this category, namely 4-hydroxybenzoic acid. The absence of positive responses for 2-hydroxybenzoic acid and in individual plate replicates accounted both for the large standard deviations and for the irregular course of the optical-density curve. Mean values at the end of EcoPlate incubation did not exceed 0.200 units. The highest value was recorded in V5 (0.176 ± 0.086), followed by V6 (0.147 ± 0.079) and V0 (0.113 ± 0.052).

The functional indices presented were used as indicators of microbial-community structure. With the exception of Margalef's richness index, which was highest in V6, all other indices reached their highest values in V5, including a value of 2.679 ± 0.174. Higher mean index values indicate greater diversity in EcoPlate substrate utilisation and a more balanced metabolism across substrate groups. V5, which received the lower soil-applied dose, may have favoured either a more diverse taxonomic structure of the microbial community or a broader substrate-utilisation range, as also suggested by the metabolic-activity data. Univariate analysis of variance detected no statistically significant differences among treatments for the calculated functional indices. Multivariate analysis of variance (MANOVA), however, indicated differences among experimental treatments for all indices, primarily between V0 and V5, with the exception of Margalef's index. Levene's test, required to verify the assumptions of the analysis, showed that homogeneity of variance was not satisfied. A treatment × year interaction was also identified, partially limiting the independent assessment of treatment effects (Field, 2009). An additional difficulty in testing for significant differences is the probable correlation among indices, including possible inverse relationships (Hubalek, 2000).

Note: indices are presented as mean ± standard deviation, n = 9.

Table 1. Functional indices describing the structure of the microbial communities formed

Treatment	H′	E	D	McI	d
V0	2.395 ±0.445	0.793 ±0.146	0.865 ±0.063	1.098 ±0.085	6.148 ±1.502
V5	2.679 ±0.174	0.889 ±0.087	0.920 ±0.019	1.185 ±0.017	6.116 ±1.541
V6	2.432 ±2.432	0.789 ±0.157	0.877 ±0.079	1.153 ±0.066	6.277 ±0.911

7. STUDIES ON THE PHYSIOLOGICAL STATUS OF THE VINES

7.1. Phenological observations

Phenometry, as a component of phenological observation, involves measuring current linear plant growth and determining its dynamics. The duration and progression of phenophases may change according to temperature, the soil water–air regime and the physiological status of the vines. Analysis of data from soil sensors operating at a depth of 1 m indicated that growth activity in the experimental vines generally began after the soil temperature had remained above 8.7 °C for 45 consecutive days. Thereafter, xylem water flow commenced together with hydrolysis of polysaccharides in the vascular tissues. Phenological observations in 2023, 2024 and 2025 showed that 11–14 days were required from the onset of sap flow to budburst. This interval was 12 days in the control (V0), 14 days in V1–V4 and, on average, 11 days in V5 and V6. Several factors contributed to this early phenological pattern, including low precipitation during the winter–spring period combined with low spring temperatures. The natural slope of the experimental site was another limiting factor. No substantial differences among treatments were observed from budburst to the first-leaf stage. Differences reappeared from first leaf to flowering, with the treatments forming distinct groups. This was assumed to result from foliar Humat Rost applications at the first-inflorescence-visible and pre-flowering stages. Thereafter, treatment-related differences in vegetative development became evident across the three years. The longest interval was recorded in V5 and V6 (49 days), followed by V3 and V4 (48 days), whereas 47 days were required in V0, V1 and V2.

In May and June, minimum temperatures declined to 6.35–10.57 °C in 2023, 6.71–13.74 °C in 2024 and 5.3–10.9 °C in 2025, causing a temporary interruption of phases III and IV of the growing cycle. Uneven precipitation during the same period further delayed transitions between phenophases. Two additional treatment-specific applications of Humat Rost were made between berry growth and the end of veraison. The effect was most evident at the highest application rates. At the end of phase IV and beginning of phase V of reproductive-organ development, the treatments again formed groups, this time favouring the foliar treatments V2, V3 and V4. All treatments were harvested simultaneously so that differences in bunch mechanical composition and must composition for winemaking could be determined. Leaf fall was prolonged in all three years because high temperatures and the absence of frost delayed leaf abscission. No treatment differences were established in the duration of leaf fall. The phenological observations indicate that, when performed simultaneously, the pruning system did not materially affect the onset or duration of individual phenophases. Delayed budburst was observed only in vines pruned later; these vines had been retained to assess winter-bud mortality during the winter–spring period. The interval from sap flow to budburst was shortest in V5 and V6 (11 days). In V1–V4, its mean duration was identical at 14 days, compared with 12 ± 4 days in V0. From budburst to first leaf, no meaningful differences were observed and the stage lasted 15–16 days. Differences were also small from first leaf to flowering and from veraison to technological maturity. In all treatments, the first-leaf-to-flowering period lasted 47–49 days. One-way analysis of the duration of phenophases, with treatment (foliar rates of 200–500 mL/da and soil-applied rates of 15–25 kg/da) as the factor and the LSD criterion, showed no significant differences among treatments.

7.2. Intensity of the principal physiological processes in grapevine leaves

Carbohydrates are formed through photosynthesis from CO_2 and water, with mineral nutrients participating in the underlying metabolic processes; this activity is closely related to chlorophyll content. Photosynthetic rate, transpiration and chlorophyll content were investigated at three phenological stages. Humat Rost was applied at different foliar and root-zone rates as an additional stimulant of the conversion of light energy into organic compounds and oxygen. Because the product was dissolved in water, it was readily available for absorption by the leaf surface. It may itself have supported photosynthetic activity by acting as an antitranspirant. This interpretation is also supported by the graphical results.

At the first measurement, on 12 June 2023, a high photosynthetic rate of 18.91 µmol CO_2 m^{-2} s^{-1} was recorded. The lowest activity occurred in V2 (13.06 µmol CO_2 m^{-2} s^{-1}) and V0 (13.97 µmol CO_2 m^{-2} s^{-1}), with no substantial difference between them. In V1, V3, V4 and V6, values in the fifth-leaf zone increased with increasing foliar and soil-applied rates. At the third measurement in this zone, V6, which received root-zone nutrition, exhibited the lowest photosynthetic activity.

This was also attributable to restricted macro- and micronutrient transport during a period of insufficient water availability in the rhizosphere. In the second experimental year, the highest value on 27 May 2024 was recorded in V6 following root-zone application of Humat Rost (16.99 μmol CO_2 m^{-2} s^{-1}), followed by V0 (16.73 μmol CO_2 m^{-2} s^{-1}). The relatively high activity of the control was partly explained by its location in the lowest and therefore better-watered part of the experimental vineyard, as reflected in the greater rainfall received in May 2024. The other treatments had similar values, in descending order: V2 (12.42), V1 (12.00), V5 (11.37) and V4 (11.26 μmol CO_2 m^{-2} s^{-1}). In 2025, activity in the fifth-leaf zone was measured for the first time on 9 June. By then, two foliar Humat Rost applications had been made and had positively affected shoot growth; the highest activities were recorded in V4 (13.46), V1 (12.86) and V3 (11.54 μmol CO_2 m^{-2} s^{-1}). During this period, the soil-applied treatments also exhibited higher activity than the control, in agreement with the findings of Stoev et al. (1952, 1966). At the second date, 11 July, the highest activity was again observed in the foliar Humat Rost treatments, which substantially exceeded the soil-applied treatments V5 and V6. V0 also performed better than V5 and V6, a response associated with high temperatures and negligible rainfall in June and July.

Measurements were also made in the tenth-leaf zone during the last ten days of May. This period was selected because shoots were growing intensively and forming both leaf area and internodes responsible for photosynthesis and transpiration. Photosynthetic rates were lower in 2023 because the leaves were less fully developed. This was largely attributable to lower spring temperatures, a shorter period of active growth and a more uneven distribution of winter–spring precipitation, consistent with reports by other authors. In 2024, photosynthetic activity was higher across the experimental treatments. On 27 May 2024, the highest values occurred in V5 (16.89 μmol CO_2 m^{-2} s^{-1}) and V2 (15.59 μmol CO_2 m^{-2} s^{-1}), followed by V0 (14.03 μmol CO_2 m^{-2} s^{-1}). The remaining treatments again formed a separate group with a comparatively high activity. Photosynthesis declined at subsequent measurement dates in both 2023 and 2024. In the third experimental year, exceptionally high activity was evident from the first measurement. This was attributed firstly to intensive shoot growth following its delayed onset in spring, and secondly to abundant rainfall combined with high temperatures. In 2025, rainfall was more evenly distributed during summer; together with canopy-opening operations and Humat Rost application, this resulted in higher photosynthetic activity.

Measurements during berry growth and technological maturity showed that soil drying restricted and eventually arrested vegetative growth. In leaves at the fifth and tenth nodes, chlorophyll degradation was accompanied by reduced photosynthesis. At the pea-size berry stage, leaf gas exchange was improved in all treatments, and photosynthetic rate was again highest in the foliar Humat Rost treatments. The control occupied an intermediate position between the soil- and foliar-applied treatments. The lower photosynthetic rate at technological maturity was associated with intensified export of photoassimilates to the ripening grapes and cessation of shoot elongation in all treatments. This process involves progressive chloroplast degradation and a decline in chlorophyll content (Pandeliev, 1987). Water and atmospheric moisture deficits, particularly during the study period, further contributed to the dynamic and variable response. Soil drought and low relative humidity strongly affect transpiration, especially during the second half of the growing season. Transpiration influences both mineral nutrient uptake and their transport through the xylem and phloem. It is generally considered that greater transpiration increases the rate at which nutrients are absorbed as ions from the soil and through the leaves. The present results showed that, during the first two stages, the rate of this process was substantially higher in all treated variants than in the control, except in the fifth-leaf zone during the first ten days of June 2025. This supported plant water supply and the dissolution and upward xylem transport of macro- and micronutrients. The high temperatures measured during the observation period further confirmed the effective anti-stress system of the grapevine as a mesophytic species. At the same time, increased transpiration on hot summer days cooled the plants and maintained temperatures conducive to normal physiological and biochemical processes. The small treatment differences in transpiration at technological maturity were attributed to senescence of the photosynthetically active leaf apparatus.

Factors limiting photosynthesis may be stomatal or mesophyll-related. Stomata primarily regulate CO_2 diffusion into mesophyll cells. The changes in photosynthetic rate, together with the absence of substantial differences in stomatal aperture among treatments, suggest that mesophyll limitations were also involved.

The chlorophyll reaction centre absorbs light energy and transfers it to other components of the photosystem. Solar energy is thereby converted into chemical-bond energy in the organic compounds synthesised during photosynthesis. Chemically, chlorophyll belongs to a group of organic pigments. Chlorophyll content was high in all treatments. At the first measurement before flowering, the fifth leaf had reached full development.

This confirms that chlorophyll content depends on the developmental stage of the grapevine leaf. During active growth, treatments receiving foliar or root-zone applications of the organic product exhibited more dynamic changes in chlorophyll content.

Analysis of chlorophyll dynamics in the tenth leaf showed that variation depended strongly on the measurement period, which in turn was related to leaf age and the availability of nutrients and water. In 2023, the highest values occurred in the foliar treatments V3 and V2 and the soil-applied treatments V5 and V6. Differences were observed during active growth in 2024 and 2025, when sufficient soil-water reserves from late-spring rainfall facilitated the transport of total protein nitrogen. Before harvest in all three years, leaf chlorophyll declined because some treatments had produced a larger vegetative canopy, while nutrient deficiency and reduced cellular water content were also present.

In 2024 and 2025, a tendency towards chlorophyll reduction was evident at the third measurement, particularly in V5 and V6, where Humat Rost had been applied to the root zone. In the absence of irrigation and natural rainfall, additional stress-induced exogenous changes were inevitably activated. Earlier formation of an abscission layer at the base of the petiole was accompanied by premature chlorophyll degradation and leaf fall, thereby impairing carbohydrate assimilation and cooling of the vines.

7.3. Dynamics of shoot growth

Grapevine growth is strongly influenced by abiotic factors, which are becoming increasingly important against a background of more frequent temperature extremes. The experimental period included a comparatively cool spring in March and April, which delayed both endogenous and externally expressed growth processes. In 2023, vegetative organs began to develop later than in 2024. A second contributing factor was insufficient precipitation during both the autumn–winter and winter–spring periods at the Training, Experimental and Demonstration Centre in Brestnik.

At the first measurement dates, V6 and V5, which had received root-zone Humat Rost, were distinguished by more rapid growth, whereas the remaining treatments showed similar growth. At subsequent measurements, V6 had produced nearly 26 cm of new growth and maintained this rate. Following the first foliar applications at the first-inflorescence-visible and pre-flowering stages, V1 reached 40 cm of shoot growth and occupied an intermediate position between V6, V4 and V5 until flowering. After the second Humat Rost application, shoot elongation changed in all treated variants. V0 separated from the others by producing approximately 45 cm less growth. As the season progressed and temperatures exceeded 33 °C, particularly during and after full flowering, rapid elongation occurred in V2–V6, which produced 85–105 cm of new vegetative growth. This group maintained an upward trend until the end of the growth period. From the end of full flowering to berry set, accelerated linear growth was observed in V1 and V2, which produced more than 120–130 cm of new growth between 21 June and 5 July 2023. The untreated control V0 produced 78 cm of shoot growth in 2023.

Higher temperatures in April 2024 promoted more active development of the winter buds. The first shoot-length measurement was made during the last ten days of April. As in the first year, V6 and V5, which received Humat Rost in the root zone at the beginning of the growth period, had an initial advantage. At this stage, no substantial differences occurred among the other treatments, although growth was lowest in V0. The same pattern was maintained at the next measurement, when only the control failed to produce new growth. Following the first foliar Humat Rost applications at the first-inflorescence-visible and pre-flowering stages, shoot length in V1–V4 changed and ranged from 90 to 105 cm. During the second and third ten-day periods of June, treatments receiving foliar or soil application formed clearly distinguishable groups. In 2024, shoot growth ceased earlier in all treatments than in 2023. The shorter growing period, particularly in 2024, was associated with low rainfall and high maximum temperatures. Despite these seasonal conditions, final shoot lengths reached 165 cm in V3, 160 cm in V2, 150 cm in V1, 145 cm in V4 and 140 cm in V0. The two soil-applied treatments, V5 and V6, produced 120 cm of growth, presumably because their more moderate growth was governed by an uneven balance between nutrient and water availability.

In 2025, the greatest early growth at the first measurement was recorded in V3, V4 and V6, owing to their better physiological condition inherited from the preceding year and to greater spring soil-water reserves. The same tendency was observed in all treatments over the next two measurements. Rising temperatures in June accelerated shoot elongation at all three measurement dates, although the soil-applied treatments produced less new growth. This relationship persisted until the final measurement on 14 July. Based on the measured climatic variables, particularly temperature and precipitation, foliar Humat Rost application can be considered to have positively affected growth, especially during thermally dynamic periods in spring and summer. Soil incorporation of solid Humat Rost as a slurry resulted in a shorter growth period and less shoot elongation in V5 and V6. This response may be attributed to soil and atmospheric drought combined with high summer temperatures. The magnitude of temperature and rainfall effects can be assessed using Branas' drought-intensity index ($i = 3\Sigma t° - \Sigma P$; Branas, 1974), for which values increasingly above unity indicate more intense drought. The index was 41.61 in 2023, 57.45 in 2024 and 23.34 in 2025, demonstrating severe water deficit and, in the driest year, approximately twice the precipitation shortage.

7.4. Dynamics of shoot maturation

Successful overwintering depends not only on meteorological conditions during autumn and winter but also on vine growth and development during the preceding growing season. Under Bulgarian conditions, when plant-available water in the upper 1 m of soil falls below 70% of field capacity, root and shoot growth are disrupted. Such changes were observed in the experiment. A gradual decline in temperature during the autumn hardening phase also plays an important role. The quantity and quality of mature cane tissue largely determine both the vegetative and reproductive potential of the shoots. Figure 41 presents the dynamics of mature growth in 2023, which is closely related to the winter hardiness of both canes and buds. Shoot maturation began later in 2023 than its usual onset around mid-July. Contributing factors included more than 95 L m^{-2} of rainfall in June and July and both foliar and root-zone Humat Rost applications. Together with the SO4 rootstock, whose roots grow at an approximately 20°

positive geotropic angle and therefore occupy deeper soil layers, these factors supported a prolonged period of growth (Roychev, 2012).

At the beginning of August, comparatively high temperatures initiated the formation of mature sectors along the shoots. As with shoot growth, a clear group pattern was observed: V1, V2, V3, V5 and V6 developed 45–65 cm of mature cane, whereas V0 produced only 20 cm. This anatomical transition continued for approximately 11–12 weeks in 2023. Shoot-maturation dynamics were directly dependent on climatic conditions. Maturation was slowest in V0 and V4, and this tendency persisted until the end of the period. The more gradual maturation of V4 was assumed to result from the considerably higher Humat Rost application rate.

V1, V3, V5 and V6 followed similar maturation trajectories throughout the period, producing 55–65 cm of mature cane, consistent with their comparable shoot-growth dynamics. V2 showed the most rapid formation of secondary anatomical structure and reached 78 cm of mature growth. Despite a pruning load of six two-bud spurs per vine, the shoots produced relatively little mature cane. Uneven rainfall, high temperatures, redistribution of polysaccharides among grapes, mature cane and winter buds, and the shorter growing season were among the factors slowing shoot maturation in August and September.

In 2024, phellogen formation at the shoot base proceeded rapidly. Rainfall during the corresponding preceding period exceeded 95 L m^{-2} in 2023 but amounted to only 26 L m^{-2} in 2024. These conditions accelerated maturation: during the first and second ten-day periods of July, vines in V0–V4 had already developed 80–105 cm of mature cane. The high temperatures and critically low rainfall in 2024 caused shoot growth to cease 10–15 days earlier and activated rapid lignification and conversion of shoots into canes. Maturation in all treatments was complete by the first half of August. The foliar treatments and the control—V4, V1, V2, V3 and V0—ceased growth after forming 95–120 cm of mature cane, whereas V5 and V6 ceased elongation at approximately 76 cm. In 2025, specific disturbances associated with low spring temperatures were observed.

The beginning of the third year was characterised by low temperatures during January, February, March and April. Temperatures close to 0 °C did not cause visible injury but delayed the growth of shoots, leaves and inflorescences. This was one reason why shoot maturation began only during the last ten days of July. Shoots were 70–90 cm long and matured sector by sector; this sequential pattern continued at subsequent measurements in July and August. By the end of the maturation period, both foliar and soil-applied Humat Rost treatments showed stable maturation, while V0 and V1 had the shortest mature portions. Drought intensified competition for water between the berries and the maturing shoots. Declining leaf photosynthesis and intensified hydrolytic reactions consequently brought growth to an end. Shoot maturation lasted 85 days in 2023, 35 days in 2024 and 34 days in 2025.

7.5. Biochemical composition of the canes in relation to the application method of the organic fertiliser Humat Rost

Successful overwintering is determined to a large extent by cold hardiness and by the genetic characteristics of the species and cultivar. Winter hardiness and vine physiological status also depend on complex anatomical, physiological and biochemical processes within the vascular system during summer. These are strongly influenced by progression through phase V of the growing cycle and into relative dormancy.

Dormant and summer pruning, combined with foliar and root-zone Humat Rost application, altered dry-matter accumulation in mature one-year-old canes. The highest amounts occurred in V6, V5, V4 and V1, while no substantial statistical differences were found among the remaining treatments. Protein content was highest in V1 (3.40 ± 1.27%), V4 (3.32 ± 0.70%)

and V2 (3.22 ± 0.90%). Protein is involved in completing periderm formation, including the cork tissue that insulates the bark and performs a protective function (Todorov, 1988). Sugar content varied widely over the three experimental years, although the mathematical analysis showed broadly similar values among cane samples. Higher values were recorded in the foliar treatments V1 (6.45 ± 0.24%) and V2 (5.60 ± 0.65%) and in the soil-applied treatment V6 (6.36 ± 0.38%), in which the organic product acted as a nutritional stimulant. The remaining treatments accumulated approximately one-third less sugar in the canes. Complete and satisfactory shoot maturation occurs when the accumulation of mobile carbohydrates—starch and soluble sugars—and the decline in tissue water content have stabilised.

In addition to its atmospheric occurrence, nitrogen is present in the proteinaceous material of all living organisms. Its fertiliser-use efficiency depends on chemical form, fertiliser type and application method, as well as susceptibility to leaching. Research in several countries has shown that foliar application at defined phenological stages can improve photosynthetic activity and growth. Nitrogen content in the analysed canes was highest in the foliar treatments V1 (0.55 ± 0.20%), V4 (0.53 ± 0.11%) and V2 (0.51 ± 0.14%), and lowest in V0 (0.43 ± 0.01%). These foliar treatments also produced greater vegetative growth during the season, indicating a positive effect of the applied organic product, consistent with Braykov (2005).

For P_2O_5, both root-zone and foliar application produced an upward change. The highest values were recorded in V2 (2.14 ± 3.07%), V4 (1.82 ± 2.48%) and V3 (1.66 ± 2.23%). Increased phosphorus reserves in canes contribute to drought and winter-cold tolerance and to rhizogenesis, which is particularly important in drought-prone areas and on south-facing slopes. Comparatively high calcium concentrations were also found in foliar-treatment samples, including V4 (5,165.81 ± 1,216 mg kg^{-1}), V3 (4,869.48 ± 1,246.061 mg kg^{-1}) and V2 (4,805.92 ± 1,144.802 mg kg^{-1}). Together with phosphorus, calcium is important for sound root development and for spring shoot and leaf growth. Calcium deficiency restricts shoot growth, causes the tips of young leaves to curl downwards and induces marginal necrosis that develops into brown scorch (Popov, 1972).

Table 2. Mass of mature cane growth, kg

Year	V0	V1	V2	V3	V4	V5	V6
2023	2.130	2.270	3.070	2.880	2.270	1.490	2.050
2024	2.300	2.990	3.440	4.050	3.260	2.200	3.130
2025	3.440	3.600	3.800	4.900	3.900	2.800	3.300
Period mean	2.62±0.71	2.95±0.67	3.44±0.37	3.94±1.01	3.14±0.82	2.16±0.66	2.83±0.68

8. BUD FRUITFULNESS

8.1. Potential bud fruitfulness

Potential reproductive capacity is a highly dynamic determinant of grape yield. Unlike annual crops, in which yield potential can be maximised through seeding rate, grapevine crop potential is more difficult to assess because fruitful and non-fruitful buds cannot be distinguished visually (Babrikov, 1983). After the treatment plots had been established, dormant pruning was carried out. In Bulgaria and other viticultural countries, vine pruning and the selection of crop load through particular fruiting units are still largely empirical, being based on cultivar biology and visual assessment of vine vigour (Babrikov, 1983). A more precise forecast of potential fruitfulness should also consider the quantity of mature cane growth produced during the season.

This parameter is continuously associated with the percentage of developed buds and mean bunch mass. Table 2 presents the quantities of mature cane removed from the vines over the three experimental years.

The mass of mature cane obtained at pruning differed statistically among treatments. Foliar Humat Rost treatments showed a dynamic response, with the greatest vegetative mass in V3 (3.94 ± 1.01 kg), V2 (3.44 ± 0.37 kg) and V4 (3.14 ± 0.82 kg), and the lowest in V5 (2.16 ± 0.66 kg). One-way analysis with foliar application rate (200–500 mL da^{-1}) as the factor and the LSD criterion showed significant differences among treatments. The different nutritional regimes applied during the three experimental years altered the amount of mature cane produced, with treatment differences reaching 1.780 kg. Across all three years, the foliar treatments produced a greater response in newly formed growth than the root-zone treatments. These findings are supported by the shoot-growth data.

To determine potential bud fruitfulness precisely, cane samples extending to the eleventh bud were collected. Before this assessment, it is appropriate to determine the percentage of buds that had died from different causes; the samples for this purpose covered the cane up to the fifteenth node (Babrikov, Braykov and Pandeliev, 1978). The percentage of dead buds in autumn 2023 was very low. A negligible value of 1.88% was recorded only in V1 before winter dormant pruning, and compensatory pruning was therefore unnecessary. This response was assumed to be related primarily to low precipitation and high temperatures during the growing season.

The appropriate approach to identifying the cause of bud injury and restoring regular vine cropping should be selected according to the training system, vine form, cultivar characteristics and severity of damage. The next sampling, in spring 2024, confirmed the preceding winter-bud analysis. Bud mortality was highest in V0 (28.13%), followed by V1 (15.63%), V2 (11.25%), V3 (7.50%) and V4 (1.88%). Under commercial conditions, the high proportion of dead buds in the control would have required a compensatory increase in pruning load. The damage was attributed mainly to limited water reserves, lack of nutritional support and the non-irrigated conditions. In the remaining treatments, bud mortality was within an acceptable range. To clarify the causes, a comparable analysis was repeated in autumn 2024 before dormant pruning. Dead buds accounted for 3.13% in V0, 1.88% in V1—identical to the preceding autumn—and 1.25% in V2. Such exogenous changes in compound-bud structure are known to result from excessive vigour, nutrient shortage or shoot crowding (Bindra and Chohan, 1975–1976).

Spring analysis of canes from the experimental treatments in 2025 showed that mechanical changes in compound winter buds had occurred during the late-autumn and winter–spring periods. These changes were influenced by soil and atmospheric moisture and by temperature dynamics during summer 2024 and winter–spring 2025. A wide range of winter-bud mortality was found: V0, 64.38%; V1, 66.56%; V2, 46.88%; V3, 55.00%; V4, 28.75%; V5, 72.50%; and V6, 48.75%. Several factors contributed, particularly sub-zero temperatures in March and April, when tissue hydration—especially within the winter buds—had increased in 2025. Another important factor was the uneven distribution of rainfall. Drought arrested root growth and function, preventing an even supply of water to above-ground organs during autumn 2024. Combined with relative humidity as low as 45%, this induced profound physiological changes as an adaptive response to the prevailing climatic conditions. The chosen dormant-pruning approach and the foliar and root-zone Humat Rost applications showed that V4 was best prepared for overwintering, with 28.75% dead winter buds. Foliar application of Humat Rost at 500 mL da^{-1} positively affected growth and cambial activity, supporting timely cessation of endogenous processes within the winter buds.

In autumn 2025, winter-bud mortality was assessed once again. The analysis confirmed that, in addition to temperature, uneven water availability was a principal cause of bud death.

This provides a basis for practical recommendations concerning pruning load and the number of fruiting units retained. Similar findings were reported by Braykov, Babrikov and Pandeliev (1976–1980), who showed that the degree of bud damage varies among periods and that mortality is induced by disturbances in thermal, water and nutrient regimes during vine fruiting. Trunk height also modifies drought effects: the greater the distance between the above-ground vine organs and the root system, the stronger the adverse effect of drought. Numerous studies have shown that buds within compound winter buds may die both at the cane base and along its length.

Investigation of this problem enabled the method for assessing potential fruitfulness to be refined. Preliminary analysis of the pooled winter-bud sample showed a very low percentage of non-fruitful buds (5.45%). The proportion of buds containing three inflorescences was significantly greatest in the foliar treatments V4 ($86.80^{b} \pm 2.10$) and V3 ($67.53^{b} \pm 9.51$), followed by the root-zone treatment V5 ($68.40^{b} \pm 1.57$). Buds with two inflorescences were most frequent in V0 ($44.63^{d} \pm 5.18$), V1 ($40.07^{bc} \pm 3.00$) and V2 ($40.53^{d} \pm 2.25$). The comparative data in Table 3 also show that inflorescences 550–750 µm long occurred most frequently in V4 ($85.10^{c} \pm 7.74$), V5 ($82.57^{abc} \pm 1.25$) and V6 ($83.40^{bc} \pm 3.61$), followed by V0 ($80.90^{abc} \pm 6.83$) and V3 ($78.50^{abc} \pm 2.00$). Accurate determination of potential fruitfulness and its realisation during the growing season depends greatly on the proportion of viable and dead buds formed during the period of intensive growth.

Differences in the realisation of potential fruitfulness were related to the nutritional effects of the foliar and root-zone doses of the organic product. Realisation also depended strongly on the degree of inflorescence differentiation within the compound winter buds. Examination of the metameric distribution and proportions of the different inflorescence-size groups showed that no inflorescences of 350 µm were recorded; the largest share consisted of inflorescences 550–750 µm long. The pooled data demonstrated significant treatment differences in the potential fruitfulness coefficient: $K = 2.70^{c} \pm 0.10$ in V4, followed by V6 ($K = 2.50^{bc} \pm 0.10$), V5 ($K = 2.40^{b} \pm 0.10$) and V3 ($K = 2.40^{b} \pm 0.10$), compared with $K = 2.00^{a} \pm 0.10$ in V0. V1 and V2 occupied intermediate positions. Inflorescence dimensions provide information on differentiation stage and expected yield. The percentage of fruitful winter buds also differed significantly: V4 had the highest value ($100.00^{c} \pm 0.00$), followed by V3 ($97.27^{bc} \pm 0.45$), V1 ($95.43^{b} \pm 0.40$) and V2 ($93.73^{ab} \pm 1.69$) among the foliar treatments. In the root-zone treatments, V5 ($96.10^{bc} \pm 1.47$) exceeded V6 ($94.27^{ab} \pm 1.15$), while the control had the lowest value ($90.70^{a} \pm 2.65$). Cross-analysis of reproductive activity showed close relationships among these parameters. Operational yield forecasting also required determination of inflorescence length within the winter buds. The differences in fruitful-bud percentage were influenced by temperature and precipitation as well as by the contrasting foliar and root-zone nutritional regimes.

Table 3. Potential bud fruitfulness of Merlot variety

Variants	Dead buds, %	K based on viable buds	K based on all buds	Non-fruitful buds, %	Fruitful buds, %	Fruitful buds with:				Inflorescence length, µm:			
						1	2	3		≤350	350–550	550–750	>750
V_0	0	$2.00^{a}\pm0.10$	$2.00^{a}\pm0.10$	$9.30^{c}\pm2.65$	$90.70^{a}\pm2.65$	$25.07^{a}\pm4.39$	$44.63^{d}\pm5.18$	$30.27^{a}\pm9.55$	0	0	$19.10^{abc}\pm6.83$	$80.90^{abc}\pm6.83$	0
V_1	0	$2.30^{b}\pm0.10$	$2.30^{b}\pm0.10$	$5.97^{bc}\pm1.17$	$93.73^{ab}\pm1.69$	$2.27^{b}\pm3.93$	$40.07^{bc}\pm3.00$	$57.67^{b}\pm1.48$	0	0	$27.60^{bc}\pm1.44$	$72.40^{ab}\pm1.44$	0
V_2	0	$2.37^{b}\pm0.12$	$2.37^{b}\pm0.12$	$4.57^{b}\pm0.40$	$95.43^{b}\pm0.40$	$1.20^{b}\pm2.08$	$40.53^{d}\pm2.25$	$58.27^{b}\pm1.00$	0	0	$29.10^{c}\pm1.01$	$70.90^{a}\pm1.01$	0
V_3	0	2.40b±0.10	$2.40^{b}\pm0.10$	$2.73^{ab}\pm0.45$	$97.27^{bc}\pm0.45$	$6.47^{b}\pm2.10$	$25.97^{b}\pm7.46$	$67.53^{b}\pm9.51$	0	0	$21.50^{abc}\pm2.00$	$78.50^{abc}\pm2.00$	0
V_4	0	$2.70^{c}\pm0.10$	$2.70^{c}\pm0.10$	$0.00^{a}\pm0.00$	$100.00^{c}\pm0.0$	$1.73^{b}\pm1.80$	$11.43^{a}\pm1.48$	$86.80^{b}\pm2.10$	0	0	$14.17^{a}\pm8.14$	$85.10^{c}\pm7.74$	0
V_5	0	$2.40^{b}\pm0.10$	$2.40^{b}\pm0.10$	$3.90^{b}\pm1.47$	$96.10^{bc}\pm1.47$	$3.57^{b}\pm1.95$	$29.27^{bc}\pm1.48$	$68.40^{b}\pm1.57$	0	0	$17.43^{abc}\pm1.25$	$82.57^{abc}\pm1.25$	0
V_6	0	$2.50^{bc}\pm0.10$	$2.50^{bc}\pm0.10$	$5.73^{bc}\pm1.15$	$94.27^{ab}\pm1.15$	$0.60^{b}\pm1.04$	$35.23^{bcd}\pm2.36$	$64.17^{c}\pm3.33$	0	0	$16.60^{ab}\pm3.61$	$83.40^{bc}\pm3.61$	0

8.2. Actual vine fruitfulness

In grape production, crop quantity and quality must be assessed before harvest. The most precise evaluation of actual vine fruitfulness is obtained immediately before harvest (Roychev, 2014). One-way analysis of the percentage of developed buds on spurs showed a clear treatment tendency. The highest value occurred in V4 (96.71 ± 1.52%), followed by V5 (95.12 ± 2.22%), V3 (94.46 ± 3.86%), V6 (94.45 ± 2.02%) and V2 (91.73 ± 4.00%); the remaining two treatments differed only negligibly. Mean values therefore indicated the strongest response in vines receiving foliar or root-zone Humat Rost. V0 had the lowest proportion of developed buds (83.67 ± 9.48%). The percentage of fruitful shoots varied less markedly, from 93.20 ± 1.17% in V6 and 93.04 ± 1.46% in V2 to 92.99 ± 4.11% in V4, 91.73 ± 3.82% in V3, 91.07 ± 3.93% in V5 and 90.11 ± 6.52% in V1. These values suggest a positive effect of organic nutrition on vigour and the realisation of differentiated inflorescences during phase IV of the growing cycle. Radulov (1961) reported that differentiation of some inflorescences within compound winter buds may continue during autumn and winter.

One-way analysis of the fruitfulness coefficient per primary shoot revealed a tendency that was also evident for the coefficient of replacement shoots. In 2024 and 2025, the replacement-shoot coefficient was 0.0 in all treatments, suggesting that high temperatures and inadequate precipitation in the preceding years prevented inflorescence differentiation in replacement buds. Drought reduces the accumulation of assimilates in perennial organs, resulting in incomplete shoot maturation. Vines consequently become more susceptible to freezing, fewer inflorescences are initiated within shoot buds and yield in the following year declines (Todorov, 1988). Actual-fruitfulness data also showed treatment tendencies in the percentages of fruitful shoots bearing one, two or three bunches. Regression analysis indicated relationships with application rate and timing. V4 had the greatest percentage of shoots with two bunches (73.38 ± 4.75%), followed by V3 (71.97 ± 7.00%), V1 (70.96 ± 1.81%) and V2 (69.67 ± 5.13%). The control had the greatest percentage of fruitful shoots bearing one bunch (35.90 ± 7.00%); corresponding values in the root-zone treatments were 34.42 ± 11.50% in V5 and 32.04 ± 7.62% in V6. The highest proportion of fruitful shoots arising from latent buds was recorded in V0 (32.27 ± 17.30%), which also had the lowest mean number of bunches per fruitful shoot (0.80 ± 0.23); a similar tendency occurred only in V3 (0.96 ± 0.07). In the remaining treatments, this coefficient was approximately 1.0 and did not differ. The lowest percentages of water shoots were formed in V4 (6.75 ± 4.60%), V3 (14.62 ± 11.10%) and V6 (15.23 ± 10.58%). These responses were assumed to result from a combination of temperature variation, insufficient soil moisture and the reserve status of perennial vine organs.

9. STUDIES ON GRAPE YIELD AND QUALITY

From bud burst until final berry formation, a series of changes may restrict the crop to different degrees. Data collected at harvest characterise grape yield within the experiment.

Treatment differences in bunch number and dimensions reflected changes occurring during bunch development. Bunch length in 2023 was substantially greater than in 2024 and 2025. In the first year it ranged from 19.83 cm in V5 to 22.19 cm in V4; in the second year it ranged from 11.54 cm in V5 to 14.18 cm in V4. In 2025, bunch length was again greatest in V4 (15.69 cm) and smallest in V5 (12.39 cm). In 2023, bunch width ranged from 8.48 cm in V0 to 10.00 cm in V4. A substantial reduction occurred in 2024, when the root-zone treatments had the narrowest bunches—5.24 cm in V5 and 5.60 cm in V6—and also the shortest bunches. Bunch width increased again in 2025 and exceeded the values from the preceding year. This pattern was influenced not only by the nutritional regime but also by the abiotic environment. Major changes in bunch dimensions begin at budburst and immediately thereafter, when parts of the young inflorescences may alter their morphology. Entire inflorescences may abscise after formation of a separation layer at their base. Another process that reduces bunch number is tendrilisation, whereby some inflorescences are transformed into tendrils and subsequently abscise. During the growing season, one wing of a bunch was occasionally transformed into a tendril while the remainder retained a typical morphology. Other authors have described abscission of some inflorescence primordia during early shoot development, both within the buds and after budburst. One-way analysis of bunch length and width at harvest demonstrated differences in treatment means.

Following foliar Humat Rost application, the largest mean bunch lengths were recorded in V4 (17.35 ± 4.26 cm), V2 (16.89 ± 3.80 cm) and V3 (16.72 ± 4.60 cm). For bunch width, the ranking differed: V2 had the greatest mean width (8.21 ± 1.31 cm), followed by V4 and V3 (both 8.13 ± 1.70 cm). V3 produced the highest mean number of bunches per vine, while differences among the other foliar treatments were small. One-way analysis using the LSD criterion showed differences among treatment means.

In 2023, mean grape yield per vine ranged from 1.54 kg in V5 to 2.43 kg in V3. In 2024, this parameter declined markedly, ranging from 0.46 kg in V6 to 0.80 kg in V3. Water-deficit stress was one of the principal causes. In 2025, yield per vine recovered to approximately 1.59–1.72 kg, with the highest values again occurring in foliar Humat Rost treatments; the more even distribution of rainfall in 2025 contributed to this response. Yield per decare was directly related to mean yield per vine. The highest mean yields per decare were recorded in V4 (542.79 ± 275.95 kg), V3 (529.47 ± 271.78 kg) and V2 (525.03 ± 276.42 kg), closely corresponding to the per-vine yield data. One-way analysis with foliar application rate (200–500 mL/da) as the factor and the LSD criterion showed differences among treatment means.

10. MECHANICAL COMPOSITION OF THE BUNCH

Mechanical analysis enables the gravimetric and percentage relationships among bunch components to be determined, including berries and rachis and, within the berry, skins, mesocarp and seeds (Roychev, 2014). In non-irrigated Merlot subjected to different foliar and root-zone nutritional regimes, analysis of bunch mechanical composition revealed changes in the components that determine the technological properties of the grape crop. The proportion of normal berries was very high in all treatments during all three experimental years.

Bunch composition showed treatment-related changes in rachis percentage. Under high temperatures and insufficient precipitation, this component tended to increase at the expense of the berries. After pooling the data and calculating the standard deviation, skin percentage was highest in V6 (4.85 ± 1.70%), followed by V5 (4.54 ± 0.90%), and lowest in V3 (3.73 ± 0.46%) and V2 (3.97 ± 0.72%); differences among the remaining treatments were small. This effect can be explained by smaller berry size caused by low atmospheric and soil moisture combined with high temperatures. During the second experimental year, phase IV ended atypically because of a climatic anomaly and abnormal berry development. Across the three years, the percentage of rotten berries was negligible, ranging from 0.02 ± 0.04% in V2 to 0.40 ± 0.69% in V3. It was highest in 2023 in V5 and V6, but remained below 1% and was more likely attributable to mechanical injury by insects before harvest. No millerandaged berries were recorded, although Merlot is prone to coulure and millerandage.

The proportions and ratio of seeds to skins in all treatments were above the values typical of Merlot. The pooled analysis showed the highest proportion of shrivelled berries in V6 (20.13 ± 11.10%) and the lowest in V0 (15.07 ± 4.24%). This was attributable to high temperatures and insufficient rainfall during ripening and was also supported by the mean-bunch-mass data. Berry ripening is accompanied by an excess of water delivered through the phloem (Keller, 2015), and berry transpiration is thought to facilitate normal ripening by removing surplus water.

The higher skin percentage was caused principally by persistent water shortage in the surface soil horizon during the growing season, compounded by low atmospheric humidity during summer. Together, these factors imposed sustained combined water-deficit stress. Seed percentage differed by as much as approximately fourfold among treatments, ranging from 12.79 ± 10.11% in V6 to 7.87 ± 2.44% in V1, compared with 3.84% reported by Radulov (1992). The higher percentage reflected a greater number of normally developed seeds and a lower proportion of mesocarp, which ranged from 76.07 ± 6.66% in V1 to 67.09 ± 21.12% in V6.

One-way analysis with treatment—foliar rates of 200–500 mL da^{-1} and root-zone rates of 15–25 kg da^{-1}—as the factor and the LSD criterion showed differences among treatment means.

Table 4. Mean values for grape yield and mechanical-composition parameters

Variant	Yield per vine, kg	Yield per decare, kg	Bunch mass, g	Mass of 100 berries, g
V0	1.05±0.51	348.54±168.23	83.19±7.48	83.01±14.37
V1	1.47±0.71	490.62±234.81	90.97±7.49	87.03±14.31
V2	1.58±0.83	525.03±276.42	118.44±16.83	99.31±21.95
V3	1.59±0.82	529.47±271.78	92.60±29.15	100.72±14.63
V4	1.63±0.83	542.79±275.95	88.94±36.29	91.93±20.32
V5	1.05±0.55	350.76±182.43	78.49±18.24	101.97±15.80
V6	1.11±0.65	369.63±214.80	80.58±37.14	90.21±24.93

11. STUDIES ON GRAPE-QUALITY PARAMETERS AND PHYSICOCHEMICAL ANALYSIS OF THE WINES

Substantial differences in grape-sample mass were observed among treatments and vintages. Although all treatments contained the same number of vines and carried the same pruning load, sample mass differed both among treatments and among years.

Yield was lowest in all treatments in 2024 and highest in 2023. These differences were probably attributable to the unfavourable climatic conditions of 2024, particularly the exceptionally hot and dry summer. Grape-sample mass was generally lowest in the control V0, with the exception of 2024, and highest in V4, V3 and V2 in every year. Differences between V0 and the root-zone treatments V5 and V6 were small, whereas differences between the control and the foliar treatments V1–V4 were substantial. Foliar application therefore had a pronounced effect on grape yield.

Sugar content and titratable acidity were very similar among treatments despite substantial differences in yield. Although apparently unusual, this was probably due to the comparatively low yield in all treatments and vintages. Lower yields are generally associated with a higher degree of grape maturity. Grapes from all treatments ripened at the same time and differed only slightly in sugar and total-acid content. Thus, neither foliar nor root-zone treatment appeared to affect sugar or acid synthesis.

Yeast-assimilable nitrogen (YAN) differed between the control and the treated variants in all three experimental years and was strongly influenced by vintage conditions. Grapes from the 2024 vintage contained more than twice as much YAN as those from 2023 and 2025. A consistent distinction was observed between the control and the treated variants: in all three years, treated grapes contained more YAN than the control, except for V2 in 2023 and 2024. Root-zone fertilisation in V5 and V6 had a stronger effect than foliar fertilisation in V1–V4.

YAN concentrations in V5 and V6 were 16–33% greater than in the control over the three-year study.

The YAN concentration of grapes is technologically important. At concentrations below 150 mg dm^{-3} of must, alcoholic fermentation slows and may stop before all grape sugars have fermented. This is because YAN availability strongly affects yeast multiplication, microbial biomass accumulation and fermentation rate. Numerous studies have shown that nitrogen deficiency in the vine canopy during the growing season alters biosynthetic pathways and promotes the accumulation of atypical grape metabolites such as indole, skatole and o-aminoacetophenone, which may substantially impair the aroma and flavour of the resulting wine.

Treatment of grapevines with Humat Rost can increase grape YAN concentration and thereby create favourable conditions for complete and trouble-free alcoholic fermentation.

Analysis of the experimental grapes showed higher anthocyanin concentrations in the treated variants than in the control. Grape anthocyanin content was strongly affected by vintage. It was lowest in 2024 and highest in 2023. The lower concentration in berry skins in 2025, and particularly in 2024, was attributable to exceptionally high summer temperatures. Anthocyanin synthesis in grapevines ceases above approximately 33 °C, and degradation may occur at still higher temperatures.

Anthocyanin content was 20–30% higher in the treated variants than in the control and was greatest in V4, V5 and V6. The larger pigment reserve in these treatments favoured wines with a more intense, saturated and vivid red colour.

The measured values were within the optimum ranges for high-quality red wines. Alcohol content was virtually identical among treatments, consistent with the very similar grape sugar concentrations. The same applied to titratable acidity and pH. Treatment with Humat Rost therefore did not affect the sugar-derived alcohol content or the acid composition of the wines.

Residual sugars ranged from 2 to 4 g/dm^3, indicating complete alcoholic fermentation. Despite the small differences, wines from V5 and V6 had the lowest residual-sugar concentrations in all three years. This was probably related to the higher YAN concentrations in grapes from these treatments. As noted above, greater YAN availability favours yeast proliferation and biomass accumulation and therefore promotes complete fermentation of grape sugars.

Volatile acidity was within the optimum range for high-quality wines, 0.4–0.6 g/dm^3, in all wines and all three years. This indicated a clean alcoholic fermentation without the development of undesirable microorganisms or bacterial infection.

Total dry extract and sugar-free extract were within the normal range of 25–30 g/dm^3. Differences in sugar-free extract among treatments were small. In most cases, the control wines had the highest sugar-free extract. This was not unexpected because extract content is strongly influenced by yield; the control generally produced the lowest yield and its wines would therefore be expected to contain more sugar-free extract. Values varied widely with vintage. All treatments produced the highest sugar-free extract in 2024, when the very hot and dry summer substantially reduced grape yield and consequently increased extract concentration in the wines.

No direct relationship was established between Humat Rost treatment and the extract content of the wines.

Wine anthocyanin content varied among vintages. It was lowest in 2024, whereas the 2023 and 2025 wines had almost identical concentrations. This pattern was explained by the exceptionally high summer temperatures in 2024: anthocyanin synthesis ceases above approximately 33 °C and degradation occurs at still higher temperatures. Anthocyanin content also varied among experimental wines. Treated variants generally contained more anthocyanins than the control, with the highest values in V4, V5 and V6 in the 2024 and 2025 vintages.

In 2023, no substantial differences in anthocyanin concentration were detected between the experimental wines and the control, despite the markedly higher berry-skin anthocyanin content in V4, V5 and V6. This apparent discrepancy probably resulted from a proportion of the anthocyanins in the treated variants being present in condensed forms, which cannot be quantified by the bisulphite method used. The higher colour intensity of the treated wines, particularly V6, supports this interpretation.

Colour hue and the percentage contributions of yellow-brown, red and blue-violet were within the normal ranges for young red wines. Wines from V6 had the lowest hue in all three years. This parameter expresses the ratio of yellow-brown to red pigments in wine colour. A lower hue indicates a higher concentration of colouring matter, principally anthocyanins, and favours a more intense, saturated and vivid red colour.

Total phenolic compounds were within the normal range of 2,600–3,300 mg/dm³. This parameter is substantially influenced by grape yield and vinification conditions, including temperature and the degree of homogenisation between the solid and liquid phases of the grape mash. The higher total-phenolic content of V5, V6 and the control in the 2024 vintage may be explained by the substantially lower grape yields in these treatments.

Table 5. Analysis of grape mash

Treatment	***Vintage***	***Sample mass, kg***	***Sugars, %***	***Titratable acidity, g/dm³***	***pH***	***Yeast-assimilable nitrogen, mg/dm³***	***Skin anthocyanins, mg/kg¹ berries***
V_0	*2023*	*46.7*	*23.05*	*5.82*	*3.58*	*115.3*	*882.0*
	2024	*27.3*	*25.37*	*5.56*	*3.37*	*261.55*	*618.63*
	2025	*41.0*	*25.02*	*5.74*	*3.45*	*131.26*	*1003.63*
V_1	*2023*	*65.1*	*22.75*	*5.87*	*3.55*	*146.59*	*987.0*
	2024	*38.0*	*25.63*	*6.92*	*3.38*	*321.91*	*628.25*
	2025	*59.7*	*25.02*	*5.78*	*3.44*	*136.25*	*915.25*
V_2	*2023*	*72.0*	*22.65*	*5.6*	*3.51*	*113.65*	*622.13*
	2024	*37.0*	*25.59*	*6.96*	*3.38*	*234.73*	*673.75*
	2025	*63.65*	*25.05*	*5.74*	*3.49*	*142.24*	*777.88*
V_3	*2023*	*72.8*	*22.6*	*5.87*	*3.51*	*143.3*	*892.5*
	2024	*40.0*	*25.86*	*6.74*	*3.44*	*301.8*	*668.5*
	2025	*61.7*	*25.8*	*5.90*	*3.47*	*140.74*	*737.63*
V_4	*2023*	*72.3*	*22.05*	*5.95*	*3.56*	*143.3*	*1101.63*
	2024	*38.0*	*25.01*	*6.74*	*3.41*	*307.7*	*716.63*
	2025	*68.4*	*25.65*	*5.86*	*3.45*	*153.32*	*772.63*
V_5	*2023*	*46.2*	*23.05*	*5.56*	*3.7*	*149.87*	*982.63*

Treatment	Vintage	Sample mass, kg	Sugars, %	Titratable acidity, g/dm³	pH	Yeast-assimilable nitrogen, mg/dm³	Skin anthocyanins, mg/kg¹ berries
	2024	22.8	25.21	6.29	3.47	311.0	714.88
	2025	46.5	25.65	5.86	3.45	127.27	784.88
V_6	2023	52.9	23.0	5.51	3.73	182.83	1145.38
	2024	23.8	24.67	6.78	3.44	348.0	789.25
	2025	43.5	25.27	5.86	3.44	152.72	619.5

12. SENSORY EVALUATION OF THE WINES

The wines were assessed blind by an expert tasting panel (Figure 1).

Wine-quality scores varied both with treatment and with vintage. The 2025 wines received the highest scores in both the control and experimental treatments, whereas the 2024 wines received the lowest. The tasting panel ranked the control and experimental wines differently in each vintage. In 2023, V5, V6 and V4 received the highest scores; V2 and V3 had identical scores and were rated even lower than the control. In 2024 and 2025, V4 and V3 were rated highest, followed by V6 and V5.

The sensory scores of V1 and V2 were close to those of the control in all three years. The Humat Rost doses used in these treatments therefore probably had no substantial effect on wine quality. The panel showed a clear preference for V4 and V3 in the 2024 and 2025 vintages, whereas V5, V6 and V4 were rated highest in 2023.

The sensory profiles of the experimental wines are presented in the radar charts (Figures 2–7). Wines were scored from 1 to 10 for colour intensity, aroma intensity, fruity aroma, aroma finesse, flavour intensity, body, harmony or balance, flavour persistence and aftertaste.

In the 2023 vintage, the root-zone treatments V5 and V6 were rated higher than the control for every sensory attribute, consistent with their highest overall scores. Among the foliar treatments, V2 received the same scores as V0 for colour intensity and aroma intensity and exceeded the control only in flavour harmony.

For all other attributes, V2 was rated below the control. V1 was preferred for aroma intensity, fruitiness, aroma finesse, flavour persistence and aftertaste, but received the same scores as the control for all remaining attributes.

V4 received the highest scores for colour intensity, aroma intensity and flavour intensity, whereas V3 was preferred for flavour harmony.

In the 2024 vintage, the root-zone treatment V6 was rated above the control for every sensory attribute. V5 received the same scores as the control for colour intensity, aroma intensity, fruitiness and body, but higher scores for aroma finesse, flavour intensity, flavour harmony, flavour persistence and aftertaste. Among the foliar treatments, V1 and V2 again tended to be rated similarly to the control and were lower for some attributes, including colour and flavour intensity. V4 received the highest scores for all attributes; for fruitiness, aroma finesse and body, its scores were similar to those of V3.

The 2025 experimental wines confirmed the tendencies observed in 2024. The root-zone treatments V5 and V6 exceeded the control for all attributes. Differences between them were small: V5 was rated higher for colour intensity, fruitiness and flavour harmony, whereas V6 was rated higher for aroma finesse, body and flavour intensity. The foliar treatments also confirmed the patterns from 2023 and 2024. V4 and V3 received the highest ratings for all attributes, with only minor differences between them.

V4 was rated higher for colour intensity, body, flavour persistence and aftertaste, whereas V3 had greater fruity-aroma expression. V1 and V2 were close to the control in sensory profile and were rated lower than it for some attributes.

Humat Rost treatment influenced the sensory characteristics and overall tasting scores of the wines. V4 and V3 received the highest ratings, followed by V5 and V6. V1 and V2 received scores similar to, and in some cases lower than, those of the control. This was probably due to the lower treatment doses, which did not produce a consistent effect on sensory quality.

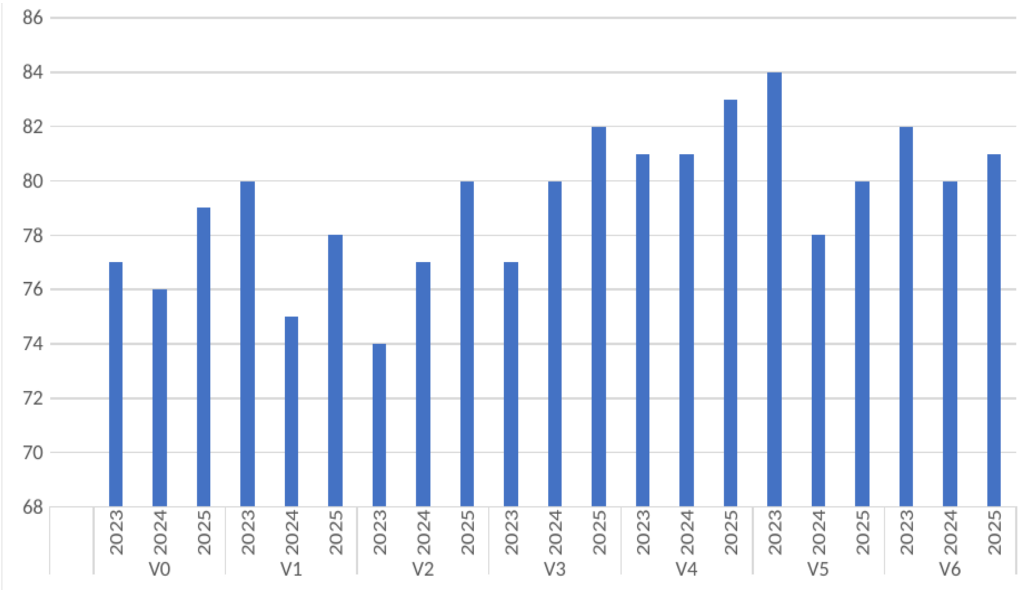

Figure 1. Overall sensory score of the wines.

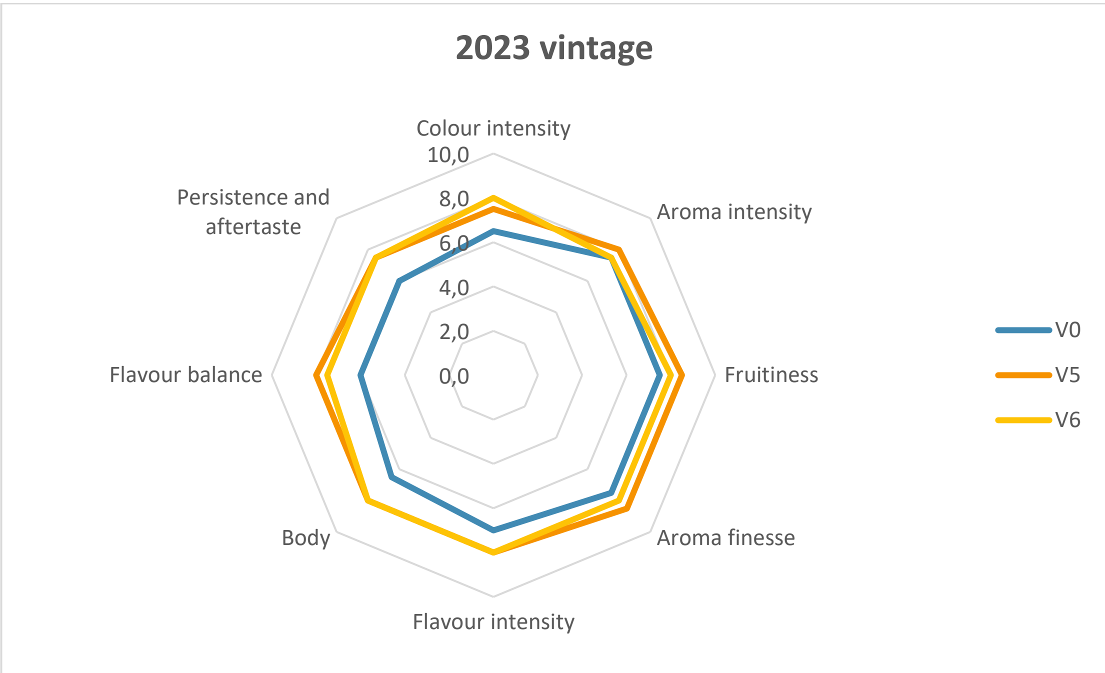

Figure 2. Sensory profiles of the wines.

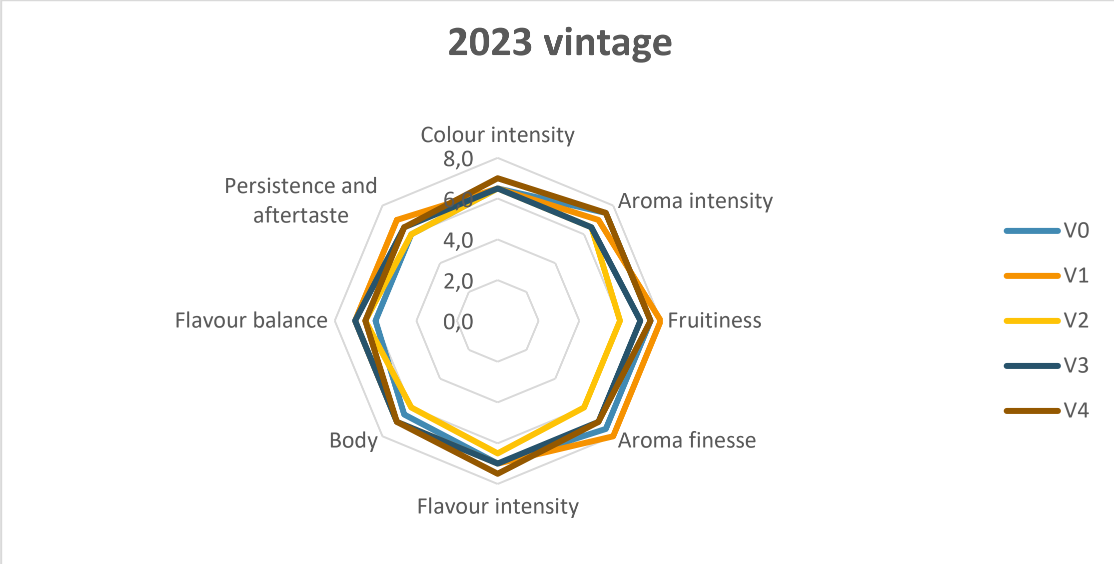

Figure 3. Sensory profiles of the wines.

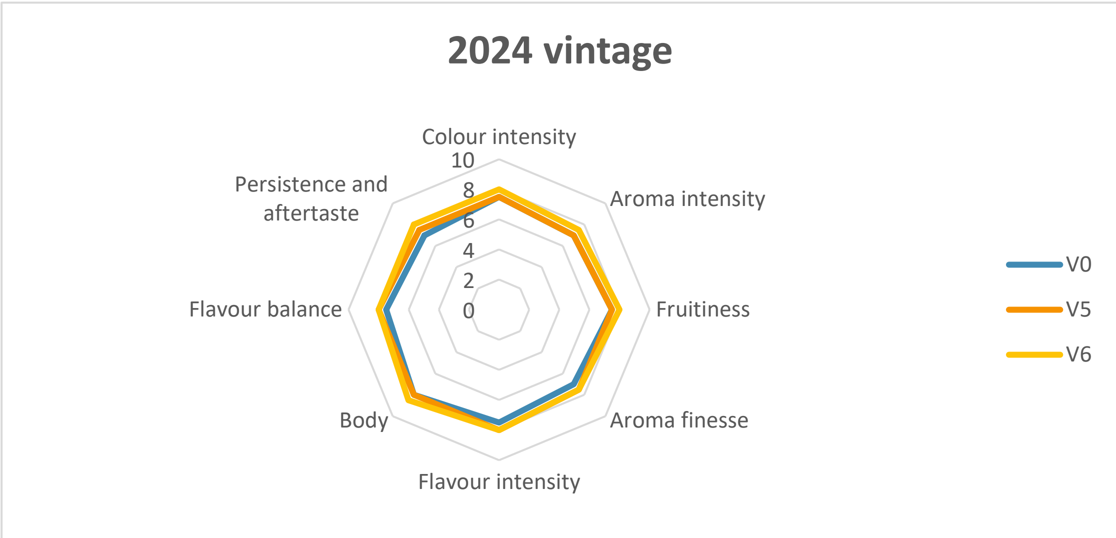

Figure 4. Sensory profiles of the wines.

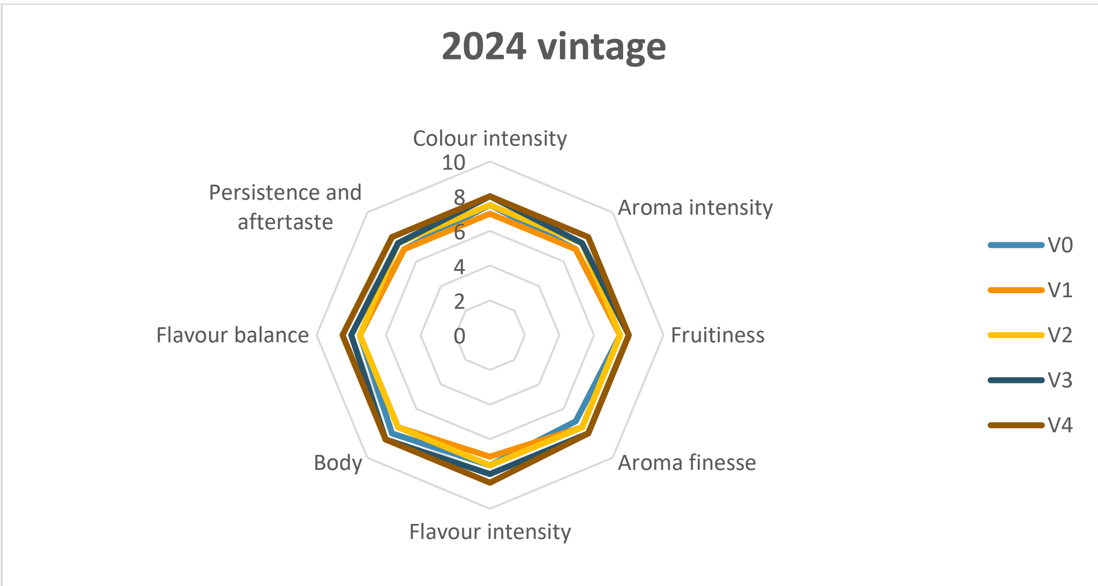

Figure 5. Sensory profiles of the wines.

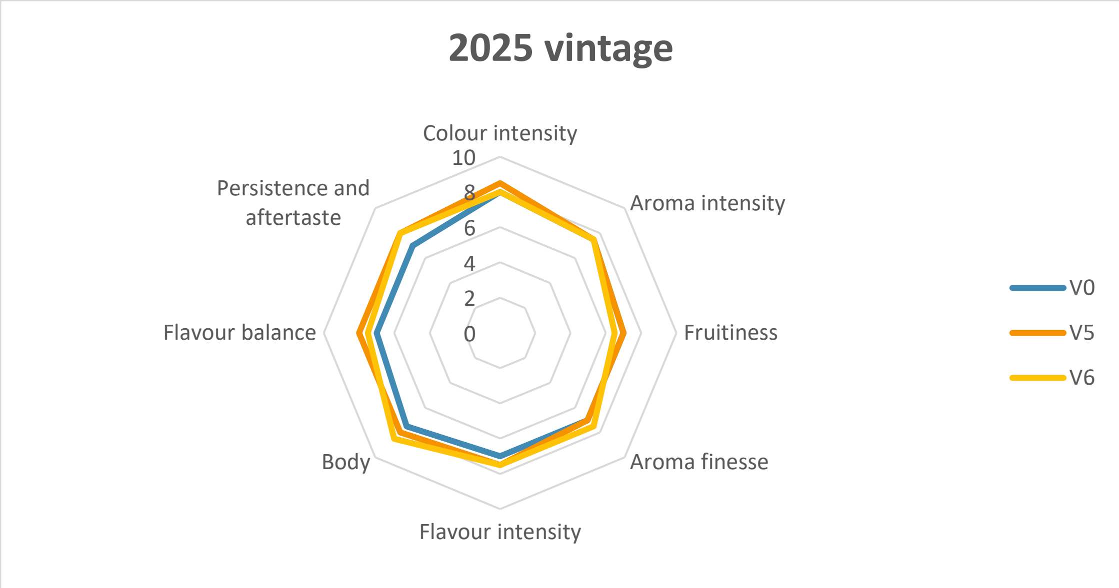

Figure 6. Sensory profiles of the wines.

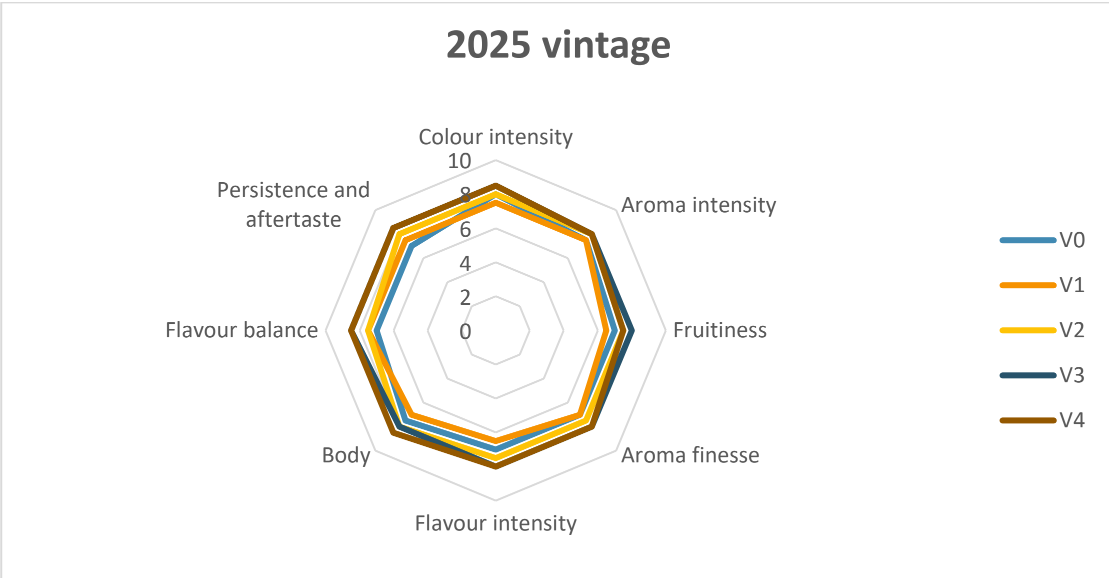

Figure 7. Sensory profiles of the wines.

13. CONCLUSIONS

1. Phenological observations showed only small temporal variation in the onset and duration of individual phenophases, including both the interval from first leaf to flowering and that from veraison to technological maturity. One-way analysis detected no statistically significant treatment differences in phenophase duration.

2. Pronounced degradation of chlorophyll structures occurred in the leaf apparatus and resulted in a substantial decline in photosynthetic activity. At technological ripeness, the reduction in photosynthetic rate was determined primarily by intensified translocation of photoassimilates towards the reproductive organs of the vine.

3. Shoot growth varied clearly with application rate. The greatest final shoot lengths were recorded in V3 (165 cm) and V2 (160 cm), followed by V1 (150 cm), V4 (145 cm) and V0 (140 cm). The root-zone treatments V5 and V6 produced substantially less growth (120 cm), probably owing to more restricted and moderate growth governed by the nutritional regime and an uneven water balance.

4. Drought increased competition for water between the berries and the maturing shoots. Intensification of hydrolytic reactions brought shoot growth to an end and created difficulties in achieving adequate phenolic maturity of grapes intended for winemaking.

5. Application of the organic product Humat Rost favourably affected the accumulation of soluble sugars in grapevine canes. Mathematical analysis identified the highest concentrations in V1 and V6, while the remaining treatments contained substantially less sugar. The treatment therefore exerted a clear stimulatory effect on vine carbohydrate status.

6. One-way analysis of bunch length and width demonstrated treatment differences. The greatest mean bunch lengths were recorded in V4 (17.35 ± 4.26 cm), V2 (16.89 ± 3.80 cm) and V3 (16.72 ± 4.60 cm). The ranking for width differed, with the highest mean in V2 (8.21 ± 1.31 cm), followed by V4 and V3 (both 8.13 ± 1.70 cm).

7. The higher proportion of skins was caused primarily by persistent water deficit in the surface soil horizon during the growing season. Differences of up to approximately fourfold reflected anatomical and structural changes in the bunch induced by prolonged combined stress from water deficit and high temperatures.

8. Yield per decare was closely related to mean yield per vine, with the highest values in V4, V3 and V2. One-way ANOVA followed by the LSD test demonstrated statistically significant differences among treatments, confirming the effect of foliar application rate on vine productivity.

9. The overall dynamics of microbial metabolic activity retained a characteristic sigmoidal form despite the low optical-density values. This confirms that, under comparable soil-treatment conditions and in the absence of inhibitory factors, soil microorganisms developed according to the pattern typical of Biolog EcoPlates.

10. Most functional indices indicated greater functional diversity and a more even distribution of microbial activity in the Humat Rost treatments. Uneven utilisation of substrate groups and variability among EcoPlate replicates resulted in high standard deviations and standard errors.

11. Humat Rost treatment did not affect grape sugar content or total acidity and consequently did not alter wine alcohol content, total acidity or pH. It did, however, increase grape yeast-assimilable nitrogen, particularly in V4, V5 and V6.

12. Humat Rost treatment stimulated the accumulation of colouring matter, principally anthocyanins, in grapes and wines and increased colour intensity and brightness.

Scientific contributions

1. The lower soil-applied dose of Humat Rost used in V5 was shown to increase the functional diversity and metabolic activity of soil microbial communities, as reflected in higher functional-index values summarised by multivariate analysis of variance (MANOVA).

2. Amino-acid utilisation by microbial communities was stimulated in the treated variants compared with the control V0. The higher metabolic activity of V5, particularly after 96 h, demonstrated more efficient amino-acid utilisation and indicated enhanced functional potential of the microbial community.

3. Novel and significant relationships were established among foliar Humat Rost treatments, vintage conditions and the sensory characteristics of the resulting wines. Sensory scores varied substantially with both vintage year and treatment dose.

Scientific and applied contributions

1. Root-zone application at 25 kg/da[1] maintained a consistently higher rate of amine utilisation than the other treatments throughout the three-year study, revealing a specific metabolic response of the soil microbiota to this treatment.

2. The results expand scientific knowledge concerning the use of organic foliar fertilisers such as Humat Rost in viticulture. A dose-dependent treatment response was demonstrated, together with a substantial influence of climatic conditions on the variability of shoot elongation and maturation in Merlot grapevines.

3. Practical guidelines were developed for improving winter-bud viability through optimisation of sampling time and organic fertilisation, thereby increasing tolerance to late spring cold events and preserving the reproductive potential of the vine.

4. Regression analysis revealed substantial relationships among nutritional regimes, application timing and the formation of grapevine reproductive and vegetative organs.

14. PUBLICATIONS RELATED TO THE DISSERTATION

Kozarov, H. (2025). Disruptions Caused by Abiotic Factors Affecting Inflorescence Development During the Short Annual Growth Cycle of the Grapevine. Journal of Mountain Agriculture on the Balkan.