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CHANGES IN THE PHENOLOGICAL AND TEMPERATURE REQUIREMENTS DURING CULTIVATION OF THE RUBIN VARIETY IN THE SOUTHERN WINE-GROWING REGION OF BULGARIA

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Abstract

This study aims to characterize the phenology and the corresponding temperature requirements of the Rubin variety grown on SO^4 rootstock. The experiment was conducted in a private vineyard under the supervision of viticulture researchers` team. The assessments of the phenology and vines temperature requirements were conducted from pruning to harvest. The climate in the studied area is transitional continental, with a slight mountain influence and a large amplitude in seasonal temperatures. In 2022, the average rainfall was approximately 572.4 mm, and in comparison, to the previous year - 755.4 mm. The total difference accounts for 183 mm with the highest precipitation in June – 103.8 mm. Such variations in precipitation significantly affect the vine growth and productivity. The study results showed the possibilities of obtaining a sufficient yield ranging from 759.24 kg to 865.80 kg per 0.1 ha due to larger individual mass of clusters - 173 and 179 g, for V_1 and V_2, respectively. Furthermore, the application of canopy technique can be useful in the production of wine grapes, especially in uneven rainfall distribution or in non-irrigated area during the vine vegetation period.

Keywords: viticulture, Rubin, climate, phenology, region, terroir

INTRODUCTION

In recent years, both in Europe and on a global scale, wine production has increased significantly (Yordanova–Dinova & Yordanov, 2020). The global consumption was estimated at 241 million hL (OIV, 2023), and in Bulgaria, it reaches one million hL per year. The area of planted vineyards in Bulgaria is around 67 000 ha, of which over 16 000 are new established vineyard areas. Annually in the country are produced 160 000 tons of wine grapes, and 1 300 000 hl wine (MZH, 2018). Climatic factors play an essential role in the development of vine plants (Wahl, 1988) and a data collected from different geographical areas revealed the relationship between grape quality, temperature and precipitation (Negrula, 1946). The global warming has caused many disturbances in viticulture ecosystems and the grape varieties are forced to shift their annual cycle of vegetation which negatively affects quality and quantity of grape and as a result the quality of produced wines deteriorate (Tănase et al., 2019). The negative effects of climate change on vineyards include the premature ripening of grapes, loss of acidity, and faster accumulation of sugar. Furthermore, the harvesting period is scheduled earlier than the usual one for the particular geographic area. In the Northern Hemisphere, this means that harvesting is done in August or September instead of October, which leads to dehydrated grapes and a decrease in yield (Cook & Wolkovich, 2016). The impact of water deficit on vine productivity and grain quality depends not only on the duration of the drought, but also on the plant phenological stage when drought occurs (Matthews & Anderson, 1988). Previous studies have reported that the water stress in the early stages of vines vegetation, either on the field or in pots experiments, has stronger negative effects on the vegetative growth, grain size and yield in comparison to the effect of the water stress that occurs later in the growing season (Ojeda et al., 1999; Chaves et al., 2007; Girona et al., 2009; Chaves et al., 2010; Basile et al., 2011; Munitz et al., 2016). The sensitivity of grains to water stress is physiologically justified by the link between roots and fruits through xylem vessels (Greenspan et al., 1994; Rogiers et al., 2001, Chaves et al., 2010; Munitz et al., 2016). The changes in precipitation and temperatures due to climate change will make the careful selection of vine rootstocks mandatory. The selection of suitable rootstocks would make vine able to grown in the terroirs that are typical for given varieties but after the change in the rainfall have fallen into the marginal cultivation zone (Willwerth et al., 2010). In general, an increasing number of grape varieties typical of northern areas are being adopted in viticulture due to the expectation that many traditional wine regions will cease to be suitable for wine cultivation (Mozell & Thach, 2014). Since the duration of phenological stages, the amount and number of shoots, and leaf area depend on the rootstocks, thus the vine vigor, fruit yield, and quality will be also affected (Koundouras et al., 2008; Soar et al., 2006). The relationship between climate variability, grape yield and vines has been studied extensively, but significant climate changes in the recent years require updated collection of data about the grape regions and the effects of vine management practices (Kliewer & Dokkozlian, 2005; Sipiora, 2009). Among the practices that can trigger differences in grape development and wine composition are two very common approaches such as winter pruning and reducing the clusters (King et al., 2015). Estimating the influence of the yield of grapes and the quality of wines, will be intertwined with the influence of variety`s characteristics, properties of the rootstock and the complex influence of climate (Lanyon et al., 2004; White et al., 2007; Leeuwen & Rességuier, 2018; White, 2020). Maintaining yield quantity, meeting quality standards and preserving the vine specific sensory characteristics are among the main challenges for viticulture production worldwide due to increasingly competitive market associated with globalization (Fraga et al., 2020). The viticulture production faced adaptation challenges since the late 19th century. The frequency of heat waves also has a negative impact on vine yield and the reduction in yield could reach 35% (Fraga et al., 2020). However, there are not sufficient data about the dependence of grape`s sugar content and temperature conditions during cultivation. Davitaia (1946) reported that for every 100 meters above sea level, a reduction of 0.65°C in the average temperature results in a decrease in grape sugar content by 0.8 to 1.0%. Despite that the high temperatures can shortened the length of phenological phases during the ripening of the grapes, they can cause accumulation of excess carbohydrates. This results in an undesirably high alcohol content in wines which do not correspond with the current consumer preferences and market demands. In addition, the higher temperatures affect the variety` s properties and ripening of the grapes, causing a discrepancy in sugar and acid contend, and also in the synthesis and ratio of the secondary metabolites. These components are responsible for the formation of color and aroma and changes in their content and composition affect the quality of wine.

Application of canopy management in wine grape production is one of the modern methods which aims to affect the growth and productive qualities of vine. Canopy management is a term that includes manipulations of shoots, foliage and clusters of the vine plant towards optimal microenvironment (Merzhanian, 1953; Uinkler, 1966; Reynier, 2000; Babrikov et al., 2000; Manis & Pandeliev, 2002). This technique delayed the bud burst till spring in order to prevent damages caused by frost and in turn this reduces the economic losses without necessity of additional measures (Popova & Angelov, 2022). The microclimate, achieved by the canopy management, allows for an optimal biosynthesis management and modelling of chemical composition of grapes. For example, with cluster thinning fruiting of the vine plant can be controlled and, thus to regulate grape quality (Popova & Angelov, 2023a). When vines are grown under non-irrigated conditions, their leaf temperature can be maintained at 4-8°C higher (Angelov et al., 2010). Popova & Angelov (2023b) found out that the production of the Syrah variety is based, to the greatest extent, on the shoots with two clusters. In relation to the ripening and quality of grapes, it was reported that the qualitative characteristics of grapes are explicitly dependent on sugars and organic acids content (Branas, 1978; Bravdo et al, 1984; Allen & Lacey, 1993).

Current study aims to characterize the phenology and the corresponding temperature requirements of the Rubin variety grown on SO^4 rootstock. The information obtained from the study could be used for establishment for a set of parameters comprised of the most important indicators determining the individual severity of the vines in the Southern wine-growing region of Bulgaria.

MATERIALS AND METHODS

The Rubin variety was used as the subject of the study. Rubin is a red wine variety created in 1944 by crossing Nebbiolo x Syrah varieties. The clusters are small in shape and weight. The berry is medium-sized, the skin is tough with dark blue color. The used in the study vineyard is located in vicinity of Kuklen village, Plovdiv municipality, Bulgaria (latitude 42.0341° N and longitude 24.7876°). The vines were formed on the stem Guyot trellis. The pruning severity of the vines was made by 2 spurs with two buds each and one cane with 12 buds. The experiment was conducted under non-irrigated conditions. The planting distances between the rows and between vines in each row were 3 and 1 m, respectively. Each variant was comprised of 30 vines.

The experiment was set in three variants: V_0 – control – only stem thinning without cluster regulation. The other two variants were subjected to shoots thinning on the stem and arms and clusters regulation in berry growth keeping 8 and 14 clusters per vine for variant V_1 and V_2, respectively.

The parameters monitored included rainfall (mm) and temperature regime (℃). Dynamic during the growth processes of the shoots and phenological observations during the vegetation of the vine. Measurements were done on yield and quality of grapes, cluster average weight (g), grape yield per vine (kg), grape yield per 0.1 ha (kg). Sugar content was measured with Dujardin mustimeter (%) and titratable acids by titration with 0.1 n NaOH, (g/dm^3) as described by Roychev et al. (2014).

RESULTS AND DISCUSSION

The monthly air temperature data was taken from the meteorological station in the vicinity of Kuklen village. The annual average air temperature for 2021 was 13.6 ºC, with the amplitude of 22.3 ºC. This value was obtained by subtracting the temperature of the coldest month, January 4.0 ºC from the temperature 26.3 ºC of the warmest month July, 2021 (Table 1).

Table 1. Average monthly air temperature, ºC.

Place	Year	Month												Average
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Kuklen	2021	4.0	6.1	6.7	11.0	18.1	21.7	26.3	25.9	19.4	11.5	8.3	4.8	13.6
	2022	3.9	5.8	5.7	13.0	18.4	22.7	25.4	25.1	19.4	15.1	10.5	6.1	14.3

In 2022, the average monthly air temperature was 14.3 ºC, with annual temperature amplitude of 21.5 ºC. The main source of water for growing vineyards under non-irrigated conditions is the rainfall. The average annual amount of precipitation in the area of the vineyard in 2021 was 755.4 mm. The largest amount of precipitation was recorded in October – 182.6 mm (Table 2).

Table 2. Monthly amounts of precipitation (mm)

Place	Year	Month												Average
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Kuklen	2021	98.2	26.6	39.8	77.6	55.6	49.6	22.8	27.4	12.0	182.6	26.8	135.4	755.4
	2022	84.2	50.8	45.0	68.8	33.2	103.8	47.6	42.0	31.2	1.4	40.2	24.2	572.4

In 2022, the average annual precipitation for the area was approximately 572.4 mm, with difference of 183 mm when compared to the previous year. The highest precipitation was recorded in June – 103.8 mm (Table 2). The amount of precipitation in the first half of the summer is necessary for the grape culture, since the stock of winter-spring precipitation is already exhausted to a large extent and the June-July period coincides with the IV phase of grain growth. The additional watering is necessary due to formation of multiple cells in the grape and grape juice accumulation. In the second half of the growing season, the amount of precipitation was not uniform and this demanded an earlier harvest due to uneven ripening of the grapes. In the early ripening varieties, this deficiency can lead to formation of berries but without the necessary phenolic maturity. Furthermore, the vineyard area is characterized by a short-lasting and relatively scarce snow cover. For its normal development the vine requires moisture no less than 70% of soil water holding capacity in the first one meter of the soil horizon and the atmospheric humidity no less than 60%. In Bulgaria these parameters fluctuate during the growing season and affect the vine physiology despite that vine is a drought-resistant mesophyte plant with a deep root system.

The specific cluster severity showed influence on the duration of the phenophases in the studied variants. The data from the phenological observations (Table 3) revealed that the phases proceeded each other relatively quickly since the budburst in the Rubin variety began in the second ten days of April. The duration of budburst was around 9 days, and flowering – 10 days. The quicker flowering was due to the favorable climatic conditions in 2021 but not in the second year of the experiment since the budburst was around 10 days, and further 13 days was necessary for the flowering phase. During the second year of the experiment the temperatures at the beginning of the growing season were lower and the rainfall was greater in volume which slowed down the buds` development. However, the vine showed an ability to compensate its phenophases for a particular period of time. Berry growing phase began during the first ten days of July and the softening of the berry took approximately 13 days in August 2021 and 10 days in August 2022. The grape harvest was carried out during the first ten days of September, regardless of the fact that in the second half of the summer there was a great dynamic in the precipitation. The results obtained from the phenological observations imply that the pruning system and the cluster severity do not have a significant impact on the timing of occurrence and the duration of individual phenophases in the studied variety. The differences in the phenological phases are mainly related to the temperature dynamics, the water supply and precipitation.

The different cluster severity led to significant changes in the speed of vine growth. The data presented in Figure 1 and Figure 2 shows that cluster severity in the phase of berry growth in V_1 and V_2 resulted in the formation of a larger average cluster weight of 173 and 179 g, respectively (data collected from 2021 and 2022 year). The experiment aimed to present the yield potential of the Rubin variety under non-irrigated conditions in the Southern vine-growing region. With evenly distributed rainfall in the Pleven region, the Rubin variety could form a yield of about 11.500 kg per hectare with a double Guyot stem training system (Radulov et al., 1985). It should be emphasized that irrigation by itself maintains the soil in turgor, but the quantity and quality of the yield are determined by the presence of atmospheric humidity, which depends on the amount of rainfall in the studied area. Every year, the optimal time for red grapes harvesting is a critical issue for farmers and winemakers, as the quality and organoleptic characteristics of the produced red wines, largely depend on the proper chosen time for harvest. When the fruits ripen, the synthesized phenolic compounds in the cells of the grape skin move from the seeds to the pulp and finally to the skin. The phenolic composition of the seeds during ripening was tracked using voltammetric techniques. These techniques allow detection of changes in the intensities and shifts in the positions of the peaks corresponding to the oxidation or reduction of phenols. Furthermore, the electrochemical responses were used to test the discriminatory ability of the sensor array using principal component analysis, while partial least squares regression (PLS) established good correlation between the electrochemical responses and the phenolic content of seed extracts with coefficients ranging from 0.93 to 0.99. The methodology provides an important monitoring of changes in phenolic composition during seeds ripening (Garcia-Hernandez et al., 2025).

Table 3. Phenological phases of development of the Rubin variety in the region of Kuklen, Bulgaria for the period 2021-2022

Variants	**Year**	**Budbreak**			**Appearance of the first leaf**	**Appearance of the first inflorescence**	**Flowering**		
		start	**mass**	**end**	**date**	**date**	**start**	**mass**	**end**
V_0	2021	18.04	23.04	27.04	30.04	03.05	09.06	13.06	18.06
	2022	20.04	26.04	30.04	02.05	05.05	07.06	15.06	19.06
V_1	2021	18.04	23.04	27.04	30.04	03.05	09.06	13.06	18.06
	2022	20.04	26.04	30.04	02.05	05.05	07.06	15.06	19.06
V_2	2021	18.04	23.04	27.04	30.04	03.05	09.06	13.06	18.06
	2022	20.04	26.04	30.04	02.05	05.05	07.06	15.06	19.06
Variants	**Year**	**Pea size berry**	**Veraison**			**Maturity**	**Leaf fall**		
			start	**mass**	**end**	**date**	**start**	**mass**	**end**
V_0	2021	09.07	04.08	13.08	17.08	06.09	22.10	08.11	10.11
	2022	08.07	05.08	12.08	15.08	06.09	25.10	11.11	20.11
V_1	2021	09.07	04.08	13.08	17.08	06.09	22.10	08.11	10.11
	2022	08.07	05.08	12.08	15.08	06.09	25.10	11.11	20.11
V_2	2021	09.07	04.08	13.08	17.08	06.09	22.10	08.11	10.11
	2022	08.07	05.08	12.08	15.08	06.09	25.10	11.11	20.11

Since the yield obtained from these variants could be considered as relevant to vine variety production capacity the reduction of clusters is highly recommended. Despite that the average weight of the cluster in variant V_1 was the lowest the obtained yield per vine was comparable with V_2.

It was found a high degree of multiple correlation between the severity, which resulted in a specified number of clusters, and the weight per cluster in IV phase. The multiple correlation coefficients (R^2) are 0.99 and 0.47 for the average weight of clusters and yield per vine, respectively, over an experimental period of two consecutive years on a 0.1 ha area.

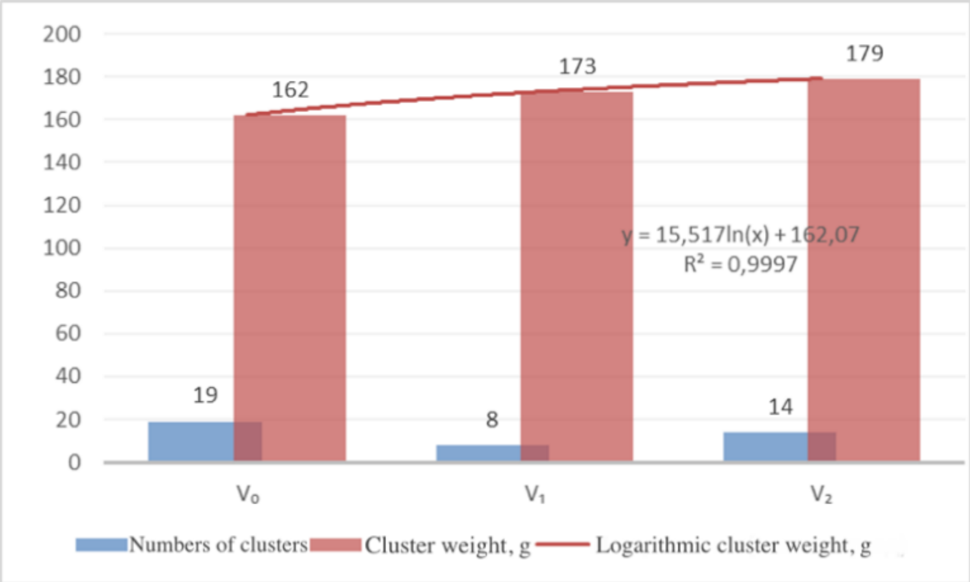

Figure 1. Change in the average cluster weight (g)

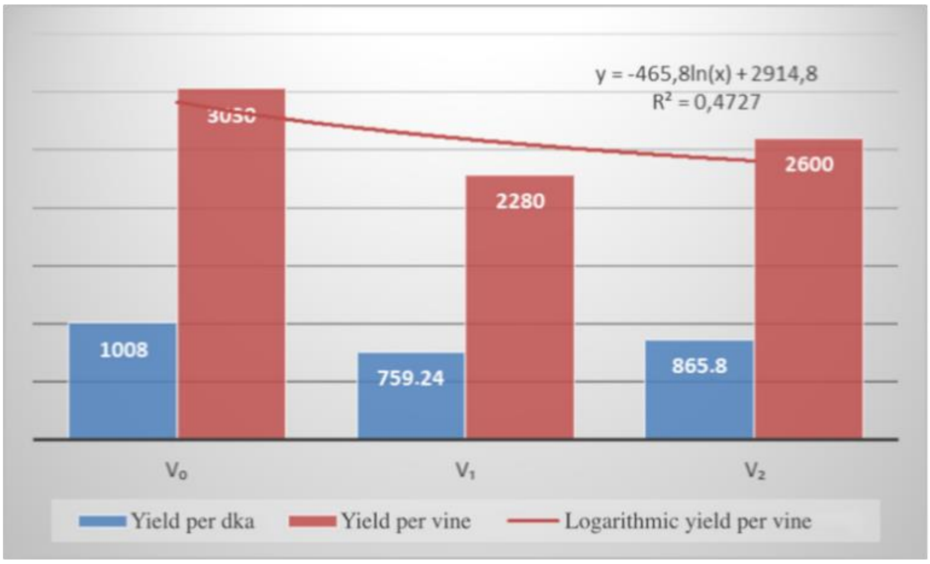

Figure 2. Quantitative change in the average yield per vine, kg/ha

The variants V_1 and V_2 showed higher average values for the length and the width of clusters (Table 4). These results indicate that cluster regulation (V_1 and V_2) provides better options for production of the Rubin variety cultivated in the climatic conditions of the area of Kuklen village. The application of these technological approaches can be useful for the production of grapes especially at low temperatures and insufficient precipitation which occur before and during flowering period, as well as under non-irrigation conditions.

Table 4. Quantitative changes in cluster sizes depending on severity

Variants	Year	Cluster size		Average cluster per vine
		Length, cm	Width, cm	
V_0	2021	14.2	10.5	Without reducing the clusters
	2022	14.0	10.0	
	Average	**14.1**	**10.25**	
V_1	2021	14.4	11.2	8
	2022	14.6	11.4	
	Average	**14.5**	**11.3**	
V_2	2021	15.2	21.1	14
	2022	15.5	21.7	
	Average	**15.35**	**21.4**	

Seasonal water management is the most important task that farmers must consider for sustainable and profitable crop production, especially in the arid and semi-arid regions of the world, where irrigation water availability is not guaranteed. The majority of high-value vineyards in Australia rely primarily on irrigation. For example, 90% of Australian vineyards depend on some form of reliable irrigation system (Australian Bureau of Statistics, 2012). Similar conditions with limited irrigation water availability are found in other arid and semi-arid regions of the world. As a result, prudent water use, long-term sustainability, and improving efficiency - i.e., enhancing the yield-to-water-use ratio - become major priorities in viticulture.

Phenolic compounds content is among the most important quality characteristics of wines. Phenolic compounds contribute to the organoleptic characteristics of wine such as color, astringency, and bitterness. Although tannins in wine can come from microbial and oak sources, the main sources of polyphenols are the grape skin and seeds. Since the 1960s, this topic has been widely studied by numerous researchers, covering various types of wines, climatic conditions, cultivation practices, and grape varieties. Because analysis and collection of data have been carried out under different conditions, they can be contradictory. Although the biosynthesis of the main proanthocyanidin units, (+)-catechin and (-)-epicatechin, is well known, the mechanism of their polymerization remains unexplained.

Table 5 presents the data about total sugars, acids and assimilable nitrogen content along with the pH value of the grape must which were measured immediately after the grape harvest. In terms of sugars content, Yoncheva et al. (2023) reported lower values for the Rubin variety - 22%. In the current study the acids content was high 7.82 – 8.05%. Usually, the acid content for this variety range was estimated as 5.5-6.0 g/dm^3 (Penkov, 2008). The estimated higher acidity could be related to the premature ripening of the grapes during the experiment and the insufficient number of days – only 19 from the full ripening until the harvest.

Table 5. Content of sugars, acids, yeast assimilable nitrogen and pH of the grape must

Variants	Sugars %	Acids g/dm^3	Assimilable nitrogen mg/dm^3	pH
V_0	25.35	8.05	327.15	3.66
V_1	31.5	7.82	453.88	3.93
V_2	29.2	7.82	406.72	3.93

High sugar content found in the berries can be due to the evaporation of water from the grapes, which concentrates the grape juice and prevents reaching phenolic maturity. From a practical point of view, the number of shoots should be reduced at an earlier stage of vegetation to preserve available water and manage nutrient sources for the vine.

CONCLUSION

The results from the study implied the existence of compensatory mechanisms of the vine plant subjected to some variable climatic conditions. The different cluster severity of the vines showed positive effect of reducing the bunches in the IV phase of the vegetative growth of the vine in terms of its production capacity. The variant V_1 with eight clusters per vine significantly overtakes the vines with 14 clusters (V_2). An increase in the average yield per vine also leads to an increase in the average yield per hectare, but did not affect the average weight per cluster since it was dependent on the yield reduction phenophases. This study showed the possibilities of obtaining a sufficient yield of 759.24 kg and 865.80 kg per 0.1 ha through a larger individual mass of clusters - 173 and 179 g, for V_1 and V_2, respectively. The application of canopy technique can be useful in the production of wine grapes, especially in uneven rainfall distribution or in non-irrigated area during the vine vegetation period.

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