



AGRICULTURAL UNIVERSITY – PLOVDIV
FACULTY OF VITICULTURE AND HORTICULTURE
DEPARTMENT OF VITICULTURE AND FRUIT GROWING

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**COMPARATIVE STUDY OF VEGETATIVE AND REPRODUCTIVE
MANIFESTATIONS OF SOME SYRAH VARIETY CLONES**

ABSTRACT

of a dissertation for awarding the educational and scientific degree Doctor"

Scientific specialty: "Viticulture"

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The research was conducted during the period 2020-2022 in the Training and Experimental Field of the Department of Viticulture and Fruit Growing at the Agricultural University - Plovdiv.

The dissertation has a volume of 174 pages, contains 28 tables and 62 figures. 257 literary sources are cited, 12 are in Cyrillic and 245 are in Latin.

The dissertation work was discussed and directed for defense at a meeting of the Department of "Viticulture and Horticulture" at the Agricultural University - Plovdiv.

The dissertation defense will take place on2023 from..... in classroom 320 of the Faculty of Viticulture and Horticulture at the Agricultural University - Plovdiv, in front of a Specialized Scientific Jury, approved by Rector's Order No. RD - 16-660/07.06.2023, composed of:

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The materials are available to those interested in the library of the Agricultural University - Plovdiv, 12 Mendelev Blvd.

I. Introduction

Viticulture and winemaking in Bulgaria have centuries-old traditions. They are strategically important, structure-determining subsectors of Bulgarian agriculture and the food industry.

The grapevine is a plant from which good results are obtained even on relatively poor soils, poorly suited for other agricultural crops.

The presence of favorable soil and climate conditions in our country is a prerequisite for stable production and high productivity.

The new vineyards are created in the natural and economic conditions most suitable for the individual varieties (clones) with certified planting material. Along with that, in the territorial arrangement of the grape varieties, the winegrowers must also take into account the traditions of growing the grape culture.

With the development of viticulture as one of the important sub-sectors of agriculture in a number of countries around the world and the implementation of new technologies in the cultivation of grape varieties, the need to study their biology also increases.

The influence of climatic regions has become an even more relevant issue with the transition in all countries, traditionally good producers of grapes and wine, to micro-zoning of grape varieties. Each variety (clone) must be located where it will show its biological potential to the greatest extent.

In this connection, the red wine variety Syrah, also known as Shiraz, is of interest. For many years, its origin was not clear, but a genetic study by the University of Davis in California in 1999 proved that the parents were the French varieties Dureza (Dureza) and Mondeuse Blanche (Mondeuse Blanche).

Through the introduction of new branches, there have been major changes in the varietal composition, which are distinguished by better economic qualities. A branch is a genetically uniform population of vine plants obtained from a single individual through vegetative propagation.

In our country and in the countries where Syrah variety and its clones are grown, high-quality red wines, extremely rich in extracts, with an excellent fruity aroma, are produced.

Today's conditions of overproduction and increased competition, the results of this study provide valuable economic information for some branches, generated by factors related to changes in the wine industry.

II. Purpose and tasks

Purpose: Research on vegetative and reproductive manifestations of clones numbered 100, 174, 470 and 524 of the variety Syrah /Syrah/, grafted on SO₄ rootstock and grown in the area of Brestnik, village as well as a general assessment of the obtained wines with a view to improving the technology of vine growing and grape processing.

Tasks:

1. Establishing the climatic and soil conditions in the region of the Rhodope collar, and their impact on the yield and grapes quality.
2. Peculiarities in the course of the main phenophases during the vegetation of the vines.
3. Studies on the indicators characterizing the actual fertility of the vines.
4. Establishing the photosynthetic activity of the leaves.
5. Determination of the dynamics of grape ripening.
6. Quantitative change in the yield and quality of grapes.
7. Technological features in vinification of grapes, through a full physico-chemical analysis.
8. Studies on the content of C13 - norisoprenoids / β - damascenone, α and β - ionone/, giving typical aromatic components of wines.
9. Organoleptic evaluation of the experienced wines in order to establish the character in the organoleptic profile of the wines, depending from the clone.

III. Research material and methods

Clones of the Syrah variety numbered 100, 174, 470 and 524, grafted onto the rootstock Berlandieri x Riparia SO₄, planted in April 2011 at the Educational and Experimental Field of the Department of Viticulture and Fruit Growing at the AU-Plovdiv, located in the territory of the town of Kuklen, on the border with the village of Brestnik, municipality of Rodopi, were used for the object of the study (Figure 1).

The vineyard is fully fruitfull. The planting distance is 3.0 m between the rows and 1,00 m between the vines in the row - 3330 plants per ha. The vines are trained high-stemmed. The training system is a double-sided cordon with the corresponding support trellis. The vines load in all variants was carried out by pruning with two buds spurs each, a total of 6 spurs /12 buds/ per vine. The inter-rows are grassed, the soil surface between the vines is kept clean by applying herbicides.

The rows direction is northwest - southeast, with a slopy terrain to the east – 3,2% /1,8% and an average altitude of -194 m.



Figure 1. Vineyard location

1. Ampelographic characteristics of the clones of Syrah variety, subject of the research

Syrah clone 100 - the clone is high-yielding, sometimes prone to overloading. The wines it produces are lower in sugar, neutral and plain (Figure 2).

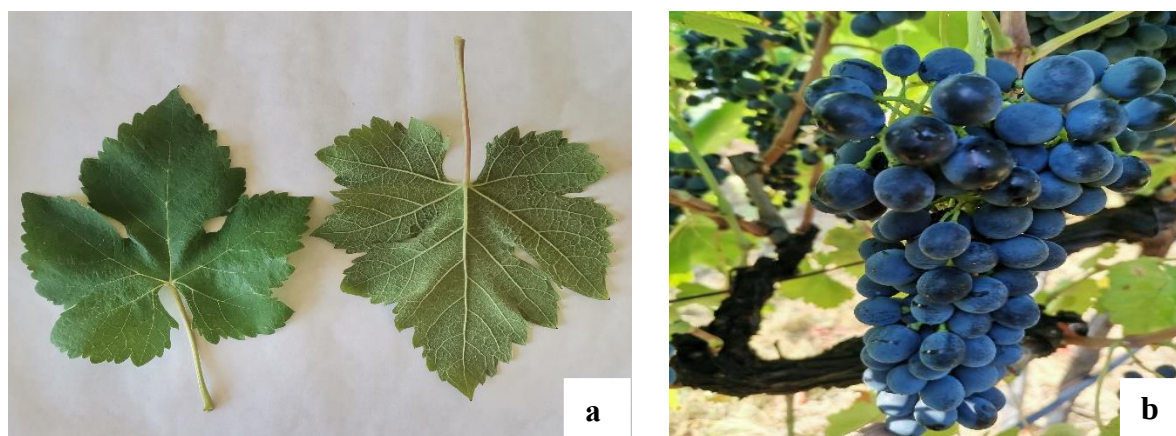


Figure 2. Clone 100 a - normally developed leaves; b - bunch

Syrah clone 174 - the clone has a limited yield, it can be applied, short and mixed pruning system. The wines are with high quality (Figure 2).

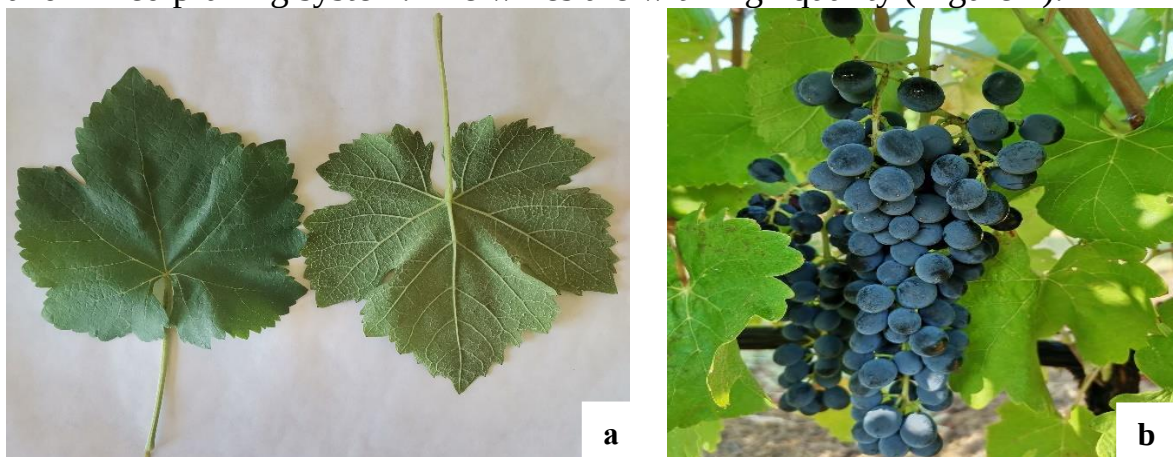


Figure 3. Clone 174 a - normally developed leaves; b - bunch

Syrah clone 470 - with this clone the yields are close to the average for the Syrah variety, but grown on poorer soils they are unsatisfactory. The wines are high quality, well colored with superior sugars and good tannin structure (Figure 3).

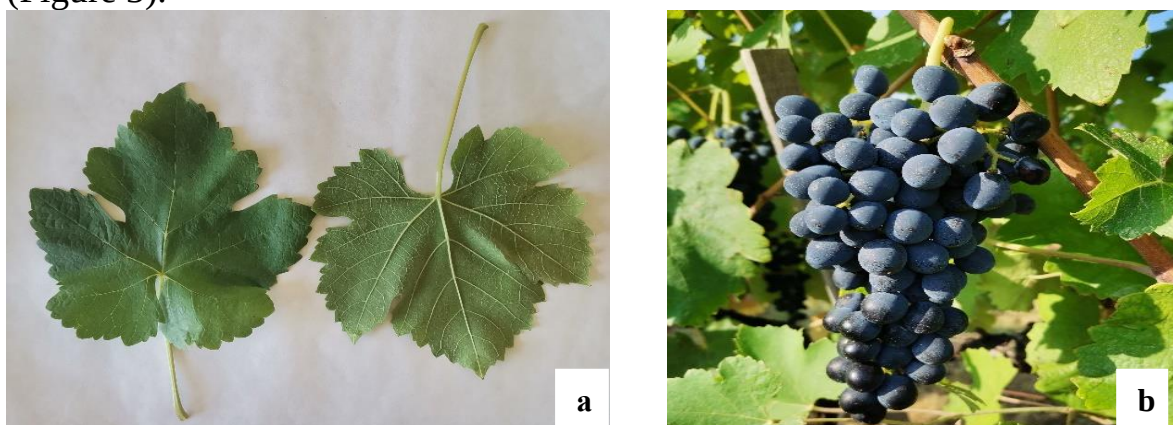


Figure 4. Clone 470 a - normally developed leaves; b -grape

Syrah clone 524 - the clone has a medium to high yield, the bunches have a lower average mass. The wines are balanced with medium to high sugars (Figure 4).

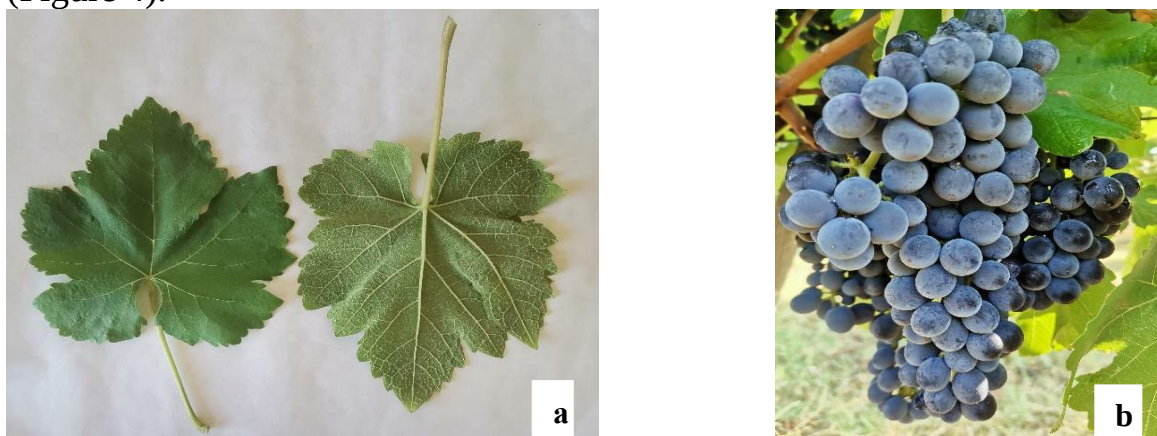


Figure 5. Clone 524 a - normally developed leaves; b - bunch

2. Scheme of the experimental work

The scheme includes the following 8 options:

V₁ - Syrah variety, clone 100 - non-reduced yield

V₂ - Syrah variety, clone 174 - non-reduced yield

V₃ - Syrah variety, clone 470 - non-reduced yield

V₄ - Syrah variety, clone 524 - non-reduced yield

The following 4 variants have a standardized yield in the “pea size” stage, in order to show the characteristics in the quality of the grapes and the resulting wine:

V₅ - Syrah variety, clone 100 - reduced yield with 8 bunches per vine

V₆ - Syrah variety, clone 174 - reduced yield with 8 bunches per vine

V₇ - Syrah variety, clone 470 - reduced yield with 8 bunches per vine

V₈ - Syrah variety, clone 524 - reduced yield with 8 bunches per vine

Each variant includes 60 vines (4 repetitions x 15 vines).

3. Research indicators:

3.1. Climatic characteristics of the studied area

3.2. Soil characteristics of the study area

3.3. Phenological observations during the growing season:

SAP movement, bud burst, first leaf appearance, first bunch appearance, flowering, berry growth /pea size/, berry softening /veraison/, technological maturity, leaf fall.

3.4. Studies on the photosynthetic activity of vines:

Intensity of leaf gas exchange;

Determination of chlorophyll content;

Dry mass and leaf water content.

3.4. Dynamics of shoot growth and maturation

3.5. Studies on the storage of one-year vine shoots with micro and macro elements

3.6. Indicators characterizing the actual fertility:

Developed buds from spurs, %, Fruit shoots, % - spurs, gluttons, fruit shoots with 1, 2 and 3 bunches. Coefficient of actual fertility /Kr/ - spurs, gluttons, replacement buds. Fertility coefficient of a fruit shoot /Kpl/ - spurs, gluttons, replacement buds.

3.7. Studies on grape production:

Average number of bunches per 1 vine, average mass per bunch (g), average mass per 100 grains (g), average yield per vine (kg), average yield per da(kg), mass of one-year vine shoots (kg).

3.8. Studies on grape quality:

Dynamics of sugars, dynamics of titratable acids, mechanical structure of bunches and berries, anthocyanins content.

3.9. Wine analyses

Experimental wines from the three harvests were made in the Experimental Wine Cellar of the AU-Plovdiv, using the same technological scheme for production of quality red wines: grape harvest, grapes acceptance, grapes processing, fermentation of the grape mash, control on the fermentation of the grape mash, separation the wine from the lees, care for young wines.

The following wine analyses were performed:

- Full physico-chemical analysis including: relative density (g/dm³); plain alcohol (%); sugars (g/dm³); total extract (g/dm³); sugar-free extract (g/dm³); titratable acids (g/dm³); volatile acids (g/dm³); free sulfur dioxide (mg/dm³); total sulfur dioxide (mg/dm³); total phenols (mg/dm³); anthocyanins (mg/dm³); color intensity; shade of color; percentage of yellow color; red color percentage; percentage of blue color;
- Organoleptic analysis;
- C₁₃-Norisoprenoids /derivatives of carotenoids/;
- Trans-resveratrol.

The physico-chemical analysis of the experimental wines was done in the modern chemical laboratory of the University wine cellar and the teaching laboratory of Winemaking at the Agricultural University - Plovdiv.

The organoleptic analysis of the experimental wines was evaluated by a 5-member expert tasting committee.

C₁₃-Norisoprenoids /derivatives of carotenoids/: β - damascenone, α -ionone and β -ionone, were studied in the laboratory of the Institute Jules Guyot, University of Burgundy, France.

The analyses for the determination of trans-resveratrol in the wine were carried out in an accredited testing laboratory at the "National Institute for the Study of Wine, Spirits and Essential Oils" EOOD - Sofia.

3.10. Statistical processing of the experimental data

The obtained data were mathematically processed by the method of variance analysis using the SPSS program, and to establish the differences between the studied variants, Duncan's multiple rank test was used, with the least significant difference (LSD) - 0,05 (5%).

III. Results

1. Climatic characteristic

The area in which the clones are grown has favorable climatic conditions for growing red wine grape varieties to obtain quality wines. During the three experimental years included in the study, no critical air temperatures were recorded, which represent a danger for the vines (Figures 6, 7 and 8).

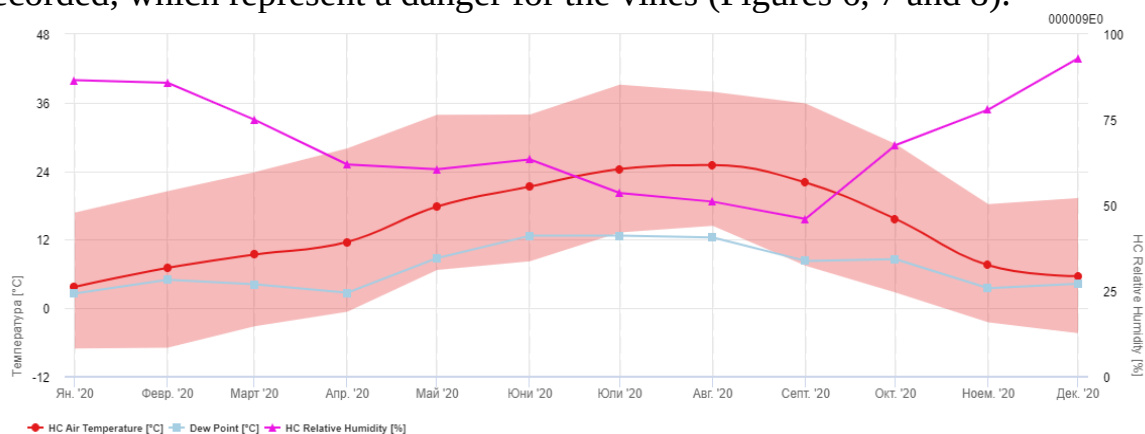


Figure 6. Changes in the average monthly air temperature (°C), for the year 2020

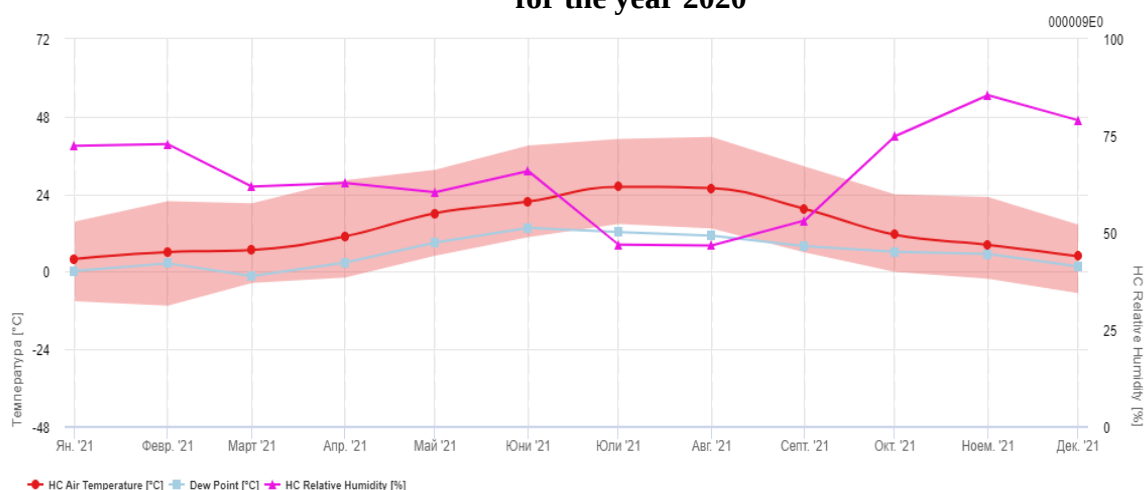


Figure 7. Changes in the average monthly air temperature (°C), for the year 2021

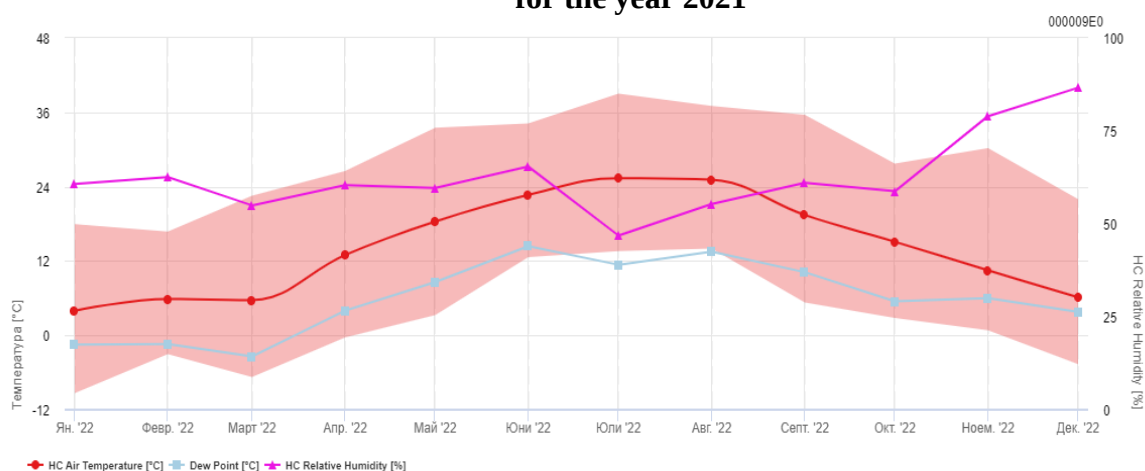


Figure 8. Changes in the average monthly air temperature (°C), for the year 2022

A better idea of the temperature stress is given by the active temperature sum, which in the first experimental year was 2075°C. This is the sum of the temperature above the vine biological zero, when more heat is needed for flowering, ripening the grapes, and the onset of physiological and technological maturity (Table 4).

Table 4. Average day-night air temperature above 10°C, number of days and temperature sum, for the period 2020 - 2022

Beginning of vegetation period	End of vegetation period	Number of days with a temperature above 10°C	Active temperature sum
21.03.2020	05.11.2020	230	2075
28.03.2021	09.11.2021	227	1971
29.03.2022	15.11.2022	232	2156

The total temperature sum for the year 2021 is 4241°C, and the active temperature sum is 1971°C.

In 2022, the total temperature sum during the growing season is 4476°C, and the active temperature sum is 2156°C.

The long, dry and relatively warm autumn during the three years of research helps to accumulate significant amounts of sugars and other active substances, especially necessary for the timely and complete ripening of shoots and grapes.

The average annual amount of precipitation in the vineyard in 2020 is 572,4 mm. The largest amount of precipitation is in the month of April – 106,8 mm (Figure 9).

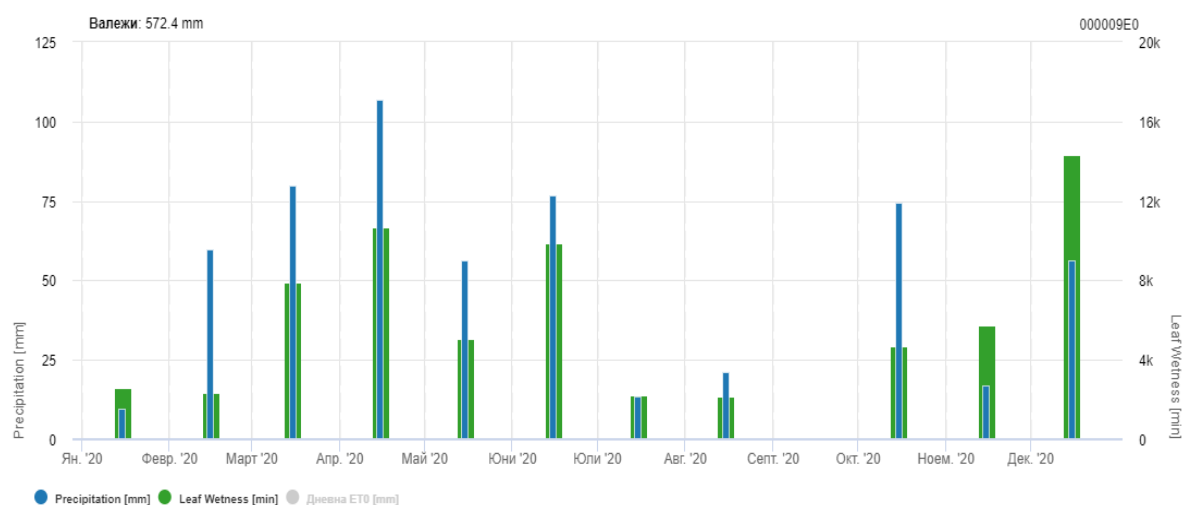


Figure 9. Precipitation amounts, in mm, in 2020

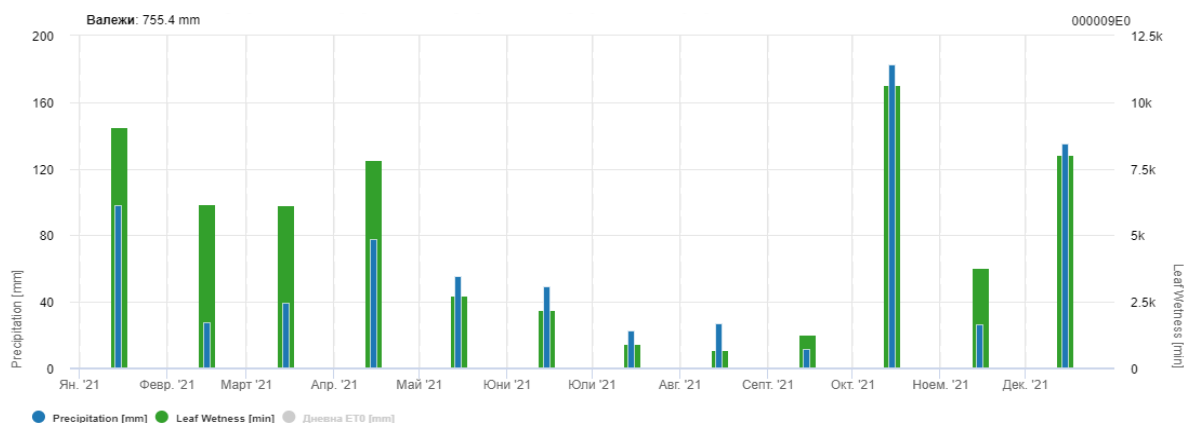


Figure 10. Precipitation amounts, in mm, in 2021

In 2021, the average annual rainfall for the studied area is approximately 200 mm more, compared to the previous year – 755,4 mm. The largest amount of precipitation is in the month of October – 182,6 mm (Figure 10).

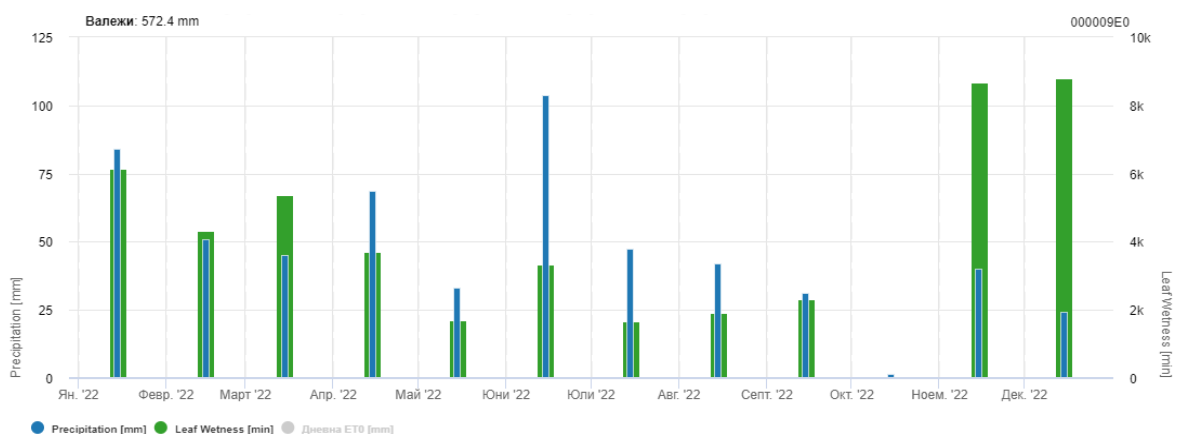


Figure 11. Precipitation amounts, in mm, in 2022

The average annual amount of precipitation in 2022 for the area is 572,4 mm. The wettest month is June – 103,8 mm (Figure 11).

Particularly useful are abundant and short-term rainfall after the flowering phase, which can significantly replenish the soil moisture reserves. During the ripening period of the grapes, excessive rainfall retards ripening, reduces the sugar content and increases the berry volume, leading to skin cracking and rotting.

The studied area is characterized by a short-lived and relatively shallow snow cover, which is characteristic of the Upper Thracian lowland.

2. Soil characteristic

The soil in the vineyard is deluvial-meadow, loamy-sandy to sandy-loamy. The profile has a depth greater than 360 cm. There is no overwetting and no surface or actual oiling. The clay content is 30 - 40%.

To establish the chemical composition and reaction of the soil /pH/ in the vineyard, a soil analysis has been made in the humus horizon from 30 to 60 cm including the main soil elements, when starting the experiment in 2020 (Table 1).

Table 1. Analysis of the main soil components

Indicator	Unit of magnitude	Substance
Soil reaction /pH/	pH units	7,6
Electrical conductivity	$\mu\text{S}/\text{sm}^{-1}$	119,70
Mobile nitrogen (sum. all forms)	mg/kg	8,55
Mobile phosphorus (P_2O_5)	mg/100g	5,46
Mobile potassium (K_2O)	mg/100g	26,27
Carbonates (total)	g/kg	62,42
Carbonates (active)	g/kg	15,00

The preliminary soil analysis establishes the content of nitrogen, phosphorus and potassium. A slightly alkaline reaction of the soil (pH =7,6) was established from the research.

Alkalinity is determined by the content of total carbonates, which help, both for the normal development of the root system, and for increasing the amount of sugars and aromatic substances in the grapes.

The content of the mobile forms of nitrogen, phosphorus and potassium show that the soil complex is very well stocked with potassium, which is typical for the country soils.

The soil and climatic conditions in the Experimental Field of the Agricultural University - Plovdiv, located near Brestnik village, are favorable for the growth and fruiting of red wine grape varieties.

3. Phenological observations during the growing season

The vine undergoes seasonal changes associated with the seasonal changes, which are repeated annually in a certain sequence. In the spring time, the development of the shoots and inflorescences begins, flowering occurs, and in the summer the knot thickens, after which the grapes and the shoots ripen. This is the period of active life, called growing season.

During the study period, phenological observations during the vegetation of 2020, 2021 and 2022 were carried out.

When summarizing the results, it was found that the vines of clone 174 (V_2 ; V_6) and clone 470 (V_3 ; V_7) started their vegetation one week earlier compared to the vines of clone 100 (V_1 ; V_5) and clone 524 (V_4 ; V_8).

After normalizing the yield during the phase of berry growth (pea size), begins the bunch removal on the vines with normalized yield (V_5 , V_6 , V_7 and V_8) occurs earlier at the end of July, compared to the vines with non-normalized yield (V_1 , V_2 , V_3 and V_4), the ripening of which occurs at the beginning of August in the three experimental years. The technological maturity of the thinned vines occurs one week earlier than the vines with non-normalized yield.

The duration of the main phenophases during the growing season was calculated in days with proven statistical differences (Tables 2 and 3).

In the non-thinned (V_1 , V_2 , V_3 and V_4) and thinned variants (V_5 , V_6 , V_7 and V_8), the period from SAP movement to bud burst in 2020 is longer than in 2021 and 2022.

When analyzing the averaged data, pairwise clustering of the clones was found: clone 100 (V_1 and V_5) with clone 524 (V_4 and V_8) and clone 174 (V_2 and V_6) with clone 470 (V_3 and V_7).

During the phenological period from bud burst to first leaf appearance, no differences were demonstrated between the clones. The same applies to the period from the appearance of the first leaf to the flowering of the vines.

The period from flowering to „pea size“ in clone 100 and clone 524 showed differences in the duration compared to the other two clones 174 and 470, in both thinned and non-thinned variants.

When summarizing the results for the entire three-year period, it was found that from the bud burst stage to the technological maturity phase, there are proven differences in the variants with thinned and non-thinned yield. Clone 100 (V_1 and V_5) and clone 524 (V_4 and V_8) have a shorter period from bud burst to technological maturity, compared to the other two clones 174 (V_2 and V_6) and 470 (V_3 and V_7).

**Table 2. Duration of phenophases in days for non-thinned variants,
for the period 2020, 2021 and 2022**

Variant	Year	From SAP movement until bud burst	From bud burst to the first leaf	From the first leaf until flowering	From flowering to pea size	From a pea size to a veraison	From veraison to technological maturity	From bud burst to technological maturity
V₁	2020	30b	14a	37a	25a	43a	38a	153a
	2021	15a	15a	36a	34b	32a	35a	146a
	2022	14a	13a	30a	30b	41a	36a	146a
	Average	20	14	34	30	39	36	148
V₂	2020	23a	18b	40b	25a	42a	42b	163b
	2021	15a	16a	36a	31a	33a	37b	149a
	2022	16b	12a	31a	28a	43b	37a	148a
	Average	18	15	36	28	40	39	153
V₃	2020	23a	18b	40b	25a	42a	42b	163b
	2021	15a	16a	36a	31a	33a	37b	149a
	2022	16b	12a	31a	28a	43b	37a	148a
	Average	18	15	36	28	40	39	153
V₄	2020	30b	14a	37a	25a	43a	38a	153a
	2021	15a	15a	36a	34b	32a	35a	146a
	2022	14a	13a	30a	30b	41a	36a	146a
	Average	20	14	34	30	39	36	148
LSD 5%	2020	2,31	2,38	2,21	1,29	2,17	2,19	8,94
LSD 5%	2021	1,98	1,96	1,28	2,96	2,48	1,98	7,92
LSD 5%	2022	1,89	1,84	1,98	1,79	1,86	2,04	8,24

*Comparative analysis with provenance $\alpha=0,05$

**Table 3. Duration of phenophases in days for thinned variants,
for the period 2020, 2021 and 2022**

Variant	Year	From SAP movement until bud burst	From bud burst to the first leaf	From the first leaf until flowering	From flowering to pea size	From a pea size to a veraison	From veraison to technological maturity	From bud burst to technological maturity
V₅	2020	30b	14a	37a	25a	39a	35a	146a
	2021	15a	15a	36a	34a	24a	33a	138a
	2022	14a	13a	30a	30b	34a	34a	137a
	Average	20	14	34	30	32	34	140
V₆	2020	23a	18b	40b	25a	39a	38b	156b
	2021	15a	16a	36a	31b	26a	36b	141a
	2022	16b	12a	31a	28a	34a	38a	139a
	Average	18	15	36	28	33	37	145
V₇	2020	23a	18b	40b	25a	39a	38b	156b
	2021	15a	16a	36a	31a	26a	36b	141a
	2022	16b	12a	31a	28a	34a	38a	139a
	Average	18	15	36	28	33	37	145
V₈	2020	30b	14a	37a	25a	39a	35a	146a
	2021	15a	15a	36a	34b	24a	33a	138a
	2022	14a	13a	30a	30b	34a	34a	137a
	Average	20	14	34	30	32	34	140
LSD 5%	2020	4,28	3,17	2,84	2,17	1,24	2,79	8,24
LSD 5%	2021	1,98	2,14	2,14	2,97	2,18	2,94	6,38
LSD 5%	2022	1,82	1,54	1,60	1,97	1,47	1,97	7,24

*Comparative analysis with provenance $\alpha=0,05$

4. Study on the physiological condition of the vines

4.1. Photosynthetic activity

The research on the photosynthetic activity of the vines during the three experimental years was carried out twice during the vegetation, before and after the flowering phase of the vines, and the following indicators were taken into account: photosynthesis, transpiration, stomatal conductance and chlorophyll content. The experimental data were obtained under comparable conditions on vine shoots of the same size, load, exposure and illumination (Tables 4 and 5).

Analyzing the results, it was found that before the flowering phase, the rate of net photosynthesis was the highest, with a proven statistical difference in clone 470 – $20,04 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, and clone 100 was with lowest rate of net photosynthesis – $17,55 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$.

The content of plastid pigments (chlorophyll) in the tissues before the flowering phase was the highest in clone 470 – $366,10 \text{ mg/g}$, in which the highest net photosynthesis was also found (Table 4).

**Table 4. Photosynthetic activity before the flowering phase
for the period 2020, 2021 and 2022**

Syrah clone	Period (Year)	A	E	Gs	Chl
100	22.05.2020 r.	16,81b	1,89a	0,04a	294,70a
	25.05.2021 r.	17,90a	2,01c	0,04a	287,00a
	23.05.2022 r.	17,94a	2,02c	0,04a	366,83c
	Average	17,55	1,97	0,04	316,17
174	22.05.2020 r.	18,85b	1,42a	0,04a	343,00b
	25.05.2021 r.	18,00a	1,50a	0,04a	326,00b
	23.05.2022 r.	18,02a	1,51a	0,04a	356,33a
	Average	18,29	1,48	0,04	341,77
470	22.05.2020 r.	18,81a	1,65b	0,04a	358,30b
	25.05.2021 r.	20,65b	1,87b	0,04a	335,00a
	23.05.2022 r.	20,66b	1,87b	0,04a	405,00d
	Average	20,04	1,80	0,04	366,10
524	22.05.2020 r.	17,30c	1,81b	0,04a	339,00b
	25.05.2021 r.	18,33b	1,90b	0,04a	334,80a
	23.05.2022 r.	18,33a	1,91b	0,04a	374,33b
	Average	17,98	1,87	0,04	349,37
LSD 5%	2020 r.	1,17	0,11	0,01	17,16
LSD 5%	2021 r.	1,74	0,14	0,01	14,71
LSD 5%	2022 r.	1,69	0,15	0,02	18,29

*Comparative analysis with provenance $\alpha=0,05$

A - Rate of photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)

E - Intensity of transpiration ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)

Gs - Stomatal conductance of leaves

Chl - Chlorophyll (mg/g)

**Table 5. Photosynthetic activity after the flowering phase
for the period 2020, 2021 and 2022**

Syrah clone	Period (Year)	A	E	Gs	Chl
100	09.06.2020 г.	11,23a	1,37c	0,03a	231,70a
	14.06.2021 г.	14,62a	1,33a	0,02a	233,08a
	10.06.2022 г.	14,62a	1,34a	0,02a	273,83b
	Average	13,49	1,35	0,02	246,20
174	09.06.2020 г.	14,03b	1,04a	0,02a	238,20ab
	14.06.2021 г.	15,74ab	1,31a	0,03a	248,50b
	10.06.2022 г.	15,74ab	1,31a	0,02a	262,66a
	Average	15,17	1,22	0,02	249,78
470	09.06.2020 г.	14,06b	1,23b	0,03a	246,50b
	14.06.2021 г.	16,44b	1,39a	0,03a	247,60b
	10.06.2022 г.	16,44b	1,39a	0,02a	321,17c
	Average	15,65	1,34	0,03	271,76
524	09.06.2020 г.	13,91b	1,17ab	0,02a	248,50b
	14.06.2021 г.	17,21b	1,27a	0,02a	234,80a
	10.06.2022 г.	17,21b	1,27a	0,02a	273,17b
	Average		1,24	0,02	252,16
LSD 5%	2020 г.	1,68	0,16	0,02	12,06
LSD 5%	2021 г.	1,11	0,15	0,01	12,83
LSD 5%	2022 г.	1,74	0,16	0,02	10,12

*Comparative analysis with provenance $\alpha=0,05$

A - Rate of photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)

E - Intensity of transpiration ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)

Gs - Stomatal conductance of leaves

Chl - Chlorophyll (mg/g)

From the summarized results after the flowering phase of the vines, when the berries are formed and accelerate all the synthetic processes related to the accumulation of biomass, the content of the investigated indicators decreases (Table 5).

4.2. Shoot growth dynamics

Vines from clones 100, 174, 470, and 524 began their shoot growth during the last ten days of April, beginning of May. At the beginning, the growth is weak and smooth, but as the vegetation progresses, it gradually accelerates, at the end of May the shoots have an increase of 130 - 150 cm. During the flowering of the vines, when the optimal temperature reaches 28-30°C, there is the most intensive growth, with an average length of more than 40 cm per week (Figure 12).

The obtained results show that the vines from clone 470 (V_3 and V_7) have the strongest growth, followed by the vines from clone 524 (V_4 and V_8), and the vines from clone 100 (V_1 and V_5) have the weakest growth.

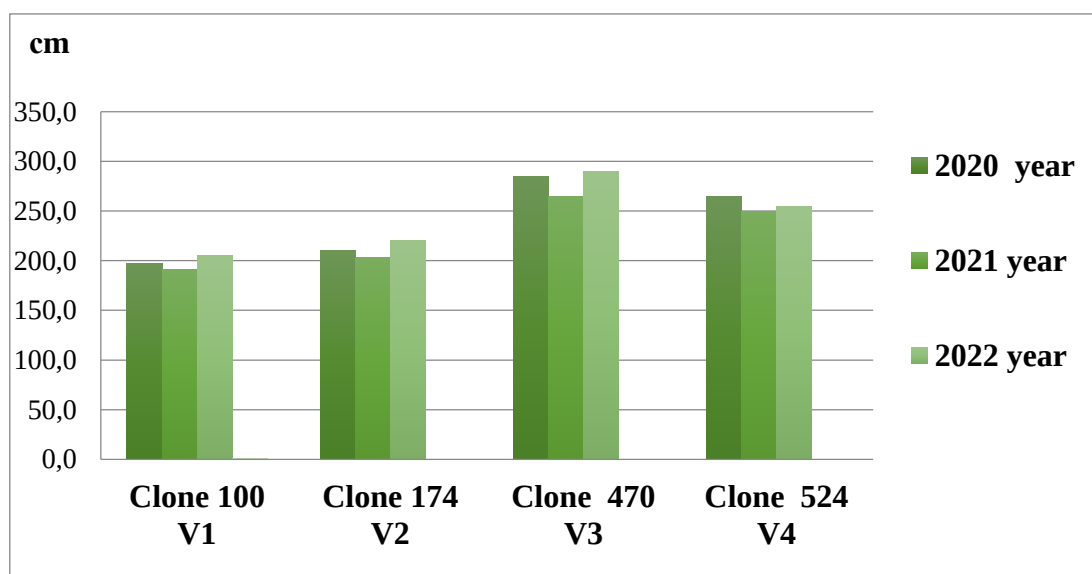


Figure 12. Shoot growth dynamics in non-thinned variants, in 2020, 2021 and 2022, cm

In the thinned variants (V_5 , V_6 , V_7 , and V_8), from the „pea size“ stage to the beginning of the veraison, shoot growth reached a greater length, compared to the non-thinned variants (Figure 13).

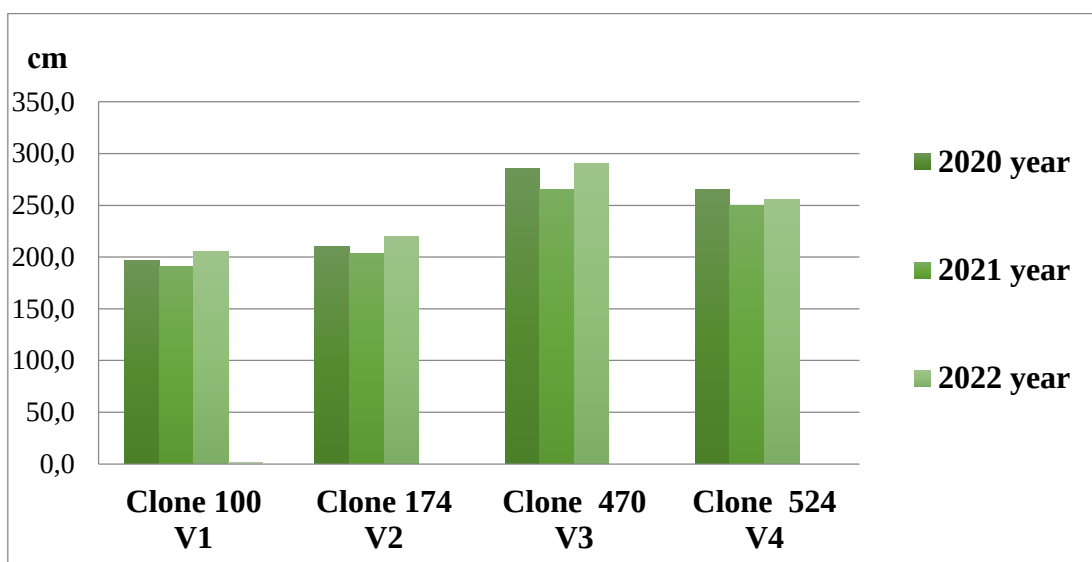


Figure 13. Growth dynamics of shoots in thinned variants, in 2020, 2021 and 2022, cm

Summarizing the results, it was found that the shoots of clone 470 (V_3 and V_7) reached the greatest length for the whole period. The vines from clone 100 (V_1 and V_5) have the weakest shoot growth.

The quantity and quality of the grapes depends on the growth of the vine shoots, which is also a biological and varietal (clone) feature.

4.3. Dynamics of shoot maturation

The ripening of the vine shoots has great practical importance, which is related to the cold resistance of the vine buds.

In the vines with irregular yield, the greatest maturation of the shoots was found in clone 100 (V_1), followed by clone 524 (V_4) and clone 174 (V_2). Shoot ripening had the shortest length in the vines of clone 470 (V_3), where the highest average weight per bunch was found (Figure 14).

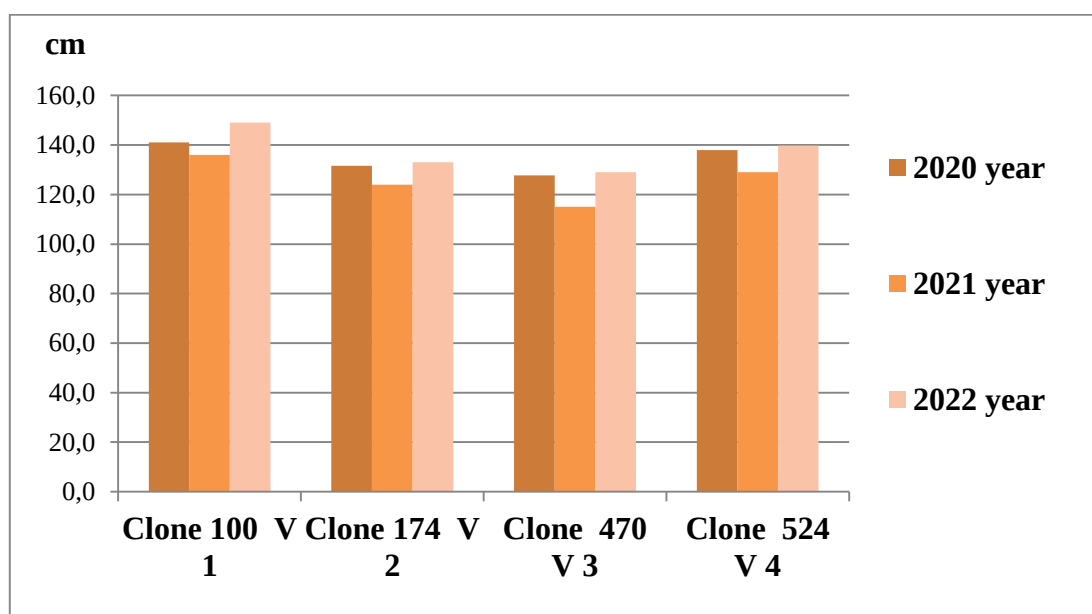


Figure 14. Dynamics of shoot maturation in the non-thinned variants, in 2020, 2021 and 2022, cm

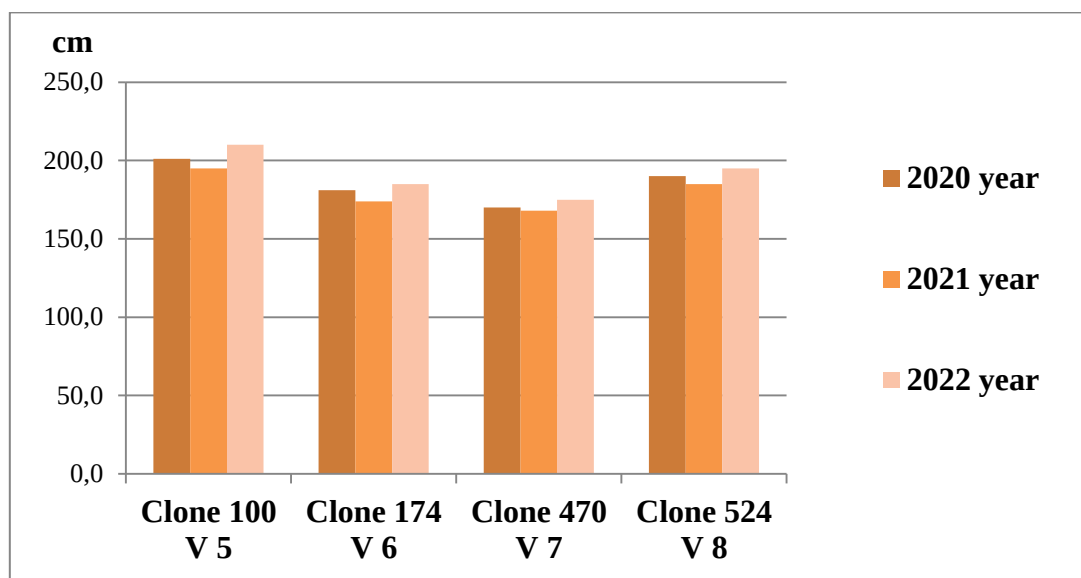


Figure 15. Dynamics of shoot maturation in the thinned variants, in 2020, 2021 and 2022, cm

The dynamics of shoot ripening in cm, for vines with normalized yield, i.e. less grape-laden vines show a higher degree of shoot ripening (Figure 15).

In the variants with normalized yield, the greatest degree of shoots maturation was found in clone 100 (V₅), which was confirmed in all three experimental years, and the least degree of maturation was in the shoots from clone 470 (V₇).

During the last ten days of August and the first week of September, the shoots ripening is at a slower pace, which is related both to reaching technological maturity and to the influence of climatic changes at the end of summer.

5. Indicators characterizing the vines actual fertility

The results obtained showed a relatively high percentage of fruiting shoots developed from spurs and gluttons in clone 100 and clone 524, followed by the other two clones 174 and 470 (Table 6).

It is of essential importance for the practice that in two clones (100 and 524) it was found approximately 50% of fruit shoots developed from dormant buds (gluttons).

The indicator showing the ratio between the total number of bunches and total number of shoots is the coefficient of actual fertility (Kr). Data from this metric are presented separately for spurs, dormant buds, and replacement buds. Clones numbered 100 (1,58%) and 524 (1,45%) have the highest coefficient of actual fertility (Kr), and clones numbered 174 (1,45%) and 470 (1,29%).

The coefficient of actual fertility per fruiting shoot (Kpl) has higher values in spurs compared to dormant and replacement buds. The data obtained from clones numbered 100 (1,76%) and 524 (1,69%) were higher than the other two clones.

The percentage of fruiting shoots with different number of clones in the three years shows, most fruiting shoots are with 2 bunches, followed by those with one bunch. A difference from the statistical analysis was found in the three years: 2020 at clone 100 (78,35%) and clone 524 (73.62%), in 2021 at clone 100 (87,15%) and clone 524 (77,25%), and in 2022 clone 100 (80,21%) and clone 524 (74,45%).

Fruiting shoots with 3 bunches in the second and third experimental years were not reported.

Table 6. Indicators of the actual fertility of the vines, for the period 2020, 2021 and 2022

Clone Number	Period (Year)	Developed buds from spurs %	Fruiting shoots, %		Coefficient of actual fertility Cr.			Coefficient of actual fertility per fruiting shoot			Fruiting shoots, %		
			Spurs	Gluttons	Spurs	Gluttons	Replacement buds	Spurs	Gluttons	Replacement buds	with 1 bunch	with 2 bunches	with 3 bunches
100	2020	76,11b	89,53b	54,63b	1,58c	0,60b	1,50c	1,76c	1,10c	1,67c	20,92a	78,35c	0,73b
	2021	69,81a	95,49b	11,43b	1,80b	0,15c	0,77a	1,89c	1,33b	1,31a	12,85a	87,15c	-
	2022	72,04a	96,66b	18,72c	1,78b	0,21b	0,90c	1,84c	1,21c	1,20a	19,79a	80,21c	-
	Average	72,65	93,89	28,26	1,72	0,32	1,06	1,83	1,21	1,39	17,85	81,90	0,73
174	2020	70,74a	87,17b	23,15a	1,45b	0,24a	0,69a	1,67b	1,04b	1,35a	29,32b	70,68ab	-
	2021	71,48a	88,60a	8,26b	1,51a	0,10b	0,71a	1,70a	1,18a	1,28a	32,13c	67,87a	-
	2022	70,93a	87,73a	12,58b	1,50a	0,08a	0,77b	1,69a	0,91a	1,41b	28,15bc	71,85a	-
	Average	71,05	87,83	14,66	1,48	0,14	0,72	1,68	1,04	1,35	29,86	70,13	-
470	2020	68,51a	82,43a	23,20a	1,29a	0,22a	1,27b	1,56a	0,93a	1,40a	33,51b	66,22a	0,27a
	2021	72,96a	90,35ab	4,29a	1,58a	0,06a	0,76a	1,75a	1,30b	1,32a	27,89c	72,11a	-
	2022	71,38a	93,25ab	5,95a	1,59a	0,07a	0,73ab	1,72b	1,18c	1,44b	30,55c	69,45a	-
	Average	70,95	88,67	11,15	1,48	0,12	0,92	1,67	1,14	1,38	30,65	69,26	0,27
524	2020	70,18a	85,75a	45,58b	1,45b	0,46b	1,57c	1,69c	1,01b	1,57b	26,38ab	73,62b	-
	2021	68,15a	90,76ab	2,95a	1,63ab	0,03a	0,72a	1,80b	1,00a	1,31b	22,75b	77,25b	-
	2022	68,70a	95,96b	3,68a	1,61b	0,04a	0,65a	1,60a	1,00a	1,22a	25,55b	74,45b	-
	Average	69,01	90,82	17,40	1,54	0,17	0,98	1,69	1,00	1,36	24,89	75,11	-
LSD5%	2020	5,01	4,12	10,26	0,14	0,19	0,15	0,10	0,05	0,15	7,26	4,93	0,27
LSD5%	2021	4,97	3,89	3,84	0,18	0,06	0,11	0,80	0,07	0,20	5,28	5,29	-
LSD5%	2022	4,21	4,01	4,12	0,13	0,09	0,12	0,13	0,08	0,21	5,14	4,24	-

*Comparative analysis with provenance $\alpha=0,05$

6. Study of the yield of grapes per vine and per da

6.1. Indicators determining the yield of grapes

Grape yield is an important indicator determining the vineyards economic efficiency. The annual obtaining of high and quality yields of grapes is possible only with optimal bud loading per each vine during pruning time.

The indicators characterizing the yield of grapes included in the study are: average number of bunches per vine, average yield per vine, average yield per hectar, average weight per bunch and average weight of 100 grains. Eight variants are included in the scheme of the experimental work. The first four variants (V_1 , V_2 , V_3 and V_4) are non-thinned vines. The second four variants (V_5 , V_6 , V_7 and V_8) are vines with thinned bunches. Bunch thinning (applying a green pruning operation) was done when the bunches were pea-sized. In this way, on each vine from variants numbered V_5 , V_6 , V_7 and V_8 , 8 bunches were left, which were the best formed (Table 7).

In 2020, the yield obtained from the non-thinned variants (V_1 , V_2 , V_3 and V_4) varies from 1013,65 kg/da (Clone 524 – V_4) to 1237,76 kg/da (Clone 100 – V_1). The average yield per vine, which is one of the main indicators, is again the highest at clone 100 – 3,72 kg (V_1), and the lowest at clone 524 – 3,04 kg (V_4).

The mass of the yield per vine in the non-thinned variants V_1 , V_2 , V_3 and V_4 is almost twice as high as the thinned variants (V_5 , V_6 , V_7 and V_8), which also explains the reduction in the number of bunches in order to increase their quality.

The highest average mass per bunch was found in the vines of clone 174 with a thinned yield of -172 g (V_6). The lowest average mass per bunch was reported for vines from clone 100 with non-thinned yield - 124 g (V_1).

The yield obtained from the non-thinned variants (V_1 , V_2 , V_3 and V_4) in the second year ranged from 925,74 kg/da in clone 470 (V_3) to 797,20 kg/da in clone 174 (V_2).

The average yield per vine, respectively, is the highest at clone 470 -2,78 kg (V_3), and the lowest at clone 174 – 2,39 kg (V_4).

In 2022, the yield obtained from the non-thinned variants (V_1 , V_2 , V_3 and V_4) varies from 1055,61 kg/da (Clone 524 - V_4) to 1268,73 kg/da (Clone 100 – V_1). The average yield per vine is again the highest in clone 100 – 3,81 kg (V_1), and the lowest in clone 524 – 3,17 kg (V_4).

The yield obtained from the variants with bunch thinning in the third year is in the same sequence as for the non-thinned variants. It is highest in clone 100 – 699,30 kg/da (V_5) and lowest in clone 524 – 599,40 kg/da (V_8).

The production of the third year (2022) is the highest compared to the previous two years (2020 and 2021). This is the result of a well-balanced year 2022 in terms of climatic indicators, average monthly air temperature and amount of precipitation.

Table 7. Grape yield per vine and per da, for the period 2020, 2021 and 2022

Option	Period (year)	Average number of bunches per vine	Average weight per bunch (g)	Average weight per 100 berries (g)	Average yield per vine, (kg)	Average yield per da (kg)
V ₁	2020	30c	124,00a	155,26ab	3,72c	1237,76c
	2021	23a	109,00a	141,03b	2,55b	850,48b
	2022	27b	162,00b	185,05b	3,81b	1268,73b
	Average	26,66	131,66	160,45	3,31	1118,99
V ₂	2020	25b	136,00b	159,86b	3,34b	1111,22b
	2021	22a	116,00ab	132,29a	2,39a	797,20a
	2022	24a	167,00b	177,14a	3,28a	1092,24a
	Average	23,67	139,67	156,43	3,01	1000,22
V ₃	2020	22a	142,00c	151,03a	3,09a	1028,30a
	2021	23a	133,00c	136,70ab	2,78c	925,74c
	2022	25a	195,00c	190,05c	3,69b	1228,77b
	Average	23,34	156,67	159,26	3,18	1060,94
V ₄	2020	24b	123,00a	154,53ab	3,04a	1013,65a
	2021	21a	129,00bc	131,70a	2,63b	875,79ab
	2022	25a	143,00a	175,30a	3,17a	1055,61a
	Average	23,34	131,67	153,84	2,95	981,68
LSD 5%	2020	2,30	5,87	5,18	0,36	79,03
LSD 5%	2021	3,05	8,09	8,41	0,11	44,6
LSD 5%	2022	2,81	9,21	9,07	0,35	81,24
Variants with a standardized yield of 8 grapes per vine in the "pea" phase						
V ₅	2020	8	158,00b	170,56c	1,33b	444,22b
	2021	8	159,00c	149,30b	1,45c	485,51b
	2022	8	258,00 c	201,25ab	2,10c	699,30c
	Average	8	191,67	173,70	1,63	543,01
V ₆	2020	8	172,00d	155,10b	1,40c	466,53b
	2021	8	154,00b	149,70b	1,38b	459,54b
	2022	8	228,00b	195,50	1,98b	659,34b
	Average	8	184,67	166,77	1,58	528,47
V ₇	2020	8	166,00c	142,33a	1,35b	448,88b
	2021	8	155,00b	146,50ab	1,42bc	473,53b
	2022	8	276,00d	198,50a	2,08c	692,64bc
	Average	8	199,00	162,44	1,62	538,35
V ₈	2020	8	144,00a	153,63b	1,17a	390,61a
	2021	8	147,00a	141,70a	1,29a	429,57a
	2022	8	222,00a	208,75b	1,80a	599,40a
	Average	8	171,00	168,03	1,81	473,19
LSD 5%	2020	-	5,87	6,42	0,04	36,5
LSD 5%	2021	-	4,18	7,10	0,07	34,80
LSD 5%	2022	-	5,26	6,92	0,09	42,91

*Comparative analysis with provenance $\alpha=0,05$

The correlation coefficient squared – R^2 (R Square) is called the coefficient of determination. It shows the variance percentage of the outcome variable is explained by the action of the factor variable. For 2020, $R^2 = 0,669$, i.e. 67% of the yield depends on the mass per one bunch (Figure 16).

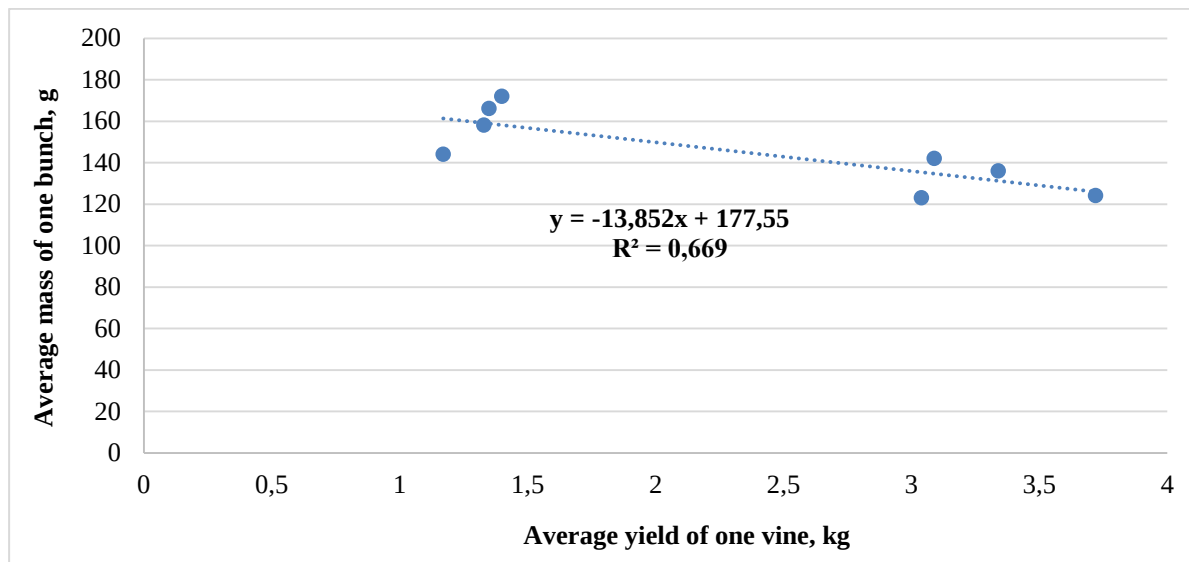


Figure 16. Linear regression model between average yield per vine and average weight of one bunch, 2020

The coefficient of determination for the year 2021 $R^2 = 0,692$, i.e. 70% of the yield depends on the mass of one bunch (Figure 17).

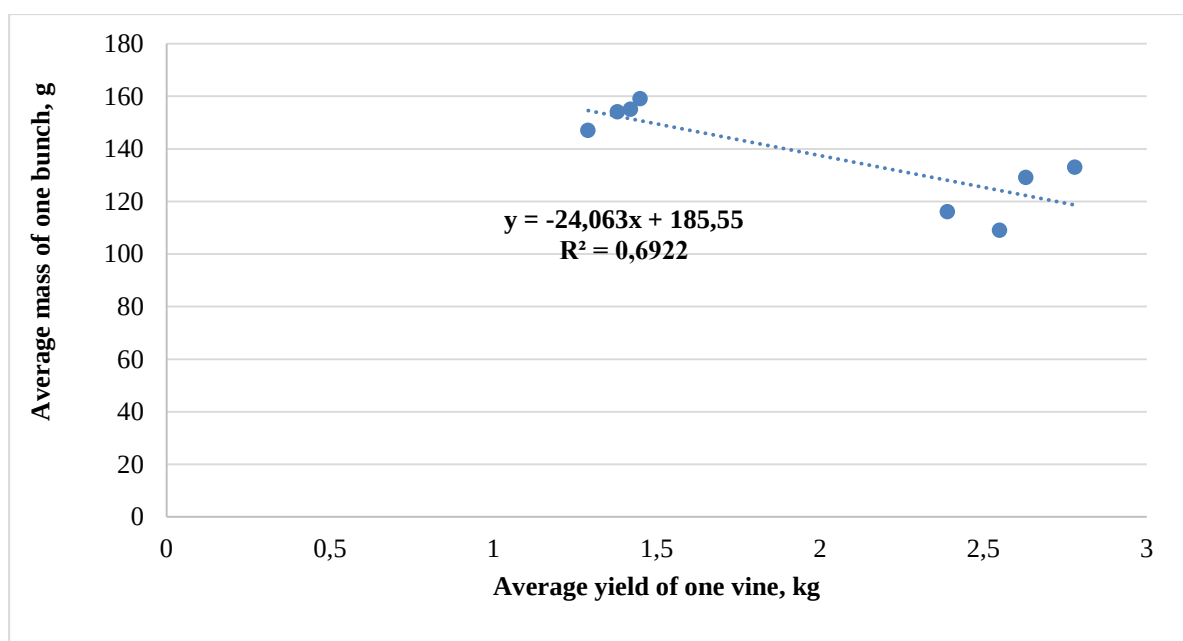


Figure 17. Linear regression model between average yield per vine and average mass of one bunch, 2021

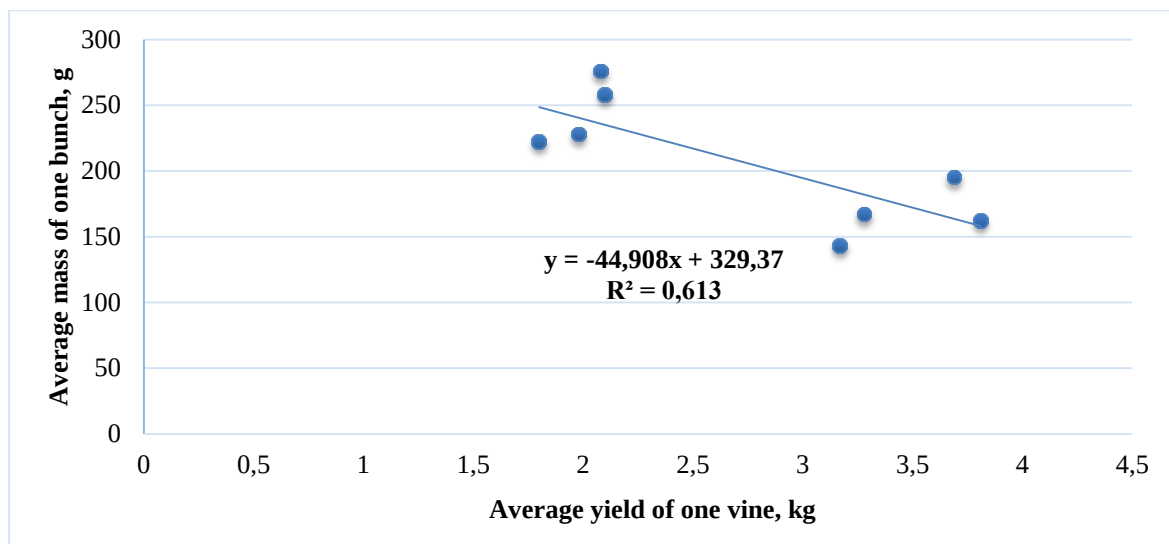


Figure 18. Linear regression model between average yield per vine and average mass of one bunch, 2022

For the year 2022, with the same considered indicators, 61% of the yield depends on the mass of one bunch, the coefficient of determination $R^2 = 0,613$ (Figure 18). We can assume that linear regression models are adequate.

6.2. Mass of one-year mature growth

Using all experimental data by variants and years, the degree of interdependence between average vine yield and average mass of mature growth in kg was determined, presented in Figure 19.

In our case, the coefficient of determination $R^2 = 0,8996$, which is evidence of a very high dependence (approximately 90%) between the two variables, i.e. the growth of vines depends very much on the yield of grapes per vine.

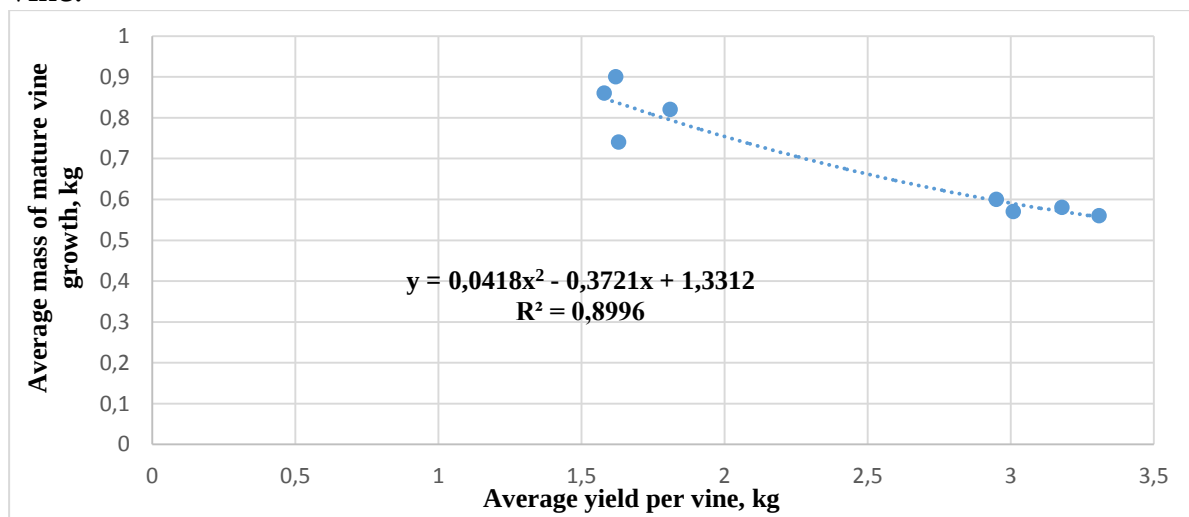


Figure 19. Dependence between vine yield and average mass of mature growth, kg

In the non-thinned variants (V_1 , V_2 , V_3 and V_4), the average yield obtained from 2,50 kg to 3,50 kg per vine, and the average weight of a mature growth about 0,60 kg. Compared to the variants with thinned yield (V_5 , V_6 , V_7 and V_8), from the average values obtained, the yield was found to be between 1,58 kg and 1,81 kg, and the average mass of mature growth was about 1,00 kg per vine. This gives us reason to argue that an increase in yield leads to a decrease in mature vine growth.

The yield according to the quality of the grapes is also determined by the proportional ratio between the vegetative and reproductive mass. The mass of the mature one-year growth is an important criterion for the growth strength of the vines.

The mass of the one-year mature growth is used to determine the load of the vines with buds and to solve questions related to yield optimization.

7. Research on the grape quality

7.1. Dynamics of grape ripening

The quality of grapes depends on the content of sugars, titratable acids, color substances, mineral salts, amino acids, etc. For wine varieties, the most important are the amount of sugars and titratable acids, which are necessary to determine the technological maturity of the grapes. During the ripening of grapes, significant changes occur in its composition. Sugars, coloring matter and varietal flavor increase, and acids decrease. These ingredients are of great importance for the future wine quality (Figures 20 and 21).



Figure 20. Dynamics in accumulation of sugars in the grapes, with non-thinned and thinned yield, in 2020, 2021 and 2022, %

When analyzing the results of the research for the three-year period of the studied indicators regarding the quality of grapes, it is confirmed that the thinned variants are distinguished by a higher intensity of accumulation of sugar content, compared to the non-thinned variants, during the grape ripening until technological maturity is reached. The content of titratable acids is inversely proportional to sugars (Figures 20 and 21).

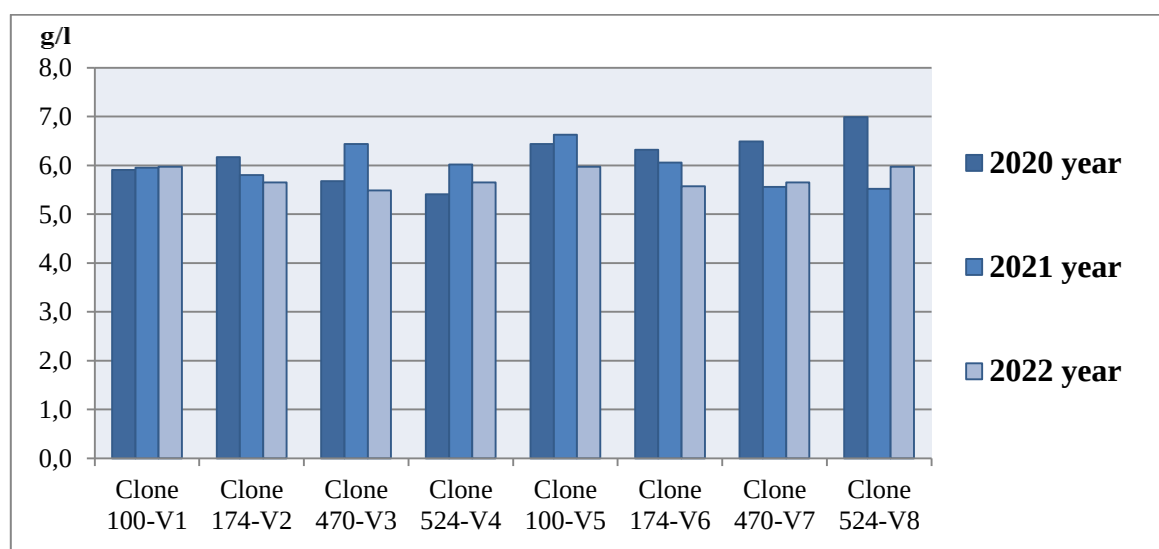


Figure 21. Dynamics of titratable acids in the grapes with non-thinned and thinned yield, in 2020, 2021 and 2022, g/l

During the three experimental years, technological maturity occurred one week earlier in the vines with thinned yield, compared to the vines with non-thinned yield.

On the harvest day, the average sugar values in the clones with thinned yield were on average 23,3%, and in the clones with non-thinned yield – 23,2%, respectively, the grape harvest was carried out when the same sugar values were reached. The content of titratable acids on the day of harvest in the thinned variants is on average 6 g/l, and in the non-thinned 6.5 g/l.

The sugar content and titratable acids depends not only from the bunch load, but also from the terroir.

When determining the date of the grape harvest, together with the chemical composition of the grapes, their sanitary condition and climatic conditions are taken into account, thus the quality of the grapes is preserved in a good sanitary condition - healthy, without rotting and acetic acidification.

7.2. Mechanical analysis of the grape and the berries

The percentage of normally developed berries in the bunches for all variants in the three experimental years was high from 93% to 96%, which is a prerequisite for very good grape quality (Tables 8 and 9). There are proven mathematical differences between the individual experimental variants in the vines with thinned yield – V_6 , V_7 and V_8 . The percentage of berries in the bunches for 2020 is from 4,20% (V_2) to 8,33% (V_5), in 2021 it is from 3,76% (V_3) to 5,76% (V_2) and from 3,72 (V_3) to 5,10% (V_1) in 2022. The largest differences were found in V_1 and V_5 in the three years of our study. The chronology is the same with the percentage of milleranded and raisined berries, with the largest proven difference in clone 100 (V_1 and V_5) during the study period.

When summarizing the results, it was found that the mass per bunch was significantly greater in the vines with thinned yield, with the biggest difference in 2022 for all experimental variants. The same sequence is preserved for the indicator - average size of each bunch. The biggest difference is proven in the variants with thinned yield V_6 and V_7 .

Regarding the percentage of mesocarp (Tables 10 and 11), there are established differences between the variants with thinned yield V_5 – 85,81% (2020), 84,84% (2021) and 87,28% (2022).

The average weight of 100 grains is greater in the variants with thinned yield (V_5 , V_6 , V_7 and V_8) compared to those with non-thinned yield (V_1 , V_2 , V_3 and V_4). From the obtained results, a difference was found in the vines with non-thinned yield V_1 and V_3 , and in the vines with thinned yield in V_5 and V_8 .

Number of seeds in 100 berries showed a difference between the non-thinned variants V_1 and V_3 , as well as with the thinned variants V_6 and V_8 . The average mass of seeds in 100 grains has a proven difference in vines with non-thinned yield V_3 and vines with thinned yield – V_8 .

Data for average berry size showed a greater difference in the vines with thinned yield, the most significant being in the variant V_5 , V_6 and V_8 in all three experimental years.

Extremely important for the quality of the wine are the sugar content (from 22,2% to 25,5%) and titratable acids (from 5,40 g/l to 6,99 g/l), which are within the normal range for obtaining of quality red wines, with greater differences being found between variants V_1 , V_4 , V_5 and V_8 .

The summary information on the indicators for grape structure, for the variants with non-thinned and thinned yield, are determined on the basis of an average sample (Tables 8, 9, 10 and 11).

Table 8. Structure and construction of a cluster in non-thinned variants, for the period 2020, 2021 and 2022

Option	Period (year)	Normal berries, %	Bunches, %	Milleranded berries, %	Raisin berries, %	Theoretical Randeman, %	Medium bunch weight, g	Medium bunch sizes, cm	
								Length	Width
V ₁	2020	95,50a	4,49ab	3,20c	1,08c	82,06b	124,00a	17,10a	9,60b
	2021	95,18a	4,82b	3,53d	1,06c	81,18b	109,00a	17,00a	10,40a
	2022	94,90a	5,10b	2,61d	0,95b	81,03a	162,00b	18,10a	10,50b
	Average	95,19	4,80	3,11	1,03	81,42	131,67	17,40	10,17
V ₂	2020	95,80a	4,20a	0,47b	0,94b	80,70b	136,00b	19,20b	8,40a
	2021	94,40a	5,60c	0,59a	1,05b	79,14b	116,00ab	18,47a	10,35a
	2022	95,67a	4,33ab	0,18b	0,45a	80,57a	167,00b	19,45b	9,75a
	Average	95,29	4,71	0,41	0,81	80,14	139,67	19,04	9,50
V ₃	2020	94,80a	5,12c	0,35a	0,81a	77,45a	142,00c	19,00b	8,80a
	2021	96,24a	3,76a	0,16a	0,99b	79,58ab	133,00c	20,50b	10,25a
	2022	96,28a	3,72a	0,12a	0,79b	80,88a	195,00c	19,50b	9,85a
	Average	95,77	4,20	0,21	0,86	79,30	156,67	19,67	9,63
V ₄	2020	95,40a	4,55b	0,34a	1,03c	77,13a	123,00a	18,90b	9,40b
	2021	95,15a	4,85b	1,17c	1,14c	77,91a	129,00bc	18,85ab	10,70a
	2022	95,18a	4,82b	0,52c	0,33a	79,14a	143,00a	19,00a	9,60a
	Average	95,24	4,74	0,67	0,83	78,06	131,67	18,92	9,90
LSD 5%	2020	4,89	0,37	0,02	0,10	3,01	5,87	1,68	0,55
LSD 5%	2021	4,16	0,84	0,06	0,11	2,01	8,09	2,31	0,99
LSD 5%	2022	5,12	0,81	0,05	0,15	2,89	10,02	1,20	0,80

*Comparative analysis with provenance $\alpha=0,05$

Table 9. Structure and construction of a cluster in thinned variants, for the period 2020, 2021 and 2022

Option	Period (year)	Normal berries, %	Bunches, %	Milleranded berries	Raisin berries, %	Theoretical Randeman, %	Medium bunch weight, g	Medium bunch sizes, cm	
								Length	Width
V ₅	2020	91,67a	8,33c	1,88d	0,95c	78,66b	158,00b	20,40a	10,70ab
	2021	94,57a	5,43c	1,74d	2,19d	80,23a	159,00c	21,70c	11,75b
	2022	94,50a	5,50d	2,10c	0,22a	82,48a	258,00c	21,15a	11,45b
	Average	93,58	6,42	1,91	1,12	80,46	191,67	21,08	11,30
V ₆	2020	94,45b	5,55b	0,43b	0,54b	79,78b	172,00d	22,10b	11,10b
	2021	95,19ab	4,81b	0,19a	0,52b	80,50a	154,00 b	20,05a	10,50a
	2022	95,61b	4,39c	0,13a	0,49a	80,96a	228,00b	21,90b	11,45b
	Average	95,08	4,92	0,25	0,52	80,41	184,67	21,35	11,02
V ₇	2020	95,33b	4,67a	0,57c	0,55b	77,76a	166,00c	22,10b	10,60a
	2021	95,67b	4,33a	0,16a	0,72b	79,55a	155,00b	22,05c	10,60a
	2022	96,92c	3,08a	0,46b	0,10b	81,78a	276,00d	21,85b	10,95a
	Average	95,97	4,03	0,40	0,46	79,70	199,00	22,00	10,72
V ₈	2020	95,00b	5,00a	0,16a	0,79b	77,61a	144,00a	21,90b	10,90ab
	2021	95,58b	4,42a	0,47b	1,37c	78,72a	147,00a	21,05b	11,55b
	2022	96,22b	3,78b	0,40b	0,13a	80,54a	222,00a	20,95a	10,60a
	Average	95,60	4,40	0,34	0,76	78,96	171,00	21,30	11,02
LSD 5%	2020	1,35	0,51	0,15	0,26	2,02	5,87	0,50	0,55
LSD 5%	2021	1,07	0,31	0,17	0,18	2,48	4,18	0,36	0,73
LSD 5%	2022	1,05	0,48	0,20	0,28	2,50	5,21	0,55	0,60

*Comparative analysis with provenance $\alpha=0,05$

Table 10. Berry structure in non-thinned variants, for the period 2020, 2021 and 2022

Option	Period (year)	Skins, %	Seeds, %	Mesocarp, %	Average weight per 100 berries, g	Number of seeds in 100 berries	Average seed weight in 100 berries, g	Medium berry sizes, mm		Chemical composition of grape juice (must)	
								Length	Width	Sugars, %	Acids, g/l
V ₁	2020	10,66a	3,41a	85,93a	155,26ab	195a	5,30a	14,20ab	13,10ab	24,57a	5,91b
	2021	11,27a	3,43a	85,30a	141,03b	160a	4,85a	14,05a	12,15a	23,27a	5,95a
	2022	11,16a	3,45a	85,39a	185,05b	225a	6,40a	15,20a	13,45a	23,40a	5,97b
	Average	11,03	3,43	85,54	160,45	193,33	5,52	14,48	12,90	23,75	5,94
V ₂	2020	12,26a	3,47b	84,27a	159,86b	205a	5,55b	14,40b	13,70b	24,05a	6,17b
	2021	12,89ab	3,27a	83,84a	132,29a	180b	5,65b	13,50a	11,80a	23,33a	5,83a
	2022	11,89a	3,89b	84,22a	177,8a	260b	6,90b	15,20a	13,50a	22,20a	5,65a
	Average	12,35	3,54	84,11	156,65	215,00	6,03	14,37	13,00	23,19	5,88
V ₃	2020	13,42ab	3,54b	81,70a	151,03a	200a	5,35a	13,80a	12,60a	26,45b	5,68ab
	2021	14,76b	3,89b	82,69a	136,70ab	225c	6,90c	13,40a	11,75a	23,15a	6,44b
	2022	12,21a	3,78b	84,01a	190,30b	245b	7,20c	15,70a	13,55a	23,00a	5,49a
	Average	13,46	3,74	82,80	159,34	223,33	6,48	14,30	12,63	24,20	5,87
V ₄	2020	14,38b	3,75b	80,85a	154,53ab	210a	5,80c	13,90a	12,60a	26,54b	5,41a
	2021	15,40b	3,74b	81,88a	131,70a	195b	5,50b	13,50a	11,85a	23,33a	6,02a
	2022	12,97a	3,88b	83,15a	175,15a	225a	6,80b	15,25a	13,45a	23,03a	5,65a
	Average	14,25	3,79	81,96	153,79	210,00	6,03	14,21	12,63	24,03	5,69
LSD 5%	2020	3,05	0,35	7,13	5,18	30,13	0,23	0,57	0,77	0,92	0,41
LSD 5%	2021	2,66	0,36	8,26	8,41	20,77	0,36	0,75	0,60	0,54	0,35
LSD 5%	2022	2,81	0,34	5,29	6,27	20,01	0,39	0,67	0,69	0,62	0,39

*Comparative analysis with provenance $\alpha=0,05$

Table 11. Berry structure in thinned variants, for the period 2020, 2021 and 2022

Option	Period (year)	Skins, %	Seeds, %	Mesocarp, %	Average weight per 100 berries, g	Number of seeds in 100 berries	Average seed weight in 100 berries, g	Medium berry sizes, mm		Chemical composition of grape juice (must)	
								Length	Width	Sugars, %	Acids, g/l
V ₅	2020	10,99a	3,20a	85,81c	170,56c	185b	5,45c	13,70a	11,50a	25,22b	6,44b
	2021	11,63a	3,53b	84,84b	149,30b	195a	5,72a	14,97c	12,60b	23,50a	6,63b
	2022	9,67a	3,05a	87,28b	201,30ab	200a	6,15a	15,65b	13,60a	24,27b	5,97b
	Average	10,76	3,26	85,97	173,72	193,33	5,77	14,77	12,57	24,33	6,35
V ₆	2020	12,34b	3,19a	84,47b	155,10b	160a	4,95a	13,80a	11,80b	24,30a	6,32a
	2021	12,09a	3,34a	84,57b	149,70b	190a	5,90ab	14,35ab	12,35ab	23,20a	6,06b
	2022	11,15b	4,17c	84,68a	195,60a	285c	8,15c	15,60b	13,85a	22,40a	5,57a
	Average	11,86	3,57	84,57	166,80	211,67	6,34	14,58	12,67	23,30	5,98
V ₇	2020	14,85c	3,58b	81,57a	142,33a	175b	5,10b	13,80a	11,40a	25,55b	6,49b
	2021	12,86b	3,99d	83,15a	146,50ab	215b	6,60c	14,00a	12,26a	23,30a	5,56a
	2022	12,18d	3,65b	84,17a	198,30A	235b	7,25b	14,10a	13,95a	23,35b	5,65a
	Average	13,30	3,74	82,96	162,37	208,33	6,32	13,97	12,54	24,06	5,90
V ₈	2020	14,71c	3,59b	81,70a	153,63b	150a	5,50c	14,30b	12,40c	25,55b	6,99b
	2021	13,96c	3,68c	82,36a	141,70a	190a	5,95b	14,80bc	12,15a	22,30a	5,52a
	2022	11,93c	4,37d	83,70a	209,00b	295c	9,15d	15,75b	13,75a	23,25ab	5,97a
	Average	13,53	3,88	82,58	168,11	211,67	6,86	14,95	12,76	23,70	6,16
LSD 5%	2020	0,74	0,15	1,29	6,42	15,24	0,18	0,59	0,31	0,61	0,15
LSD 5%	2021	0,67	0,15	1,25	7,10	18,62	0,27	0,68	0,32	0,71	0,20
LSD 5%	2022	0,71	0,21	1,42	7,20	19,02	0,42	0,72	0,42	0,91	0,26

*Comparative analysis with provenance $\alpha=0,05$

8. Wine analyses

8.1. Physico-chemical analysis

The results from physico-chemical analysis of the experimental wines from the three years study demonstrate significant differences in their analytical composition, both depending on the farming technique (thinned and non-thinned yields) and on the clone as well (Tables 12, 13 and 14).

Analyzing the results, it was found that the thinned variants (V₅, V₆, V₇ and V₈) had higher content of total and sugar-free extract, higher content of anthocyanins, total phenolic substances and higher color intensity compared to non-thinned variants (V₁, V₂, V₃ and V₄) from the same vintages. It is known that yield standardization is one of the most important tools in the production of quality wines with a controlled designation of origin, and the results of the study clearly illustrate this.

As a rule, the wines obtained with reduced yield (V₅, V₆, V₇ and V₈) from all four investigated branches received higher tasting ratings, compared to the wines from the variants with non-reduced yield (V₁, V₂, V₃ and V₄), under the conditions of hidden and anonymous tasting.

The content of alcohol and titratable acids is within optimal limits, and the differences between the thinned and non-thinned variants are minimal, since all experimental variants were selected at approximately the same technological maturity, which was reached earlier in the reduced yield variants.

High day and night temperatures combined with low humidity are responsible for the synthesis and accumulation of the highest amounts of total phenolics in the grapes and wine of this vintage. It is noteworthy that the high content of total phenolic substances in the experimental wines from the 2020 vintage is not correlated with the anthocyanin content, compared to the wines from the 2021 and 2022 vintages. The lower anthocyanin content and lower color intensity in the 2020 vintage wines is likely due to the very high summer temperatures. It is known that at temperatures above 33°C, the synthesis of anthocyanins is inhibited and their destruction is observed.

A higher content of total and sugar-free extract was found in the wines from the 2020 and 2022 vintages, compared to 2021 (especially in the variants with reduced yield). This result is probably due to the significant rainfall during the ripening period of the 2021 grapes, which leads to dehydration of the grape juice and is the reason for the lower content of total and unsweetened extract of the wines from 2021 vintage, compared to the wines from 2020 and 2022 year. For the same reason, wines from 2021 vintage have almost the same (unified) content of sugar-free extract in the normalized and non-normalized variants, while the wines from 2020 and 2022 vintages, a clear tendency is observed for the wines from the thinned variants to have a higher extract content and a denser, more concentrated and more voluminous body, respectively.

The obtained aggregated results of the three years show differences in the physico-chemical indicators of the wines from the studied clones at each harvest. It can be definitely concluded that the wines from clone 100 from three vintages have the highest anthocyanin content, consequently the highest color intensity, the highest percentage of red color and the lowest color shade. The above is an indication that the wines of clone 100 are with most intense dense and sparkling color with the maximum dominance of red over yellow-brown and blue shades in it.

For wines from clone 100 (V_1 and V_5), a higher alcohol content was reported, which correlated with a higher sugar content of the grapes, for both the thinned and non-thinned variants. This is valid to the greatest extent for the wines from the 2022 vintage and allows us to make the assumption that the grapes from this branch have the highest energy of sugar accumulation.

The wines from clone 100 have the highest content of total phenolic substances compared to the wines from all other clones, which makes these wines better structured, but at the same time rougher and astringent.

The wines from clone 174 (V_2 and V_6) demonstrated the lowest alcohol content (respectively the lowest grape sugar content during harvest). They are characterized by the lowest content of coloring matter (anthocyanins) and therefore have the lowest intensity of coloring. In two of the vintages (2020 and 2021), wines from clone 174 contained the lowest amounts of total phenols, which is why these vintages were characterized by tasting as not particularly well structured, with insufficient concentration and volume.

In the 2022 vintage, wines from clone 174 (V_2 and V_6) have a better concentration of total phenols and are rated as harmonious, soft with good body and length.

Wines from clone 524 in the thinned (V_8) and non-thinned (V_4) variants in the 2020 and 2022 harvest have a slightly higher content of total and sugar-free extract, which favorably affects their volume and flavor density.

Analyzing the results, the wines from clone 524 (V_4 and V_8) ranked behind clone 100 in terms of anthocyanin content, high color intensity, bright and sparkling color. The moderate (but sufficient) content of total phenols makes the wines of this branch well-structured, yet soft and harmonious, which is why they also received the highest tasting ratings during the three years study.

Table 12. Physico-chemical analysis of the wine - 2020 harvest

Variant		V 1 Clone 100	V 2 Clone 174	V 3 Clone 470	V 4 Clone 524	V 5 Clone 100 thinned	V 6 Clone 174 thinned	V 7 Clone 470 thinned	V 8 Clone 524 thinned
Research INDEX									
RELATIVE DENSITY		0,9917	0,9931	0,9937	0,9927	0,9924	0,9920	0,9920	0,9926
ALCOHOL, Vol. %		15,10	14,30	15,91	15,91	15,28	14,92	15,37	15,19
SUGAR, g/dm ³		2,62	3,58	8,48	4,34	2,11	1,94	2,00	2,00
EXTRACT, g/dm ³	TOTAL	28,70	30,00	36,20	33,60	31,00	28,90	30,20	31,30
	SUGAR FREE	26,08	26,42	27,72	29,26	28,89	26,96	28,20	29,86
pH		3,53	3,55	3,69	3,71	3,72	3,72	3,72	3,80
TITRABLE ACIDS, g/dm ³		6,86	7,02	6,54	6,62	7,02	6,94	7,02	6,86
VOLATILE ACIDS, g/dm ³		0,73	0,77	0,64	0,43	0,61	0,51	0,32	0,48
SULFUR DIOXIDE, mg/dm ³	FREE	34,24	30,58	30,58	39,14	11,07	8,56	12,25	10,40
	TOTAL	127,19	133,92	126,58	128,42	42,19	38,52	47,70	46,11
ANTHOCYANS, mg/dm ³		756,00	575,75	660,63	682,4	794,50	622,40	718,32	744,28
TOTAL PHENOLS, mg/dm ³ , such as gallic acid		2514,40	2083,36	2370,72	2375,21	2765,84	2330,31	2440,80	2406,64
COLOR INTENSITY		16,48	12,21	14,76	15,16	21,42	17,42	17,17	17,67
NUANCE OF COLORING		0,566	0,617	0,633	0,661	0,588	0,598	0,607	0,602
COLOR, %	YELLOW-BROWN	31,61	33,49	33,47	34,70	32,49	32,72	33,14	32,94
	RED	55,89	54,30	52,91	52,51	55,28	54,71	54,63	54,67
	BLUE	12,50	12,21	13,62	12,79	12,23	12,57	12,23	12,39
TASTING EVALUATION		80	78	81	82	80	79	83	85

Table 13. Physico-chemical analysis - 2021 harvest

Variant		V 1 Clone 100	V 2 Clone 174	V 3 Clone 470	V 4 Clone 524	V 5 Clone 100 thinned	V 6 Clone 174 thinned	V 7 Clone 470 thinned	V 8 Clone 524 thinned
Research INDEX									
RELATIVE DENSITY		0,9928	0,9930	0,9932	0,9932	0,9932	0,9936	0,9933	0,9935
ALCOHOL, Vol. %		14,3	14,12	14,12	14,12	13,85	13,59	13,67	13,32
SUGAR, g/dm³		2,12	1,76	1,88	2,51	1,38	1,65	2,26	1,23
EXTRACT, g/dm³	TOTAL	29,2	29,2	29,7	29,7	28,9	29,2	28,7	28,1
	SUGAR FREE	27,08	27,44	27,82	27,19	27,52	27,55	26,44	26,87
pH		3,35	3,48	3,45	3,49	3,58	3,67	3,53	3,62
TITRABLE ACIDS, g/dm³		7,88	7,11	7,31	7,19	7,12	6,69	7,12	6,69
VOLATILE ACIDS, g/dm³		0,42	0,36	0,44	0,38	0,31	0,32	0,47	0,34
SULFUR DIOXIDE, mg/dm³	FREE	8,11	13,51	16,21	14,86	13,51	13,51	14,86	8,11
	TOTAL	35,13	41,88	59,44	41,21	43,23	40,53	42,56	40,53
ANTHOCYANS, mg/dm³		764,75	623,88	679	668,5	829,5	722,75	742,88	728,00
TOTAL PHENOLS, mg/dm³, such as gallic acid		1580,48	1495,17	1558,03	1454,76	1840,9	1516,20	1620,80	1544,20
COLOR INTENSITY		24,57	17,88	18,41	18,11	23,9	20,46	20,59	19,05
NUANCE OF COLORING		0,475	0,505	0,514	0,522	0,512	0,541	0,510	0,540
COLOR, %	YELLOW-BROWN	28,65	30,09	29,88	30,2	29,70	30,60	30,01	30,71
	RED	60,36	59,56	58,12	57,87	57,99	56,55	58,86	56,85
	BLUE	10,99	10,35	12,00	11,93	12,31	12,85	11,13	12,44
TASTING EVALUATION		74	77	76	78	76	81	80	83

Table 14. Physico-chemical analysis - harvest 2022

Variant		V 1 Clone 100	V 2 Clone 174	V 3 Clone 470	V 4 Clone 524	V 5 Clone 100 thinned	V 6 Clone 174 thinned	V 7 Clone 470 thinned	V 8 Clone 524 thinned
Research INDEX									
RELATIVE DENSITY		0,9927	0,9939	0,9936	0,9933	0,9931	0,9946	0,9943	0,9946
ALCOHOL, Vol. %		14,3	13,15	13,41	13,67	14,56	13,24	13,5	13,59
SUGAR, g/dm³		1,27	1,21	1,12	0,94	1,12	1,02	0,75	0,81
EXTRACT, g/dm³	TOTAL	28,9	28,7	28,7	28,7	30,7	30,7	30,7	31,8
	SUGAR FREE	27,63	27,49	27,58	27,76	29,58	29,68	29,95	30,99
pH		3,57	3,69	3,65	3,65	3,77	3,84	3,81	3,82
TITRABLE ACIDS, g/dm³		7,13	6,25	6,69	6,9	7,3	6,64	6,51	7,13
VOLATILE ACIDS, g/dm³		0,49	0,37	0,37	0,42	0,35	0,35	0,35	0,36
SULFUR DIOXIDE, mg/dm³	FREE	17,96	16,47	14,97	13,47	20,96	19,46	19,46	17,96
	TOTAL	58,38	53,14	59,88	57,63	68,86	59,13	60,63	59,88
ANTHOCYANS, mg/dm³		706,13	537,25	582,75	615,13	812,88	555,63	604,63	648,12
TOTAL PHENOLS, mg/dm³, such as gallic acid		1616,40	1477,21	1373,94	1257,2	1714,50	1562,22	1484,30	1386,40
COLOR INTENSITY		14,76	8,64	9,59	10,67	14,94	8,87	9,94	10,90
NUANCE OF COLORING		0,521	0,576	0,541	0,545	0,557	0,639	0,605	0611
COLOR, %	YELLOW-BROWN	30,22	32,13	31,17	31,02	31,59	35,36	33,0	33,8
	RED	57,99	55,77	58,6	56,89	56,76	55,32	54,53	55,3
	BLUE	11,79	12,10	10,23	12,09	11,65	9,32	12,47	10,9
TASTING EVALUATION		77	80	78	81	78	84	80	83

8.2. Organoleptic wine analysis

For a more detailed study of the differences in the organoleptic wine profiles from the studied clones, radar (spider) diagrams were made, from which the differences in the organoleptic profile could be seen. The studied clones and variants were evaluated from 1 to 10 points according to the following indicators: color intensity, aroma intensity, fruitiness, aroma finesse, flavor intensity, body, flavor harmony/balance, flavor length and aftertaste (Figures 22 and 23).

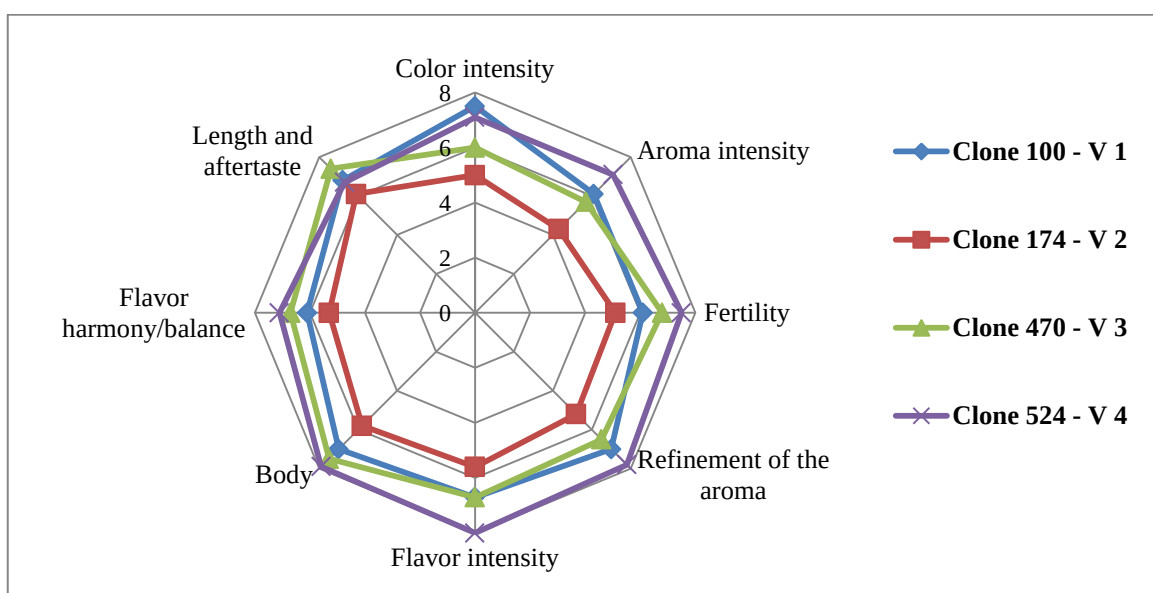


Figure 22. Organoleptic wine characteristics non-thinned variants in average for the period 2020, 2021 and 2022

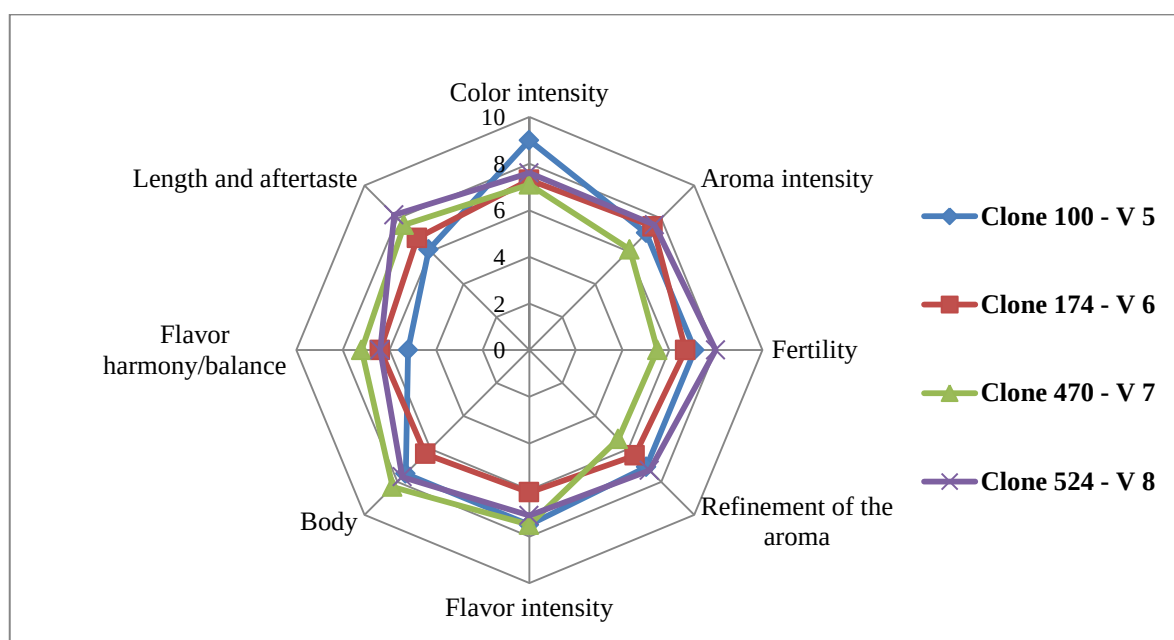


Figure 23. Organoleptic wine characteristics thinned variants, in average for the period 2020, 2021 and 2022

Regarding the fruitiness of the aroma, the wines from clone 524 received the highest score again, but only in the non-thinned variants. In the thinned variants, wines from clone 470 received the highest score for this indicator, followed by clone 174.

The tasting committee gave preferences regarding the subtlety of the wine aroma from clone 524 and clone 470 (equal scores), but only for the wines from the thinned variants. In the case of non-thinned variants, according to this indicator, wines from clone 174 are preferred, followed by clone 524.

8.3. Analysis of C₁₃ - norisoprenoids in wines

For more precise study and to enrich the information of the experimental wines obtained from the studied clones of the Syrah variety, studies were made by measuring the amounts of C₁₃ - norisoprenoids (β - damascenone, α and β - ionone), which are leading compounds depending on the effect of terroir on the typicality and aromatic wine.

Variants with non-reduced yield, where there are more bunches per vine, have a higher concentration of β -damascenone. The correlation was reversed for the thinned variants (V₅, V₆, V₇ and V₈), for which a lower concentration of β -damascenone was reported, due to the fewer bunches left on one vine.

8.4. Analysis of trans-resveratrol in wines

Grapes and wine, with their diverse chemical composition, refer to foods and beverages possessing certain characteristics and qualities that have a beneficial effect on human health. Trans-resveratrol is an extremely rare substance.

Summarizing the obtained results, it was found that clone 524 (V₄) with non-reduced yield and clone 100 (V₅) with reduced yield have the highest concentration of trans-resveratrol in the wine.

Clone 174 had the lowest trans-resveratrol content in the wine, both in the non-reduced yield variant (V₂) and in the reduced yield variant (V₆).

For this special highlight, trans-resveratrol, which gives the wine its antioxidant properties, we can say that its amount in the wine directly depends on the clone and the grape harvest. Factors such as climate region and terroir are known to influence the polyphenolic compounds in wine and therefore its quality.

To obtain wine with higher levels of trans-resveratrol, viticultural practices and oenological technologies are very important.

IV. Conclusions

Based on the research results, the following important conclusions could be drawn:

1. The growing area of the clones 100, 174, 470 and 524 from Syrah variety has favorable climatic conditions for obtaining red wines. On average for the studied period, the total temperature sum is 4364°C, the active temperature sum is 2067°C, the number of days with a temperature above 10°C is 230. The average amount of precipitation for three years period is 618 mm, which characterizes it as moderately dry.
2. The soil is deluvial-meadow, loamy-sandy to sandy-loamy. The profile has a depth greater than 360 cm. There is no overwetting and no surface or actual oiling. The clay content is 30 - 40%, and the soil reaction is slightly alkaline (Ph 7,6). The availability of nitrogen and phosphorus in the soil is low, and the potassium content is sufficient. The amount of active calcium is also low - 15 g/kg. This defines the soil complex as favorable for growing red wine varieties.
3. The vines vegetation period in clones 174 and 470 begins earlier compared to the vines from clone 100 and clone 524. This should be taken into account when planting them on terrains with frequent recurrence of late spring frosts. The phenological period in days from the beginning of bud burst to technological maturity in the variants with yield reducing on average is 142 days, and in the variants with non-reduced yield is 151 days.
4. The obtained results of the three experimental years prove that clone 470 is the most vigorous growing, reaching the greatest average shoot length, both in the variants with thinned and non-thinned yield. This is directly related to the higher photosynthetic leaves activity and the higher water content in the plant tissues during the months of May and June. From a practical point of view, this should be taken into account when choosing a clone to plant vines under non-irrigated conditions on dry terrains.
5. Variants with reduced yield in the pea size phase (V_5 , V_6 , V_7 and V_8), the highest degree of ripening is reached by the shoots of clone 100, and the lowest by the shoots of clone 470. By overloading the plants with grapes causing the incomplete shoot maturation.
6. The studied clones are distinguished by high actual fertility of the vines /Kr – 1,72 for clone 100 and 1,54 for clone 524/. The fertility coefficient of the fruiting shoots is also high /1,83 in clone 100 and 1,69 in clone 524/. The yield is formed to the greatest extent by shoots with two bunches /81,90% in clone 100 and 75,11% in clone 524/. Both clones have the ability to produce a higher percentage of fruiting shoots derived from dormant buds, which would be beneficial for plant recovery and partial yield after late spring frost damage.
7. The average coefficient of determination $R^2 = 0,658$ shows that 66% of the yield depends on the mass per one bunch. The yield mass per vine in the non-thinned variants (V_1 , V_2 , V_3 and V_4) is almost twice as high as the thinned variants (V_5 , V_6 ,

V₇ and V₈), which also explains the reduction in the number of bunches in order to increase their quality.

8. The reduction in the number of grapes has an impact on extremely important indicators for the wine quality: the dynamics of the sugar content and titratable acids in the grapes. Within the experimental period, all variants reached the technological maturity stage relatively early (beginning of September). The grape harvest of the vines with reduced yield (V₅, V₆, V₇ and V₈) precedes those with non-reduced yield (V₁, V₂, V₃ and V₄) by approximately one week.

9. We could categorically conclude that the largest amount of coloring matter (anthocyanins) is contained in the wines from clone 100, which is due to the higher color intensity into the wines from this branch.

10. The physico-chemical composition and organoleptic wine qualities are significantly influenced by the branch. Standardized yield variants (V₅, V₆, V₇ and V₈) have higher total and sugar-free extract, higher anthocyanin content, total phenolics and higher color intensity, which accounts for the higher tasting scores compared to the unstandardized yield variants (V₁, V₂, V₃ and V₄).

11. In terms of tasting, the wines from clone 524 received the highest marks, followed by 174 and 470, and the wines from clone 100 received the lowest marks. The wines from clone 524 were awarded with the highest intensity of aroma, finesse and fruitiness, followed by the wines from clones 174 and 470. The wines from clone 524 are preferred, as the best also according to the indicators - intensity of taste, body, harmony, length of the taste, followed by the wines from clones 174 and 470.

12. The content of C₁₃-Norisoprenoids (β -damascenone, α -ionone and β -ionone) in wines are significantly influenced by the harvest, the degree of bunch openness to direct sunlight, but also by the clone. The highest content of norisoprenoids was found in the experimental wines from clone 470, followed by 174 and 524.

13. The content of trans-resveratrol in wine depends on the climatic characteristics of the region, as well as on the variety. Based on the results obtained, the wines with the highest content of trans-resveratrol are distinguished from clone 524 with non-reduced yield and clone 100 with reduced yield. The wines obtained from clone 174 with reduced and non-reduced yield had the lowest content of trans-resveratrol.

V. Scientific - applied contributions

1. Scientific contributions

1.1. For the first time, the reaction of clones 100, 174, 470 and 524 from Syrah variety, grown in the conditions of the Rhodope collar, high-stemmed, with a short pruning system and loading with 12 buds per vine, was determined. The Syrah 100 and 524 clones have shown their biological potential to the greatest extent.

1.2. The content of C₁₃-Norisoprenoids (β -damascenone, α -ionone and β -ionone) in wines varies significantly, depending on the clone biology and the cultivation technology. The wines under the soil and climate conditions of the Rhodope collar are distinguished by a rich aromatic profile.

1.3. Syrah clone 524 wines with a reduced yield are distinguished by the highest organoleptic qualities - high content of total and sugar-free extract, content of anthocyanins, total phenolic substances, higher color intensity, aroma, finesse, body, harmony, length of taste and fruitiness.

2. Applied contributions

2.1. Syrah 524 and Syrah 100 wines with a reduced yield are distinguished by the highest content of coloring matter and trans-resveratrol, which makes them suitable for the pharmaceutical industry in the production of drugs against cardiovascular, cancer, neurodegenerative and other diseases.

2.2. The quality of the wines obtained from the clones 100, 174, 470 and 524 is much higher when summer pruning operations are applied, such as pruning, thinning the number of bunches and others, which necessitates the determination of the optimal number of bunches, as a compulsory practice.