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Influence of growth biostimulators used for the production of vine planting material

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Abstract: Growth biostimulators activate some physiological processes that increase the efficiency of the usage of nourishing substances, stimulate the development of plants and allow reduced fertiliser consummation. Most biostimulators are capable of reacting against the effect of biotic and antibiotic stresses as they increase the crop production of the cultures and represent an opportunity of growing quality and healthy food. The article affects the results of the influence of RadiCifo L24 and Euroradix over the growth and development of grafted cuttings in vine rootings. The study was carried out through the dessert seedlees grapevine variety 'Zornitsa' grafted over the Berlandieri × Riparia (SO4) pad. Before rooting, paraffin is applied to 2/3 of the stratified grafted cutting's length and is then immersed in a 2% solution of RadiCifo L24 (for 20 minutes) and Euroradix (for 5 minutes), after that the cuttings are rooted to 15–17 cm in depth (to the non-paraffined part). According to the biometrics rating (number of developed shoots, number of roots), the variant with the growth stimulator RadiCifo L24 seems to be a more effective method for the production of first-class vines of the 'Zornitsa' variety, thus it can be recommended for the production of vine planting material.

Keywords: vine rooting; common increase; root forming; fertilisers

The viticulture and wine industry contribute to the conomy and reputation of many countries all over the world. With the predicted climate change, a negative impact on the grapevine physiology, growth, production, and quality of berries is expected. Climate change and disproportionate anthropogenic interventions, such as the excess of phytopharmaceutical products and continuous soil tillage, are jeopardising viticulture by subjecting plants to continuous biotic and abiotic stresses. In light of this scenario, it is inevitable that sustainable techniques and sensitivity approaches for environmental and human health have to be applied in viticulture. Sustainable viticulture can only be made with the aid of sustainable products. Biostimulants (PB) applications (including resistance inducers or stimulators) have been used to control vine diseases and improve grape quality (Cataldo et al. 2022; Monteiro et al. 2022).

The creation of profitable vine rootings depends on the quality of the initial planting material. Improvement in the production techniques of the vine planting material is the premise for studying fertilisers and

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growth stimulators that increase the yields of the standard planting material. The mineral nutrients, humic acids, vitamins, enzymes, proteins and phytohormones used for pre-sowing seed treatment of field crops and to stimulate the root system and leaf mass in the vine (Prodanova-Marinova 2016; Staneva, Gospodinova 2018; Iliev, Pachev 2020).

Dimitrova et al. (2010), Tsvetanov et al. (2014) ascertained positive effects by applying "Immunocitofit" and "Humustim" to the rooting of cuttings into the vine rooting. Many scientists have proven that the usage of biostimulators and biofertilisers is an opportunity to increase the yield and quality of the production for different cultures (Koteva et al. 2013; Delchev, Stoyanova 2015; Vlahova, Popov 2018). According to Pachev and Prodanova-Marinova (2016) the „Root-Most" growth regulator stimulates the growth of the shoots and the formation of a mature annual increment with greater length, weight and diameter of internodes of the grafted vine cuttings. Polat et al. (2012) and Pacholczak et al. (2013) announced the positive effect on the root system of plants in which leaf fertilisers that contain humic acids are used. The usage of organic agents that have the characteristics of growth biostimulators, microfertilisers, and anti-depressants increases the yield of standard rooted vines, helps the reduction of losses linked with the production of vine planting material, and achieves a better economic effect (Titova 2014; Otoo et al. 2016; Cioroianu et al. 2017).

The purpose of the present study is to determine the effect of the growth biostimulators RadiCifo L24 and Euroadix on the root system and the growth dynamics of grafted vines used for the production of the vine planting material of the 'Zornitsa' variety.

MATERIAL AND METHODS

In the period 2018–2021, in the experimental vine nursery of the Institute of Agriculture and Seed Science (IASS) "Obraztsov Chiflik" – Ruse, Bulgaria, on the soil type leached black soil, the influence of two growth biostimulants of natural origin – RadiCifo L24 and Euroradix on the root formation, growth dynamics and total improvement of the dessert seedless grapevine variety 'Zornitsa' grafted onto the rootstock Berlandieri × Riparia SO4 was tested. The two growth biostimulants tested are characterised by a complex organo-mineral composition.

RadiCifo L24 (Cifo, Italy) is an innovative product designed specifically to stimulate the rooting process and plant growth. The preparation contains macrocystis Integrifolia, amino acids, humic and fulvic acids, L-tryptophan and chelated zinc. Thanks to the synergistic effect of these components, RadiCifo L24 improves the root formation and growth, nutrient uptake and soil structure, increasing the fruit size and uniformity.

Euroradix (Eurovix S.p.A., Italy) contains natural enzyme components (cellulase, hemicellulases, alpha-amylases, beta-amylases, pentosanases, glucoamylases, proteases, phosphorylases, pulullanases, pectinases, beta-glucanases, lipases, endochitinases, exochitinases, polyphenoloxidases, etc.), beneficial microflora (Glomus spp. Gigaspora, Rhizopogon, Trichoderma spp., Bacillus spp.) and oligopeptides – inducing the development of microbes of natural origin, carbohydrates and nourishing minerals.

The preparation improves the soil fertility, reduces any crop damage resulting from chemical residue and soil salinisation. Thanks to the rhizosphere bacteria and mycorrhizal fungi involved, the plants optimally absorb nutrients from the soil. The content of Trichoderma and Bacillus is a prerequisite for the effective protection against a wide range of pathogenic organisms.

The active fertility of the soil is characterised as containing a good amount of potassium (33.17 mg/100 g soil), not enough nitrogen nutrients (16.84 mg/100 g soil) and bad phosphoric nutrients (6.15 mg/100 g soil). The physico-chemical properties are characterised as a low acidic soil reaction (Sabev, Stanev 1963; Dimitrov 1990). The experiment included 472 grafted vines planted in four replicates (118 each replicate), with an experimental plot area of 3.96 m^2 and were compared with a control (non-treated) variant with grafted vines of the same variety planted for rooting with the same replicate size. Before rooting, the stratified grafted cuttings were paraffined up to 2/3 of their length and immersed in a 2% solution of RadiCifo L24 (for 20 minutes) and Euroradix (for 5 minutes). The paraffinised grafted cuttings were rooted to a depth of 15–17 cm (to the non-paraffinised part) according to the technology of open cultivation, adopted by IASS "Obratzov Chiflik" (Todorov 2005). The planting scheme is in two-row beds with a distance of 50 cm between the rows and 7–8 cm in the rows. During the experiment, the following options were tested: *con-*

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trol – soaking the roots in water; *V1* – by soaking the roots in a 2% solution of RadiCifo L24 and *V2* – by soaking the roots in a 2% solution of Euroradix. After the cuttings were stacked in the vine roots, to combat weeds, spraying with the soil herbicide Stomp Nov 330 EC (pendimetalin 330 g/L) was carried out at a dose of 600 ml/da, and the experimental plots were kept free of weeds by hand weeding throughout the growing season. The grafted cuttings of all the variants were fertilised equally with nitrogen, phosphorus and potassium fertilisers. The following biometric indicators were tracked during the experiment's output: overall mature growth length (cm); overall mature growth mass (g); diameter of the internodes (mm); length of the internodes (cm); number of roots, including those with a diameter of less than two mm and more than two mm; average yield of the first-class rooted vines, in % of grafted cuttings. A comparative analysis was carried out on the indices of the 'Zornitsa' variety vine based on the results of the experiment over the four-year test period (SPSS; version 19.0) (Ganeva 2016). To establish the influence of the growth stimulators on the examined indices, a two-factored disperse analysis with statistical significance at a level P = 0.05 was applied. To determine the statistical and reliable differences between the examined variants, an analysis of variance (ANOVA), by calculating the least significant difference (LSD), Fischer's test, was applied.

RESULTS AND DISCUSSION

By estimating the complex influence of the climactic factors (amount of rainfall and average month temperatures) during the vegetation of the set for the rooting vines, the years were relatively good for their development (Table 1). The average monthly temperature of the air according to the multi-year norm (a period of 109 years) was 15.8 °C. The data state that the examined years had higher air temperatures than the multi-year norm and vary from 13.5 °C to 25.3 °C. June 2021 and July 2018 were the exception, as the measured average temperatures were below the average for the multi-year period with a difference of –0.22 °C and –0.21 °C, respectively. The yearly change in the temperatures shows that years can be classified as hot and are distinguished with notably higher average monthly air temperatures compared to the multi-year period. The period of active vegetation of the set for the rooting vines (from May to October), during the years of the study, clearly expressed a trend of higher temperature differences compared to the multi-year norm (+ 0.04 °C to + 8.0 °C). The months June 2021 and July 2018 had a lower temperature difference compared to the multi-year norm (–0.22 ºC to –0.21 °C), but they did not have any influence on the development of the set for the rooting vines.

The average amount of rainfall during the vegetation of the vine for the multi-year period is

Table 1. Average monthly values for the main meteorological elements at the time of the experiment

Period		Vegetation period						Average V–XI
		V	VI	VII	VII	IX	X	
Average monthly air temperature (°C)								
Average for MP		16.4	20.2	22.5	23.9	17.9	7.2	15.8
Diversion (± °C from MP)	2018	5.0	1.7	–0.2	0.0	1.3	6.3	2.4
	2019	0.6	2.2	0.5	0.5	1.7	6.6	2.9
	2020	0.1	0.4	1.6	1.4	2.7	7.5	2.4
	2021	0.4	–0.2	2.1	0.0	–0.2	8.0	2.0
Monthly rainfall sum (mm)								
Average for MP (1896–2005)		66.2	80.9	66.7	48.4	43.0	40.0	55.7
Diversion (% from MP)	2018	53.0	130.3	251.6	27.5	153.0	77.3	127.0
	2019	106.3	159.6	29.2	21.7	10.2	88.5	75.5
	2020	101.4	155.7	1.6	42.4	111.2	165.5	89.8
	2021	111.0	149.6	1.5	58.7	11.4	165.5	79.9

MP – multi-year period

The period from 1896 to 2006 was chosen for the multi-year period as the climate norm, as this period contains decades with differences in the meteorological factors

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55.7 mm. The rainfall for the period of 2019 to 2021 did not exceed the rainfall of the multi-year norm which was from 75.5% to 89.9%. The wettest year was 2018, during which the rainfall (72.4 mm) exceeded the rainfall of the multi-year period norm (55.7 mm) by 127%. The amount of rainfall during the summer months of July and august in the years 2018 to 2021 are way below seems the rainfall of the multi-year period. As a percent relationship, compared to the norm, the rainfall varied from 1.5% to 58.7%. An exception was July 2018, whose rainfall largely exceeded the rainfall of the norm (by 251.6%). September 2019 and September 2021 were drier, as the rainfall was 11% less than the norm. During the other months, an increasing trend in the rainfall could be observed. During the other months, an increasing trend in the rainfall could be observed, and according to a Alexandrov (2005), the equivalent of soil drought (insufficient precipitation) is 50 mm per month.

Two-way analysis of variance was applied to determine the effect of growth biostimulants on the studied parameters during the study period. In this analysis, the growth biostimulants and the year of the experiment participated as control factors, and all the studied indices participated as parameters. Each parameter was determined in triple iteration. For convenience, the analysis is presented in tabular form. Tables 2 and 3 show the calculated F – criteria and probability P values that refer to the controlled factor. Probability P is mainly used in the analysis and is compared to the statistical significance at $\alpha = 0.05$. In the case of the situation where the condition $P \leq \alpha$ is used for the controlled factor, the factor is accepted as having a great influence over the parameter. In Table 2, it can be seen that probability P is lower than the statistical significance α for the two controlled factors of two of the parameters (number of roots and roots with a diameter > 2 mm). Therefore, the two controlled factors influence the change of the parameters, number of roots and roots with diameter > 2 mm. For the parameter, roots with diameter < 2 mm, the influence of the factor year shows definite levels of the growth biostimulator factor, which is shown in the Table 2 as a mixed interaction (year x growth biostimulators).

The comparative analysis of the indices is shown in the Table 3 and its essence is an analysis of variance for which the hypothesis for the equality between the indices of the studied growth biostimula-

Table 2. Results from the analysis of variance of the first-grade rooted vines, the average for the period of 2018–2021

Variants	Roots			Roots with diameter < 2 mm			Roots with diameter > 2 mm		
	Mean square	F	P	Mean square	F	P	Mean square	F	P
Intercept	47 219.8	2 165.8	0.000	21 221.3	3 541.1	0.000	5 906.2	1 141.9	0.000
Year (Y)	115.3	5.3	0.001	286.3	47.7	0.000	472.8	91.4	0.000
Growth biostimulators (C)	122.3	5.6	0.004	8.3	1.3	0.250	40.3	7.7	0.000
Y × C	27.2	1.2	0.280	16.5	2.7	0.012	46.8	9.0	0.000
Fault	21.8	–	–	5.9	–	–	5.2	–	–

F – empirical value of F-statistic; P – value

Table 3. Results from the analysis of variance for the length and mass of the ripe growth of the grafted vine cuttings for the period of 2018–2021

Variants	Length of the ripe growth (cm)			Mass of the ripe growth (g)		
	Mean Square	F	P	df	F	P
Intercept	183 453.4	2 695.1	0.000	7 596.8	473.07	0.000
Year (Y)	523.1	3.65	0.027	403.9	2.21	0.111
Growth biostimulators (C)	248.8	7.68	0.000	35.6	25.15	0.000
Y × C	250.4	3.67	0.001	36.0	2.24	0.037
Fault	68.0	–	–	16.0	–	–

F – empirical value of F-statistic; P – value; df – degrees of freedom

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Table 4. Number of steplike roots for the period of 2018–2021

Variants	Roots	Roots with diameter < 2 mm	Roots with diameter > 2 mm
Control	12.11[a]	7.71[a]	4.40[a]
RadiCifo L24	10.99[b]	7.60[a]	3.39[b]
Euroradix	12.73[a]	8.20[a]	4.53[a]
LSD (0.05)	1.11	0.70	0.79

[a,b]Data correspond to the average values from four iterations, each consisting of 118 cuttings
The different letters in the columns show significant differences ($P < 0.05$) according to Fisher's test

tors is checked (Table 4). The highest root count is reported for the variant with the applied Euroradix – 12.73, the lowest one is the variant with the applied RadiCifo L24 – 10.99. As an average for the studied period, the values of the index for the roots treated with RadiCifo L24 are higher than the ones from the control. For the reported roots with diameter < 2 mm and > 2 mm, the same trend is observed as follows: highest root count with diameter < 2 mm and > 2 mm is reported for the variant with Euroradix and the lowest one is the variant with RadiCifo L24 (Figure 1). In preliminary studies, Dya-

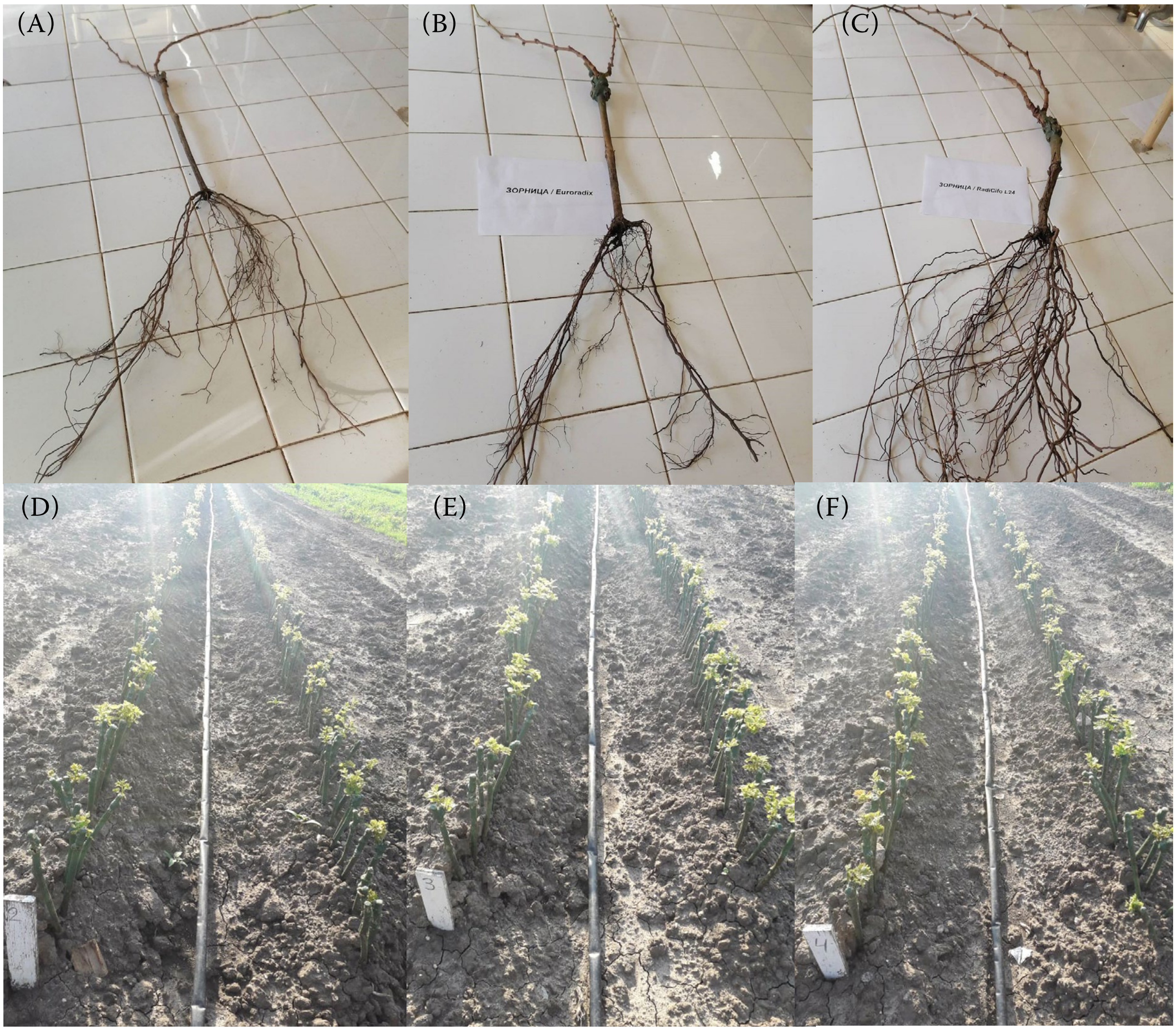

Figure 1. Vine planting material of the 'Zornitsa' variety
Rooted vine: control variant (A), RadiCifo L24 (B); Euroradix (C); Establishment of the open-field trial: control variant (D), RadiCifo L24 (E), Euroradix (F)

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kova et al. (2018b) and Dyakova et al. (2019) reported that the application of biologically active preparations used as rooting agents produced more vigorously developed first-class vines with a greater number of taproots. The same trend is proved by other authors who state that biostimulation products have a positive influence on the growth and development of the studied cultures (Asik et al. 2020; Caliskan et al. 2021; Sefaoglu et al. 2021).

The most important index for the quality of the planting material is the mature part of the vine shoots of the vines' shoots whose data are presented in Tables 3, 5 and 6. In Table 4, it can be seen that *P* is lower than the set statistical significance α for both of the observed indices. The year has an influence over one of the studied indices only – the length of the ripe growth, for which $P = 0.027$ and is less than the set statistical significance. For the other two indices – the diameter and length of the distance between the nodes – the strong influence of the growth biostimulators that were determined for all the studied indices can again be observed (Table 5).

The variant with the applied RadiCifo L24 is reported as having the longest length and largest mass at 24.22 cm and 5.01 g, respectively, compared to the control variant, and the variant with the applied Euroradix at 21.91 cm and 4.95 g (Table 6), respectively. The average for the period, by means of the length of the ripe growth, the difference between RadiCifo L24 and the control variant is 2.64 cm and by means of the mass the difference is 0.33 g. After the application of Euroradix, by means of the index for the length of the ripe growth, the difference between the length and mass of the ripe growth is 0.93 cm and 0.87 g, respectively. Presented as a correlation between the tree variants, the variants had 12.2% (RadiCifo L24) and 1.5% (Euroradix) longer growth and 22.8% (RadiCifo L24) and 21.3% (Euroradix) bigger mass than the control variant.

The diameter of the second distance between the nodes varies between 2.41 mm for the control variant to 2.72 mm for the variant with the applied growth stimulator RadiCifo L24. The measured higher value of the diameter of the distance between the nodes for the variant with the applied Euroradix, compared to the control, is so minimal that it was not statistically proven. The vines that have the shortest distances between the nodes are the ones from the variant with the shortest length of the ripe growth (control variant – 2.09 cm). The average for the period, compared to the control variant, for the di-

Table 5. Results from the analysis of variance for the length and the diameter of the distance between the nodes for the grafted vine cuttings for the period of 2018–2021

Variants	Diameter of distance between nodes (mm)			Length of distance between nodes (cm)		
	df	*F*	*P*	df	*F*	*P*
Intercept	2 218.7	2 004.87	0.000	1 635.0	2 429.23	0.000
Year (Y)	399.8	1.71	0.182	11.1	1.53	0.217
Growth biostimulators (C)	1.9	361.27	0.000	1.0	16.48	0.000
Y x C	0.7	0.600	0.731	2.9	4.31	0.000
Fault	1.1	–	–	0.7	–	–

F – empirical value of F-statistic; *P* – value; df – degrees of freedom

Table 6. Length and mass of maturing of the increment, diameter and length of the distance between the nodes for the period of 2018–2021

Variants	Length of the ripe increase (cm)	Mass of the ripe increase (g)	Diameter of the distance between nodes (mm)	Length of the distance between nodes (cm)
Control	21.58^a	4.08^a	2.41^a	2.09^a
RadiCifo L24	24.22^b	5.01^a	2.72^a	2.31^b
Euroradix	21.91^a	4.95^a	2.47^a	2.19^{ab}
$LSD_{(0.05)}$	2.20	1.14	0.55	0.21

^{a,b}All the data correspond to the average values of four iterations, each presented by 118 cuttings; the different letters in the columns show significant differences ($P < 0.05$) according to Fisher's test

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ameter of the second distance between the nodes has a eported bigger difference – 0.31 mm (12.9%) for the variant with the applied RadiCifo L24 and 0.06 mm (2.5%) for the one with the applied Euroradix. A similar trend is observed for at the diameter of the second internode, where the positive difference between it and the control is minimal. For the variant with the applied RadiCifo L24, this difference is 22 mm (11.9%) and 0.02 cm (4.8%) for the applied Euroradix variant (Table 5). Dyakova et al. (2018a) proved, in their studies, that the application of different bioagents leads to more intensive growth and quality of the one-year increase, which provides some understanding of the growth power of the vine and for the production of the vine planting material – for its quality.

CONCLUSION

The applied growth biostimulators do not have a positive effect on the development of the root system of the 'Zornitsa' variety grafted vine cuttings. The reviewed lower values of the indices, roots with a diameter < 2 mm and > 2 mm that form the total root count of the grafts, compared to the control variant, do not have a negative influence on he quality of the planting material.

The growth of the diameter of the second internode for the treated variants with RadiCifo L24 and Euroradix is more intensive than the growth of the control variant throughout the four years of the experiment. The active vegetation in the rooting ended with difference of 2.31 cm in their length (11.9%) for the variant with the applied RadiCifo L24 and with a length of 2.11 cm (4.8%) for the Euroradix variant – compared to the control variant. The common growth of the rooted vines correlates with the result of the growth dynamics, which confirms the observed trend of the positive influence of the biostimulators. The diameter of the second distance between the nodes of the main summer raised also shows a positive difference in favour of the treated vines, which was 2.64 cm (12.2%) for RadiCifo L24, while the difference is minimal at 0.33 cm (1.5%) by applying Euroradix compared to the control variant.

The higher average for the studied period, as a more effective method for the production of first-class vines, is provided by the variant with the growth biostimulator RadiCifo L24. According to the biometrics rating, this variant can be recommended in the practice used for the production of vine planting material.

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