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Effects of some herbicides on the leaf apparatus of young vine (*Vitis vinifera* L.)

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Abstract

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The study was carried out at the Experimental Base of the Institute of Viticulture and Enology, Pleven, Bulgaria. The soil type where the plantation was located was leached chernozem, formed on clay loess. Vines of Cabernet Sauvignon variety grafted to Berlandieri X Riparia Selection Openheim 4 (SO4) rootstock were used in the experimental work. The vines were planted in stages in the spring of 2015 and 2016. The objective of the study was to determine the impact of the herbicides Lumax and Gardoprim plus Gold in combination with Stratos Ultra on the leaf apparatus of grapevines during the first year after their planting. The treatment with Lumax and Gardoprim plus Gold was carried out on the soil, immediately after the formation of the protective piles, without incorporation, while with Stratos ultra between the thirties and the sixtieth day after that. The size of the leaf area was determined by the method proposed by Carbonneau and improved by T. Slavcheva.

Vines from the herbicide-treated plots had been found to form larger-sized leaves and had a larger leaf area than those grown on areas where weed control was only by mechanical removal. Deformities and changes in the coloration of the leaf blades as a result of the phytotoxic response were not observed. Of the factors, sources of variation during the study period (dose – A, herbicide – B and the combination between them – A+B), the set of active substances used for weed control was of the greatest significance for the obtained results, i.e. the factor – herbicide (B).

Keywords: vines; newly-planted vineyard; herbicides; leaf area

Introduction

The chemical method of weed control has been an essential element of the grapevine growing technology. Reducing the labor force involved in viticulture on the one hand, and increasing the demand for the various produce of this branch of agriculture, has turned this method into an important means for getting higher yields. Despite the environmental hazards that have provoked the growing development of the organic production, the treatment of the areas with the conventional herbicides has remained the most common way of controlling unwanted vegetation in vines.

According to some authors, to eliminate the annual species in the newly planted vineyards, trifluralin, orisaline, oxyfluorfen, etc. might be applied; however they were not sufficiently efficient for the perennials (Lange et al., 1970; Spasov et al., 1999; Tonev, 2000). Pendimethalin, napropamide, s-metolachlor, applied immediately after planting had exhibited high levels for the annual species control (Bordelon, B., 2011). Foliar herbicides for control of the gramineous weeds during the growing season had been also recommended: fluazifop-P-butyl, quisalofop ethyl, cycloxidime, haloxyfop R, etc. (Spasov et al., 1999; Tonev, 2000; Tonev et al., 2007). In many cases, the chemicals for controlling the unwanted vegetation in the fruit-giving vineyards had been completely inapplicable in young plantations and their use could cause severe phytotoxic reactions (Al-Khatib et al., 1993). Young vines were particularly susceptible to the harmful environmental impact. Their poorly developed root system increased their susceptibility to both the competitive agrophytocenosis and the applied weed control measures. The incorrect selection of active substances in all plant species as well as in vine could cause abnormal growth through morphological, anatomical and cytological effects (Berova et al., 2004; Bondada et al., 2006). The application of a number of herbicidal agents (acetochlor, aminotriazole, diuron, flazasulfuron, fluoroglycofen, glyphosate, chlorosulfuron, 2.4 D, trichloroacetate) could lead to physiological and morphological changes in vine, often manifested as deformities, hyper- or decoloration of the leaf blade and vine shoot, growth inhibition, etc. (Leonard & Lider.; 1961; Abrasheva & Chelebiev, 1991; Itoh et al.,1997; Bhattia et al.,1998; Radetski et al., 2000; Groupe de travail regional Midi-Pirenes, 2002; Magné et al., 2006; Prodanova-Marinova, 2012, 2013; Chambre Regionale d'agriculture Langedoc Roussillon, 2014; Tan et al., 2014; Chambre d'agriculture Charente – Maritime, 2017). Leaves, as the main organ where the three vital processes of the plant occurred (photosynthesis, respiration and transpiration), played a decisive role for grapevine growth, development and fertility, thus the leaf blade anomalies affected the whole plant.

The objective of the study was to determine the impact of the herbicides Lumax and Gardoprim plus Gold in combination with Stratos Ultra on the leaf apparatus of grapevines during the first year after their planting.

Material and Method

Plant Material

Vines of Cabernet Sauvignon variety grafted to Berlandieri X Riparia Selection Openheim 4 (SO4) rootstock were used in the experimental work. The propagation material was produced for this purpose at the IVE, Pleven in 2014 and 2015. By the time of planting it was stored in a specialized room under controlled conditions.

Setting of the Trial

The vines were planted in stages in the spring of 2015 and 2016. The distance between the rows was 3 m, while the intra-row distance – 1.2 m. The trial was two-way, set by the long plots method in 5 replicates. Each replicate had a plot size of 5 m^2 and included 4 vines (20 vines per variant).

The variants of the trial were:

– Factor A (dose): A1 – low dose (V1, V3) and A2 – high dose (V2, V4);

– Factor B (herbicide): B1 – Lumax (V1, V2) и B2 (V3, V4) – Gardoprim plus Gold;

– К – untreated, cultivated (technological) control;

Herbicides

Application Details

The herbicides (Table 1) were introduced into the intra-row stripe once, with a backpack sprayer at a consumption rate of working solution 40 l/ha and the nozzle pressure Pmax 300 kPa. The treatment with Lumax and Gardoprim plus Gold was carried out immediately after the planting of the vines and the formation of the protective piles, without incorporation. For the eradication of Johnson grass germinated from seeds and partially preserved rhizomes during the vegetation, spraying with Stratos ultra between the thirties and the sixtieth day after the vine planting was performed. The distances between the rows were treated mechanically 4 times during the year.

Analysis

The leaves (main and from the lateral shoots) per vine from repetition were measured to evaluate the effects of the herbicides and the applied doses on the vine leaves. The size of the leaf area – per leaf and per vine – was determined by

Table 1. Herbicides, their doses and time of application

Variants	Herbicides applied (formulated product)	Time of application	Active substance (g a. i. L^{-1})	Doses (g a. i. ha^{-1})
1	Lumax (Syngenta)	preemergence	375 a. i.L^{-1} s-metolachlor + 125 a. i.L^{-1} terbuthylazine + 37.5 a. i.L^{-1} mesotrione	215
	Stratos ultra (Basf)	postemergence	100 a. i.L^{-1}	20
2	Lumax (Syngenta)	preemergence	375 a. i.L^{-1} s-metolachlor + 125 a. i.L^{-1} terbuthylazine + 337.5 a. i.L^{-1} mesotrione	322
	Stratos ultra (Basf)	postemergence	100 a. i.L^{-1}	20
3	Gardoprim plus Gold (Syngenta)	preemergence	312.5 a. i.L^{-1} s-metolachlor + 187.5 a. i.L^{-1} terbuthylazine	200
	Stratos ultra (Basf)	postemergence	100 a. i.L^{-1}	20
4	Gardoprim plus Gold (Syngenta)	preemergence	312.5 a. i.L^{-1} s-metolachlor + 187.5 a. i.L^{-1} terbuthylazine	300
	Stratos ultra (Basf)	postemergence	100 a. i.L^{-1}	20

the method proposed by Carbonneau (1976) and improved by Slavcheva (1983, 1990). It referred to the non-destructive ampelometric methods and took into account the natural asymmetry of the leaves. The theoretical curves used made it possible to determine both the area per leaf and the size of the leaf area of individual shoots and vines with sufficient accuracy.

The statistical processing of the obtained results was performed by two-way analysis of variance ANOVA, (Dimova & Marinkov, 1999). The power of impact of the factors was calculated by the method of Plohinski (Lakin, 1990). One-way analysis was applied to determine the significance of the differences from the untreated controls and two-way analysis to clarify the effect of the dose and herbicide composition.

Soil and Climate Characterization of the Region

The study was carried out at the Experimental Base of the Institute of Viticulture and Enology, Pleven, Bulgaria. The vineyard was located at 43.42°N 24.62°E and 140 m altitude.

The soil type where the plantation was located was leached chernozem, formed on clay loess. By mechanical composition it was heavy sandy loam, with good aquatic physical properties, fully satisfying the biological requirements of the vine (Krastanov & Dilkova, 1963).

Data on precipitation and temperature changes were obtained from the automatic weather station iMetos, located on the territory of the Production Experimental Base of IVE – Pleven (Figure 1). The period from April to the end of September 2015 was characterized by low precipitation – 206 mm. The drought was particularly severe in July (27.4 mm) and during the month of May (30.4 mm) – critical for the plantation. Poor precipitation rates were recorded for 2016 too – in June 97.8 mm, July 10.4 mm and August 35.8 mm. The average daily temperatures for these months did not differ significantly for both years of the experimental work.

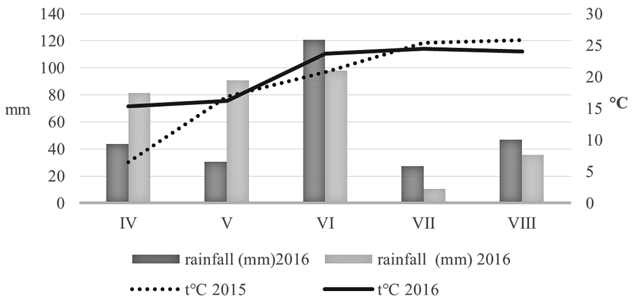

Fig. 1. Weather characteristics for the period April - September 2015 and 2016

Results and Discussion

The two herbicides (Lumax and Gardoprim plus Gold) included in the study maintained a low degree of weeding and more effective control over the unwanted vegetation in the vineyard compared to the manual treatments. The duration of their action exceeded ninety days and, in combination with the action of Stratos ultra against Johnson grass, allowed the young vines to absorb nutrients from the environment with reduced competition (Prodanova-Marinova et al., 2019). During the visual inspections at the time of the growing season, no visible manifestations of phytotoxicity were registered – in neither of the treated variants it was found grapevine growth suppression. The petal coloration was uniform, without spots. No signs of chlorosis, necrosis, or other type of herbicide-induced damage had been identified. The shape of the leaf blade was typical for the variety. The deformations caused by Lumax in other varieties (Bolgar, Muscat Plevenski) observed by us in previous studies (Prodanova-Marinova, 2014; Prodanova-Marinova, 2016) were not found here.

The leaf size (the leaf blade area) varied over the years, both in the main leaves and those from the lateral shoots (Table 2). In 2015, the vines from the variants treated with Gardoprim plus Gold at a dose of 300 g a. i. ha^{-1} (V4) and Lumax at a dose of 215 g a. i. ha^{-1} (V1) had the largest main leaves. In V4, the area per leaf from the lateral shoots was significantly smaller compared to the other variants – only 2.34 cm^2 and practically was equal to that of the control. For V1, V2 (Lumax 322 g a. i. ha^{-1}) and V3 (Gardoprim plus Gold 200 g a. i. ha^{-1}), the leaf size from the lateral shoots varied the least, with the difference between the two Lumax treated variants being particularly small. The smallest area of the main leaves and those from the lateral shoots was recorded in the control (19.99 cm^2 for the main leaves and 2.21 cm^2 for those from the lateral shoots). The main leaves of the treated variants were significantly larger and the mean area ranged from 21.44 cm^2 in V3 to 33.05 cm^2 in V4).

In 2016 again, the main leaves of the treated variants were larger than the leaves in the control (7.1 cm^2). The average area per main leaf of the vines from the herbicide-treated plots ranged from 17.68 cm^2 in V1 (Lumax at a dose of 215 g a. i. ha^{-1}) to 26.13 cm^2 in V2 (Lumax at a dose of 322 g a. i. ha^{-1}). That year the control vines did not develop lateral shoots therefore a comparison could be made only for the treated variants – the largest leaves of the lateral shoots (13.4 cm^2) were measured in V2.

The most significant influence on the size of the main vine leaves in 2015 had the interaction between the two factors (dose A and herbicide B) – Table 3. The combination

Table 2. Area per leaf (cm²)

Variants	Main leaves			Lateral shoot leaves		
	2015	2016	$\bar{X}$	2015	2016	$\bar{X}$
V1	26.95 *	17.68 ***	22.32	9.35 *	4.51	6.93
V2	22.24 ns	26.13 ***	24.19	9.87 *	13.4	11.64
V3	21.44 ns	20.23 ***	20.84	8.84 *	0.77	4.81
V4	33.05 ***	20.64 ***	26.85	2.34 ns	5.51	3.93
K	19.99	7.10	13.55	2.21	0	1.10

Proven respectively at: 5% – (*); 1% – (**); 0.1% – (***) and < 5% – (n.s) – unproven

Table 3. Effect of the factors herbicide and dose on the size of the main leaves for the year 2015

Source of variation	SS	df	MS	F	F crit (5%)	Power of influence (%)
Dose (A) n.s. (< 5%)	39.67	1.00	39.67	2.46	4.35	–
Herbicide (B)** (1%)	269.91	2.00	134.96	8.38	3.49	26.46 ** (1%)
Interaction *** (0.1%)	352.93	2.00	176.46	10.96	3.49	34.59 *** (0.1%)
Errors	321.95	20.00	16.10			31.56

Proven respectively at: 5% – (*); 1% – (**); 0.1% – (***) and < 5% – (n.s) – unproven

Table 4. Effect of the factors herbicide and dose on the size of the lateral shoot leaves for the year 2015

Source of variation	SS	df	MS	F	F crit (5%)	Power of influence (%)
Dose (A) n.s. (< 5%)	29.34	1.00	29.34	1.37	4.35	–
Herbicide (B) ** (1%)	274.64	2.00	137.32	6.41	3.49	27.91 ** (1%)
Interaction *** (0.1%)	75.54	2.00	37.77	1.76	3.49	–
Errors	428.34	20.00	21.42			43.54

Proven respectively at: 5% – (*); 1% – (**); 0.1% – (***) and < 5% – (n.s) – unproven

of the active substances with which the areas were treated immediately after the vines were planted (factor B) had the second major impact on the main leaves development. The effect of the dose (factor A) was unproven. That year, factor B was the most significant factor in the formation of the leaves from the lateral shoots while the influence of factor A and the combination of the two factors was unproven (Table 4). In 2015, factors unrelated to weed vegetation control played a significant role in achieving the reported leaf blade size – a significant source of variation (31.56% for the main leaves and 43.54% for the leaves from the lateral shoots) represented side effects for the trial (temperature, precipitation, etc.) – Tables 3 and 4.

In 2016, factor B had the greatest influence on the formation of the main leaves (Table 5). Factor A and the combination of dose and herbicide had significantly less impact and their power of influence was unproven. The side factors were a much weaker source of variation that year. The reported error, formed under their influence, in that case was 11.61%. The type of the herbicide (factor B) – 36.04%, followed by the dose of application (factor A) had the highest power of influence for the leaves from the lateral shoots – Table 6. The error was 31.09%, i.e. the factors not related to the herbicide and its dose of application had a significant effect on the size of the leaves from the lateral shoots.

In 2015 and 2016, the vines of the herbicide-treated variants had a larger leaf area than the control vines (Table 7). The differences in 2016 were particularly expressive, when the leaf area of the control vines was 40.77 cm² on the average. In 2015, the most developed leaf apparatus was found in V1 (Lumax at a dose of 215 g a. i. ha^{-1}), and in 2016 – in V2 (Lumax at a dose of 322 g a. i. ha^{-1}). On the average for the

Table 5. Effect of the factors herbicide and dose on the size of the main leaves for the year 2016

Source of variation	SS	df	MS	F	F crit (5%)	Power of influence (%)
Dose (A) * (5%)	65.48	1.00	65.48	6.22	4.35	3.61 n.s. (< 5%)
Herbicide (B) *** (0.1%)	1331.32	2.00	665.66	63.22	3.49	73.38 *** (0.1%)
Interaction * (5%)	113.62	2.00	56.81	5.40	3.49	6.26 n.s. (< 5%)
Errors	210.59	20.00	10.53			11.61

Proven respectively at: 5% - (*); 1% - (**); 0.1% - (***) and < 5% - (n.s) – unproven

Table 6. Effect of the factors herbicide and dose on the size of the lateral shoots leaves for the year 2016

Source of variation	SS	df	MS	F	F crit (5%)	Power of influence (%)
Dose (A) ** (1%)	154.27	1.00	154.27	8.60	4.35	13.49 ** (1%)
Herbicide (B) *** (0.1%)	412.06	2.00	206.03	11.48	3.49	36.04 *** (0.1%)
Interaction n.s. (< 5%)	98.41	2.00	49.21	2.74	3.49	–
Errors	358.85	20.00	17.94			31.39

Proven respectively at: 5% – (*); 1% – (**); 0.1% – (***) and < 5% – (n.s) – unproven

Table 7. Area of the leaf surface per vine (cm²)

V	Main leaves			Lateral shoot leaves			Leaf area per vine		
	2015	2016	$\bar{X}$	2015	2016	$\bar{X}$	2015	2016	$\bar{X}$
V1	358.60 ^{ns}	181.95**	270.28	49.96 ^{ns}	4.53 ^{ns}	27.25	408.56**	186.47**	297.53
V2	252.60 ^{ns}	347.47***	300.04	85.84*	69.40***	77.62	338.44 ^{ns}	416.86***	377.66
V3	301.20 ^{ns}	226.31***	264.26	7.87 ^{ns}	1.54 ^{ns}	4.71	309.07 ^{ns}	227.86**	269.00
V4	285.27 ^{ns}	291.38***	288.33	48.98 ^{ns}	19.00 ^{ns}	33.99	334.25 ^{ns}	310.38***	322.32
K	238.08	40.77	139.43	6.63	0	3.39	244.71	40.77	142.75

Proven respectively at: 5% - (*); 1% - (**); 0.1% - (***) and < 5% - (n.s) – unproven

period of the study, the leaf area per vine was the highest in the variants treated with higher doses (V2 – 377.66 cm² and V4 – 322.32 cm²).

In the analysis of the results on the average for the period, it was found that the size of the leaf area of the vine depended to a great extent on the main leaves. The data showed that in the variants with the largest leaf area, on the average over the duration of the study (V2 and V4), it was also found the largest area of the leaves from the lateral shoots. That increased their percentage participation in the formation of the total leaf area – respectively 20.55% for V2 and 10.55% for V4 (Figure 2).

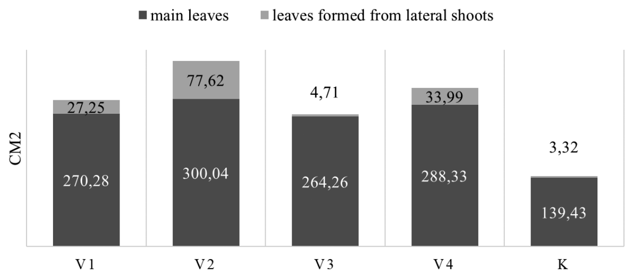

Fig. 2. Leaf area ratio per vine from main leaves and leaves formed from lateral shoots, on the average for 2015 and 2016

There had been a well-established correlation between the leaf area of the vine and its photosynthetic activity – the larger surface was a prerequisite for more intense photosynthesis and more active plant growth (Plakida & Voznesenskiy, 1977; Yonev & Mineva, 1980; Petrie et al., 2000; Vasconcelos et al., 2000, etc.). Achieving effective control of weed vegetation and minimizing the competitive relationships in the newly planted vineyard created the conditions for the growth of larger leaf apparatus and thus enhancing the photosynthesis productivity.

In 2015, only factor B (herbicide) had a proven effect on leaf surface formation, i.e. the combinations of the active substances that the plots were treated after planting the vines (Table 8). As with the size of the leaves, the factors unrelated to weed control were of particular importance that year – 39.78%

In 2016, the main source of variation was again factor B – Ii had the highest power of influence (54.79%). The impact of the other two factors was significantly less (11.06% - A and 9.23% - A + B) and unproven (Table 9). The error caused by the influence of the side factors for the experiment was 16.14%.

The weather factors were likely to play a significant role in the formation and differences over the years of the errors found in the two-way analysis of variance. According to a number of authors, the average daily temperature and the air humidity and soil moisture had a particularly significant influence on the growth of the leaf apparatus. Lowering the temperature below a certain threshold resulted in a decrease in the growth force of the leaves. The higher air and soil moisture provided greater physiological moisture in the leaf and hence a larger surface of the leaf blade (Todorov, 1969, 1977; Bulgarian Ampelography, 1990; Matuzok et al., 2013). The temperature course during the two vegetation periods included in our study was approximately similar, but the precipitation during the two months of the most active

Table 8. Effect of the factors herbicide and dose on the leaf area per vine for the year 2015 (cm^2)

Source of variation	SS	df	MS	F	F crit (5%)	Power of influence (%)
Dose (A) n.s. (< 5%)	7565.55	1	7565.55	1.106894	4.351244	–
Herbicide (B) ** (1%)	84001.83	2	42000.92	6.145037	3.492828	24.45 ** (1%)
Interaction n.s. (< 5%)	6304.359	2	3152.18	0.461187	3.492828	–
Errors	136698.7	20	6834.933			39.78417098

Proven respectively at: 5% - (*); 1% - (**); 0.1% - (***) and < 5% - (n.s) - unproven

Table 9. Effect of the factors herbicide and dose on the leaf area per vine for the year 2016 (cm^2)

Source of variation	SS	df	MS	F	F crit (5%)	Power of influence (%)
Dose (A) ** (1%)	81597.02	1	81597.02	13.6975	4.351244	11.06 n.s. (< 5%)
Herbicide (B) *** (0,1%)	404229.7	2	202114.8	33.92856	3.492828	54.79 *** (0.1%)
Interaction * (5%)	68132.65	2	34066.32	5.718636	3.492828	9.23 n.s. (< 5%)
Errors	119141.4	20	5957.072			16.14869595

Proven respectively at: 5% - (*); 1% - (**); 0.1% - (***) and < 5% - (n.s) - unproven

growth (June and July) might have had a significant effect on the size of the leaf area and the difference in the area in 2015 and 2016. That difference was particularly pronounced in the technological control, where the effect of the two factors studied by us was excluded – dose (A) and herbicide (B).

Conclusions

The herbicides tested during the first year after planting of Cabernet Sauvignon vines did not cause changes in the coloration and the shape of the leaf blade due to the phytotoxic response.

The vines from the plots treated with the herbicides Lumax and Gardoprim plus Gold, in combination with Stratos ultra, formed higher size leaves and had a larger leaf area compared to those grown in plots where the weed control was done only through mechanical removal.

The analysis of the influence of the factors dose (A) and herbicide (B) on the formation of the vine leaf apparatus in the first year after planting showed that the combination of the active substances used was of the greatest importance for the results obtained in 2015 and 2016 for weed control, i.e. the factor herbicide (B).

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