

# Relative Leaf Expansion Rate as an Indicator of Compensatory Growth of Defoliated Vines (*Vitis vinifera* L. cv. Prokupac)

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**Defoliation is a common practice in vineyards, with numerous benefits for vine microclimate conditions, and it can significantly improve the composition of grapes. In addition, early defoliation reduces the active leaf area, which affects the modifications of the source-sink balance and decreases whole-vine photosynthesis. The reaction of grapevines to early defoliation is to mitigate the effects through compensatory growth, resulting in more lateral shoots with a greater number of leaves. In this study, we evaluate the use of non-destructive and continuous measurements of mean and lateral leaf area on the same shoots for the purpose of monitoring leaf area development and calculating relative leaf expansion rate (RLER) during active growth. The results show that the grapevine's ability to recover its leaf area after defoliation depends mainly on the time of defoliation. Vines defoliated early had time to compensate for the removed leaves by producing a greater number of lateral shoots with more leaves, resulting in a larger total leaf area. With a decrease in shoot growth during vegetation, the recovery ability decreases, and compensatory growth is therefore not enough to restore the reduced leaf area. Based on the value of RLER, it is shown that, if defoliation is performed in the period of intensive shoot growth, it retards the emergence of new shoots and leaves over several days, followed by a period of regrowth. Very slow or no growth of shoots and leaves occurred with defoliation after the véraison stage.**

## INTRODUCTION

Leaf removal from shoots in the fruiting zone is becoming common practice in vineyards with high-quality wine cultivars in Serbia. The main aim of defoliation is to improve vine microclimate conditions inside the canopy, especially light conditions, along with temperature and humidity (Smart *et al.*, 1985; Poni & Civardi, 2008). Improved microclimate conditions prompt the accumulation of dry matter in the must, as well as anthocyanins and polyphenol compounds in the berry skins (Kliwer, 1970; Hunter *et al.*, 1991; Sabbatini & Howell, 2010; Baiano *et al.*, 2015). Improved aeration of the canopy and better penetration of fungicides reduce the degree of damage caused by diseases, especially by grey rot (Gubler *et al.*, 1991; Molitor *et al.*, 2011; Gambetta *et al.*, 2020).

The effect of defoliation depends mainly on its intensity and the time of application. Early defoliation, carried out during the intensive shoot-growing phase, causes photosynthetic shock due to the reduction in the photosynthetically active area and decreases whole-vine photosynthesis (Petrie *et al.*, 2003; Palliotti *et al.*, 2011). The level of total shoot photosynthesis can be reduced by up to 70%, which stops the development of sink organs (Poni *et al.*,

2006). These modifications of the source-sink balance can affect the bunch and berry structure (Coombe, 1992; Intrieri *et al.*, 2008; Sabbatini & Howell, 2010). The most pronounced changes in the composition of bunch and berry occur when defoliation is performed during the phenological stages of flowering and fruit set, when intensive divisions of the cells of young berries take place (Poni *et al.*, 2009). During fruit set, the number of pericarp cell layers is determined and each reduction of the inflow of assimilants results in decreased cell numbers. In most defoliation studies, it is necessary to assess the consequent effect of leaf removal on change in leaf area. Leaf area is an important element in the study of plant physiology, particularly when exploring the photosynthetic activity, canopy light conditions and water balance of the plant, and also when assessing the effect of cultural practices (Bešlić *et al.*, 2013). Furthermore, unfavourable weather conditions, especially hail, diseases and pests, can result in a loss of leaves and a reduction of leaf area.

The natural reaction of grapevines to defoliation is to reduce its effects through compensatory growth. Compensatory growth is defined as the restoration of morphological and physiological changes that occur in

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plants following defoliation (Collin *et al.*, 2000). Grapevines have a strong capacity for compensation by producing more lateral shoots with a greater number of leaves (Candolfi-Vasconcelos & Koblet, 1991; Petrie *et al.*, 2000; Kurtural *et al.*, 2013), which is a response to the disturbed source:sink relationship and a balancing act of the grapevine canopy upon manipulation (Hunter, 2000). The most common method of quantifying is compensation, by comparing the performance of defoliated and normal plants (Hilbert *et al.*, 1981; Anten *et al.*, 2003). In this study, compensatory growth is defined as an increase in relative leaf expansion rate (RLER) of defoliated vines relative to normal vines. Non-destructive and continuous measurements of mean and lateral leaf area on the same shoots enabled the measuring of leaf area development and the calculation of RLER during vegetation.

The main objective of this study was to evaluate the changes in RLER caused by different defoliation times and to quantify its role as an indicator of growth compensation.

## MATERIALS AND METHODS

The study was conducted from 2014 to 2016 in a commercial vineyard of Prokupac (*Vitis vinifera* L.) grafted on Kober 5BB (*V. berlandieri* × *V. riparia*) rootstock. Experimental plots were located at the Toplicki Vinogradi Winery (lat. 43.12057° N; long. 21.25031° E; alt. 359 m) in the vine-growing region of Toplica, Prokuplje wine district, Serbia. The area has a temperate continental climate with an annual mean air temperature of 11.4°C and a seasonal mean temperature of 17.0°C. Total annual rainfall averages 556.7 mm, with 347.4 mm of rainfall during the growing season. The vineyard soil type is a Cambisol, which has favourable physical characteristics. The vineyard was planted in 2009 at a planting distance of 2.5 × 0.8 m (5 000 vines per ha). The training system is a double Cordon Royat with a trunk height of 60 cm. At pruning, six two-node spurs were kept on the permanent cordon, corresponding to a bud load of 12 nodes per vine. Standard vineyard management practices, except for main and lateral shoot tipping, were carried out in the study plots. The trial was a complete random block design, with three blocks and four treatments per block. Defoliation was carried out by hand removal of six basal leaves. The vines were tagged and randomly assigned to the following treatments: (K) non-defoliated (control); (v1) removal of the first six basal leaves at full flowering, phenological stage 65 (50% of cap fall), according to the BBCH scale (Lorenz *et al.*, 1995); (v2) removal of the first six basal leaves at phenological stage 73 (groat-sized berries, ovary diameter 3 to 5 mm); (v3) removal of the first six basal leaves at stage 81 (véraison, berries begin to colour).

The single leaf area, main shoot leaf area and lateral shoot leaf area were estimated according to Bešlić *et al.* (2010). From 15 to 31 May each year, 50 leaves were collected randomly from vines in all the experimental plots. The leaves were immediately placed in plastic bags and kept and transported in a field refrigerator. Leaf area (LA) and the length of two inferior leaf veins (l) were measured using a computer scanner and Adobe Photoshop 7.0 under laboratory conditions. These data were used to calculate the regression between l and LA. The obtained formula (LA = -111.3242

+ 14.4764 × l;  $r^2 = 0.98$ ) was used for non-destructive calculation of the leaf surface of the basis of leaf vein length data collected in the vineyard. Furthermore, in the period from 15 to 31 May, 30 shoots were randomly labelled for each treatment and used for calculating the leaf area during vegetation. The main shoot leaf area (MLA) was calculated individually for all labelled shoots. Leaf number (NL), the largest (Lmax) and the smallest leaf area (Lmin) were then determined for each main shoot. Multiple regression analysis was used to calculate the relationship between the dependent variable MLA and three independent variables (NL, Lmax and Lmin). The obtained formula (MLA = -1 688.43 + 128.36 × NL + 4.83 × Lmax + 14.02 × Lmin;  $r^2 = 0.892$ ) was used to calculate the leaf surface area for the main shoots. For the lateral shoot leaf area (LLA), the following analogous formula was used: LLA = -520.212 + 50.462 × NL + 4.806 × Lmax + 3.739 × Lmin;  $r^2 = 0.974$ ). Based on the figures obtained, MLA, LLA and total leaf area (TLA = MLA + LLA) were calculated for four periods: 70 to 75 days after bud break (DAB); 85 to 90 DAB; 100 to 110 DAB; and 125 to 130 DAB. Continuous LA measurement during these intervals was used for calculating the relative leaf expansion rate (RLER), using the following formula (Dzamic *et al.*, 2001):  $RLER = (\ln LA_2 - \ln LA_1) / (t_2 - t_1)$ ; where: LA<sub>1</sub> – leaf area at the beginning of the observation (t<sub>1</sub>), LA<sub>2</sub> – leaf area at the end of the observation (t<sub>2</sub>).

Data were processed and analysed by standard statistical methods using software package Statistica v. 9.0 (StatSoft Inc., Tulsa, OK, USA). Differences between treatments were determined by F and Duncan's multiple range tests.

## RESULTS AND DISCUSSION

During the period of the investigation, defoliation reduced the lateral leaf area (LA) in v1 vines 40% more than the values found in the other treatments and the control plants in the first measurements (DAB 70 to 75) (Table 1). The first measurements of LA were carried out about 20 days after defoliation of the v1 vines. At that stage, the balance between source:sink organs after the removal of the basal leaves (which are the source organs) had still not been established, resulting in stagnation of the vegetative development and delayed lateral shoot emergence. The removal of the photosynthetically most active leaves from the fruiting zone during flowering causes a significant decrease in whole-vine photosynthesis and modifies the source:sink relationship (Ollat & Gaudillere, 1998; Petrie *et al.*, 2003; Poni *et al.*, 2006; Frioni *et al.*, 2018). In a similar investigation of the defoliation effect on Prokupac, Bešlić *et al.* (2013) emphasised a growth stagnation of up to 30 days after basal leaf removal at stage 65 (BBCH scale). The next measurements were carried out in the second half of July (DAB 85 to 90), during intensive shoot growth and 20 days after v2 defoliation. This removal of leaves from the six basal nodes was reflected in the LLA of the v2 vines, which was reduced by 50% compared to the control vines. Differences between v1 and v3 and the control vines, respectively, were also statistically significant. A similar relationship between the values of LLA was found for the third measurements, which were carried out in a period of decreased growth of the main and lateral shoots.

TABLE 1  
Mean values of lateral leaf area (LLA) for the period 2014 to 2016.

DAB period Days after budbreak	LLA (m <sup>2</sup> )			
	V1	V2	V3	Control
I (70 to 75)	0.100 <sup>a</sup>	0.177 <sup>b</sup>	0.194 <sup>b</sup>	0.193 <sup>b</sup>
II (85 to 90)	0.353 <sup>b</sup>	0.195 <sup>a</sup>	0.414 <sup>c</sup>	0.408 <sup>c</sup>
III (100 to 105)	0.523 <sup>b</sup>	0.415 <sup>a</sup>	0.615 <sup>c</sup>	0.629 <sup>c</sup>
IV (125 to 130)	0.689 <sup>b,c</sup>	0.602 <sup>a,b</sup>	0.532 <sup>a</sup>	0.759 <sup>c</sup>

a, b, c – Values were grouped based on Duncan's multiple range test ( $\alpha = 0.05$ ), where different letters within the same row denote significant differences between treatments.

LLA: Lateral shoot leaf area

V1: Basal leaf removal at flowering

V2: Basal leaf removal at groat berry size

V3: Basal leaf removal at véraison

Many investigations of grapevine growth in temperate climates show that the intensity of shoot growth decreases from mid-summer (Mullins *et al.*, 1992). The third measurements were performed before defoliation (v3), so no reduction was detected in the LLA of v3 vines. The fourth measurements of LLA were carried out after defoliation at véraison (v3), when the final leaf area had almost been attained. Defoliation of the v3 vines reduced their LLA by 30% in comparison to the control vines. At the end of the observation period, significant differences in LLA were measured between the control and the v2 and v3 vines, respectively. The v1 vines had a significantly larger LLA compared to v3. It is evident that vines defoliated early (v1, v2) had time to compensate for the removed leaves by producing more lateral shoots with a greater number of leaves, resulting in a larger total LLA. As the intensity of shoot growth decreased in the vegetation, compensatory growth was not sufficient to recover the reduction in total leaf area. The growth of new shoots and leaves was caused by the loss of source organs.

Many studies have shown that early defoliation causes an increase in both main and lateral leaf area as a compensatory response (Weaver, 1963; Candolfi-Vasconcelos & Koblet, 1990; Hunter, 2000; Kurtural *et al.*, 2013). Under similar agroecological and experimental conditions, Stefanović (2021) obtained a significant increase in lateral leaf area on early defoliated Cabernet Sauvignon, compared to vines defoliated at véraison and non-defoliated vines. The author emphasised that the vines defoliated early were able to recover their leaf area as a compensatory response to leaf removal.

Non-destructive and continuous measurements of MLA and LLA area of the same shoots enabled the monitoring of LA development and the calculation of the RLER during shoot growth. Following the second LA measurements, the main, lateral and total shoots on the vine had the greatest RLER-1 values on the v1 vines and the lowest values on the v2 vines.

The RLER-1 of lateral shoots on v1 vines was 72% higher than that of v2 vines and 41% higher than that of v3 and control vines. The reason for the high value at v1

and lower at values v2 lies in the time of defoliation and the time of LA measurements. The second measurement of LA was performed about 40 days after v1 and 20 days after v2 defoliation. During that time, v1 vines passed through a period of slow growth of the main shoots caused by carbon assimilation depression (Ollat & Gaudillere, 1998; Petrie *et al.*, 2003). This was followed by a period of lateral shoot emergence and the intensive growth of these shoots as compensation for the removed leaves. These results are consistent with previous experiments on defoliation at the flowering stage (Candolfi-Vasconcelos & Koblet, 1991; Pastore *et al.*, 2013; Acimovic *et al.*, 2016). Unlike the v1 vines, the v2 vines were still in the phase of slow growth caused by the recently performed defoliation. The third measurement of LA and the calculation of RLER-2 took place 30 days after v2 and 10 days before v3 defoliation. Fig. 1. shows that the v2 vines had the highest RLER values compared to that of the other treatments, but that the differences were not as pronounced as for the v1 measurements. The values of the v2 vines were about 30% higher compared to that of the other treatments.

As mentioned above, the third measurement was performed during the period of slower growth of the main and lateral shoots, thus the level of compensatory growth was lower compared to that of the shoots whose leaves were removed in the phase of intensive growth. The last measurement and RLER-3 calculations were performed about 10 days after v3 defoliation (in the véraison phase), when the growth of shoots is very slow or stops. This result is in accordance with previous studies (Pastore *et al.*, 2013; Stefanović, 2021), which emphasise a significant reduction in total leaf area of defoliated vines at véraison because there was no leaf regrowth after véraison. A similar result was recorded for the calculated RLER based on the first and last leaf area measurements. The RLER of the main, lateral and all shoots on the vine were highest on the v1 vines compared to all other treatments, between which there were no significant differences. This is consistent with findings that show that vines defoliated early had time to compensate for the removed leaves by producing more lateral shoots with more leaves.

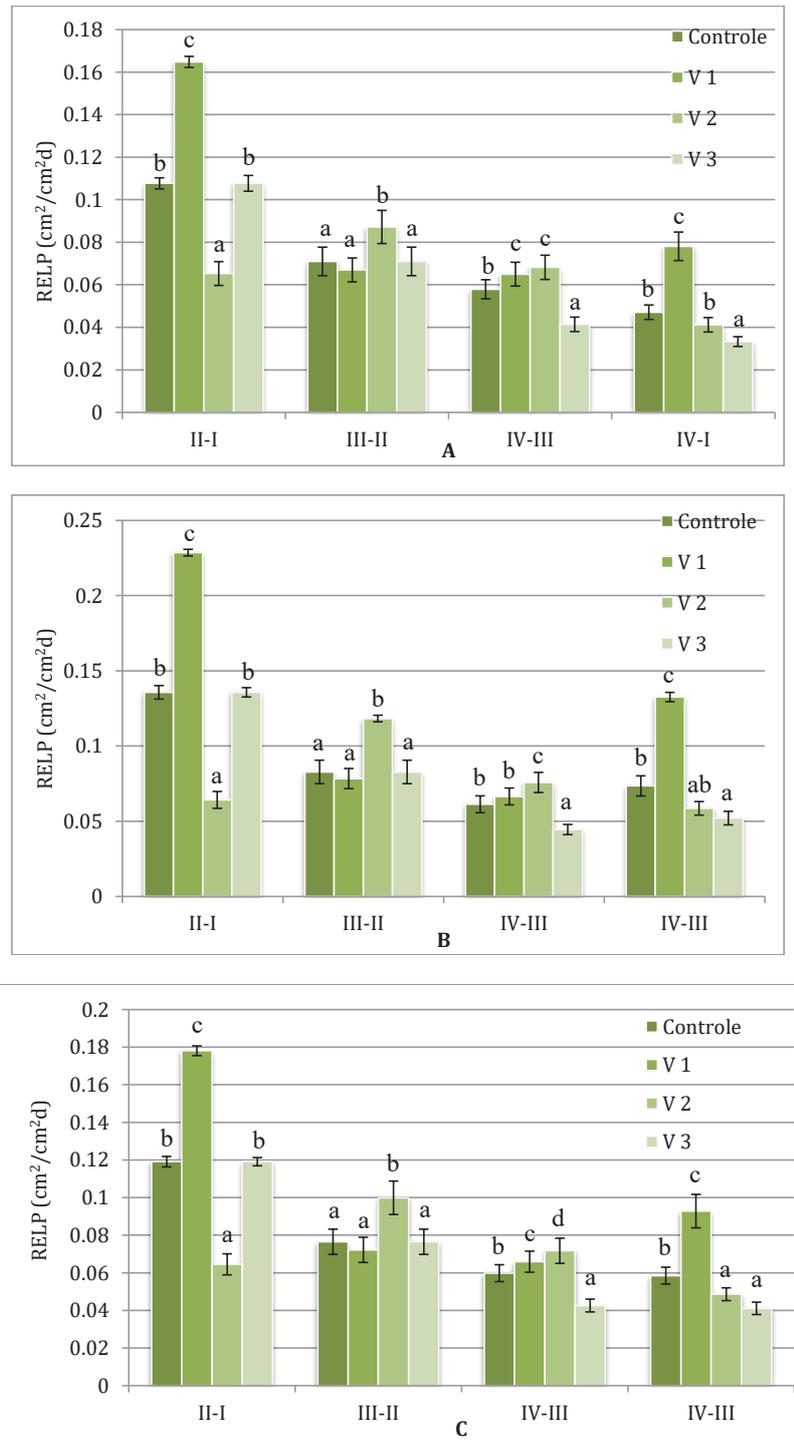


FIGURE 1  
Relative leaf expansion rate (RLER) of main (A), lateral (B) and total shoots (C), 2014 to 2016

CONCLUSIONS

Non-destructive and continuous measurements of leaf area on the same shoots enabled the monitoring of leaf area development and the calculation of relative leaf expansion rate. The grapevine’s ability to recover leaf area after defoliation depends mainly on the time when defoliation occurs. This study has shown that vines defoliated early had time to compensate for the removed leaves by producing more lateral shoots with a greater number of leaves, resulting in larger total leaf area. Moreover, the results show that, with

a decrease in the intensity of shoot growth during vegetation, the vine’s recovery ability decreases, and the compensatory growth is not enough to restore the reduced leaf area. Based on the values of the relative leaf expansion rate, it can be concluded that defoliation in the period of intensive shoots growth retards the emergence of new shoots and leaves for several days, followed by a period of regrowth. Very slow or no new growth of shoots and leaves occurs after defoliation at the véraison stage.

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