

# Effects on Berry Shrinkage in *Vitis vinifera*. L cv. ‘Merlot’ From Changes in Canopy/Root Ratio: A Preliminary Approach

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**A trial was conducted to find a possible relationship between the canopy/root ratio and the incidence and severity of premature berry shrinkage, and to propose an alternative to avoid this phenomenon in ‘Merlot’ grapevines. The ratio was changed by cutting foliage at a certain height 15 days before véraison, and by delaying the removal of trunk shoots. Treatments were the control (T1), 50% foliage area of control (T2), 75% foliage area of control (T3), and delayed trunk shoot removal (T4). Foliage area and the canopy/root ratio were lower in the T2 and T3 treatments. T4 was ineffective in changing the parameters. The incidence of berry shrinkage was lower for the T2 and T3 treatments, with the percentage of affected plants dropping from the 52% of the control to 22.9% and 31.3% for T2 and T3 respectively, and from 52.4% of the affected bunches to 16.6% and 21.2% for the same treatments respectively. The percentage of affected bunches falling into the range of moderate to severe damage fell from the 24% of the control to 5.2% and 3.9% for T2 and T3 respectively. Therefore, it is possible to avoid the incidence and severity of berry shrinkage by decreasing the canopy/root ratio in ‘Merlot’ grapevines.**

## INTRODUCTION

For many years, ‘Merlot’ and ‘Carménère’ cultivars were confused in Chilean vineyards, so that, when a distinction was made between them in the 1990s, the viticultural behaviour in the vineyard of each of them became clear. In this way, it was observed that, in much of the ‘Merlot’ vineyards, berry shrinkage occurred, which affects the yield and wine quality at harvest (Moreno & Vallarino, 2011). On the other hand, the growth curve of the grape berry (*Vitis vinifera* L.) has a double sigmoid shape, with two periods of significant growth being observed. The first one falls between fruit set and véraison, and the second one between véraison and maturity (Mathews *et al.*, 1987; Coombe, 1992; McCarthy, 1997; Rogiers *et al.*, 2000). However, in this second phase of grape berry development, when the concentration of sugars is still too low to harvest, a rapid loss of weight can be registered, causing berry shrinkage. This phenomenon has been described in *Vitis vinifera*. L cv. ‘Syrah’ (McCarthy, 1997; Rogiers *et al.*, 2000, 2001; Carlomagno *et al.*, 2018), where yield losses are estimated at around 25% of the total production (Rogiers *et al.*, 2000; Krasnow *et al.*, 2010).

Currently, publications on the subject are from the perspective of the damage to the conductive vessels that supply the grape berries. Some authors conclude that the premature dehydration of berries could be due to damage to the xylem inside the berry after véraison, a phenomenon that has been described in cultivars such as ‘Riesling’, ‘Merlot’, ‘Muscat Gordo Blanco’, ‘Pinot Noir’ and ‘Syrah’ (Düring

*et al.*, 1987; Findlay *et al.*, 1987; Creasy & Lombard, 1993; Rogiers *et al.*, 2001). Others authors have reported that this phenomenon could be due to the fact that sugar accumulation in the berry apoplast may lead to the decline in xylem water influx during ripening, causing berry dehydration (Keller *et al.*, 2006, 2015). Then there also are authors who showed the presence of phytoplasmas in grapevines affected with the berry shrinkage phenomenon (Matus *et al.*, 2008).

On the other hand, an inverse relationship between the daily gain in weight by the grape berries and the evaporative demand of the environment, even in berries close to harvest, has been reported (Rogiers *et al.*, 2000). Water potential becomes more negative when the absorption of water is slower than transpiration. Thus, excessive transpiration, low absorption or both can lead to a water deficit in the plant (Tardieu & Parent, 2017). It therefore could be expected that plants that possess a large surface area of leaves transpiring and a small number of roots supplying them (high canopy/root ratio) have a lower water potential and therefore extract more water from the fruit. In this way, there could be a balance between the leaf area and the roots of a plant that ensures that it does not lower its water potential too much in the face of a high evaporative demand from the environment. Due to this, the aim of this work was to provide an alternative through a preliminary approach to avoid berry shrinkage in ‘Merlot’ grapevines by making changes in the canopy/root ratio in one study season.

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## MATERIALS AND METHODS

### Study site

The field study was conducted in a commercial vineyard cv. 'Merlot' (from a local massal selection) of six years old, located in Los Niches-Curicó, Maule Valley, Chile (35°05' S.L., 71°03' O.L.). The vineyard was planted at a distance of 2.5 m between rows and 0.9 m between plants, with rows oriented from north to south. Grapevines were trained to a vertical shoot positioned (VSP) trellis system and pruned according to the Guyot system, leaving about 20 to 22 buds per vine. The vineyard has drip irrigation, with an output per plant of 4 L/hr. The climate is sub-humid Mediterranean type, with a prolonged dry season with an average annual temperature of 12.7°C, annual rainfall of 953 mm, and potential annual evapotranspiration of 921 mm. During the six warmest months (October to March), an average temperature of 16.5°C was registered, with a thermal sum of 1 030 degree days and a relative humidity of 73%, while the rainfall for the period from December to February was 33 mm. The soil corresponds to the series Huecán (Mollisol), which is deep, of colluvial origin, with good drainage and silty clay loam (clay 27.4%, sand 35.3%, lime 37.3%, organic matter 3.72%, bulk density 1.26%, field capacity 33.5%, and a permanent wilting point of 16.7%). The water provided for the period from véraison to harvest was 1 115 m<sup>3</sup>/ha. During the season, the vineyard presented premature dehydration of berries, causing losses of approximately 80%, leaving some sectors more affected without harvesting.

### Grapevine treatments

The field trial involved a comparison of the incidence of berry shrinkage in treatments with different canopy/root ratios from véraison to harvest, for which this relationship was altered by the direct reduction of leaf area or by delayed trunk shoot removal (Table 1). Foliage was cut approximately 15 days before véraison, so that the plants already showed very little or no growth of shoots. Before performing T2 and T3, the leaf area of 30 representative shoots was measured with a leaf area meter (model LI-3100, LI-COR, Lincoln, NE), establishing the average contribution of each bud to the total leaf area of the grapevine and, subsequently, the cutting height required to leave 50% and 75% of the original foliar area for the T2 and T3 treatments respectively was calculated. Delayed trunk shoot removal was carried out when the shoots reached 1 m average length. The assumption was made that the root system remains the same for all treatments during the critical period when berry shrinkage occurs, since from the stage at which the treatments were established until 70 days later, there would not have been any

important development of the roots (McKenry, 1984; Van Zyl, 1984). The field trial was conducted in a homogeneous sector. The statistical design corresponded to random blocks with six repetitions, and blocking was done according to the proximity to a drainage channel. Each experimental unit consisted of 12 plants.

### Grape berry measurement

From 30 days before véraison, the berry diameter was measured once a week until harvest. For this purpose, 18 berries were collected from two homogeneous grapevines. Once a week, 36 berries were collected from two homogeneous grapevines. Soluble solids were measured on seven dates, while pH and titratable acidity were measured three times in the obtained musts. The evolution of the incidence of berry shrinkage was evaluated once a week in 10 previously marked clusters from five grapevines per repetition, while at harvest, clusters from eight plants were evaluated per repetition. The incidence of berry shrinkage was expressed as the percentage of affected grapevines and clusters, and the severity was established by classifying each cluster according to the percentage of affected berries. Four categories were used: 1) healthy cluster: 0% affected berries; 2) slight damage: 1% to 32% affected berries; 3) moderate damage: 33% to 65% affected berries; 4) severe damage: 66% to 100% affected berries.

### Stem water potential ( $\Psi_{\text{stem}}$ ) and soil humidity measurements

$\Psi_{\text{stem}}$  was measured six times until harvest: 1) post-flowering, 2) immediately before establishing T2 and T3, 3) 10 days after establishing the treatments, 4) at véraison, 5) pre-harvest and 6) harvest, using a pressure chamber (Soil Moisture Equipment Corp, Santa Barbara, U.S.A.) according to the method described by Acevedo-Opazo *et al.* (2013). The moisture content of the soil was determined using a TDR (Soil Moisture Equipment Corp., Santa Barbara, U.S.A.), on the same dates that the stem water potential was measured.

### Canopy and root measurements

After harvest, the total leaf area of one vine was measured, in duplicate. From each leaf, the pedicel was removed, after which the leaf area was measured with a leaf area meter (LI-3100, LI-COR, Lincoln NE, U.S.A.). Subsequently, a root count was carried out according to the methodology of Hunter and Le Roux (1992). The roots were classified into five groups according to the classification of Richards (1983): fine (< 1 mm), extended (1 to 2 mm), permanent (2 to 5 mm) and structural (5 to 10 mm and > 10 mm). Canopy/root ratio was calculated by dividing the leaf area of the grapevine by its number of roots with a diameter equal to or less than 2 mm in the depth under the influence of the dropper (60 cm).

### Statistical analysis

The statistical analysis of the parameters analysed was performed using variance analysis (one-way ANOVA) (Statgraphics Centurion XVI.I, Warrenton VA, U.S.A.). Differences between samples were compared using the Duncan test at a 95% probability level.

TABLE 1  
Description of the treatments, Los Niches-Curicó.

Treatment	Description
T1	Control
T2	Defoliation at 50% leaf area of control
T3	Defoliation at 25% leaf area of control
T4	Delayed trunk shoot removal

## RESULTS AND DISCUSSION

**Canopy/root ratio and stem water potential ( $\Psi_{\text{stem}}$ )**

Foliar reduction gave rise to final differences in foliar area per grapevine between treatments with and without defoliation. However, no differences were found between 50% foliage area of control (T2) and 75% foliage area of control (T3), resulting in 58% and 67% of leaf area with respect to the control (T1) respectively. The T2 and T3 treatments decreased the canopy/root ratio. Thus, comparing leaf area obtained, differences were found in canopy/root ratio between the control and the defoliation treatments (T2 and T3), without finding differences between the latter two (Table 2). At seven days after defoliation, stem water potential ( $\Psi_{\text{stem}}$ ) was higher in the T2 and T3 treatments compared to T1 and delayed trunk shoot removal (T4), which indicates that these grapevines (with a lower canopy/root ratio) are able to respond better to the evaporative demand of the environment (Table 3). These changes in  $\Psi_{\text{stem}}$  imply that the canopy/root ratio is able to determine a change in the water status of the grapevine. This result matches those reported by Reynolds *et al.* (1996), who compared the grapevine water potential of different canopy/root relationships. It has been reported that the physiological and morphological alteration of plants under partial root-zone irrigation may bring more benefits to crops than improved water-use efficiency where carbon redistribution among organs is crucial in the determination of the quantity and quality of the products (Kang & Zhang,

2004).

Although there were no extreme water potentials, reaching only -0.6 MPa close to harvest in the control grapevines, it is possible to think that, under an extremely high evaporative demand of the environment and with low soil moisture, a grapevine with a low canopy/root ratio should have a less negative water potential and therefore extract less water from the grape berries. Increased transpiration and decreased phloem influx have been suggested as causes for berry shrinkage (McCarthy & Coombe, 1999; Rogiers *et al.*, 2006). However, certain authors have provided clearly evidence that several varieties of grape berries remain hydraulically connected to the parent grapevine (Bondada *et al.*, 2005; Chatelet *et al.*, 2008), and therefore may lose water back to the parent grapevine late in ripening, as well as to the dry ambient air (Tyerman *et al.*, 2004; Keller *et al.*, 2008; Krasnow *et al.*, 2010). Although the total leaf area of the treatments was very different from each other, which could have conditioned different water consumption between them, there were no differences in the moisture content of the soil between the treatments during the experiment (Table 3). This could be explained by the different water status of the treatments, where it is possible that the treatments with lower water potential could have closed their stomata for longer periods during the day to avoid gas exchange compare to the others treatments.

TABLE 2

Foliar area (cm<sup>2</sup>), foliar area per gram of fruit (cm<sup>2</sup>/g), percentage of effective foliar area of the control (%), and canopy/root relation (leaf cm<sup>2</sup> /No. of roots thinner than 2 mm in the first 60 cm): Post-harvest measurements, Los Niches-Curicó.

Treatment	Foliar area cm <sup>2</sup> /vine	cm <sup>2</sup> leaf/g fruit	% of effective foliar area of control	Canopy/root ratio (cm <sup>2</sup> leaf/No. roots < 2 mm, 60 cm depth)
T1: Control	43.224a	16.5	100.0	117.6a
T2: Defoliation at 50% of T1	25.132b	8.9	58.1	70.4c
T3: Defoliation at 75% of T1	28.971b	10.2	67.0	80.2bc
T4: Delayed trunk shoot removal	43.486a	16.3	100.6	107.4ab

For each parameter, different letters in the same column indicate significant differences between treatments ( $p \leq 0.05$ )

TABLE 3

Stem water potential (MPa) before and after the establishment of treatments 2 and 3, and soil water content (volumetric %) of the last evaluation, Los Niches-Curicó.

Treatments	Stem water potential (MPa)				Soil moisture (% v/v)
	January 25 (before treatments)	January 31 (1 week after treatments)	February 23 (véraison)	March 29 (first symptoms)	March 29 (first symptoms)
T1: Control	-0.37a	-0.44a	-0.41a	-0.6a	26.3a
T2: Defoliation at 50% of T1	-0.4a	-0.34b	-0.25b	-0.38b	27.2a
T3: Defoliation at 75% of T1	-0.4a	-0.37b	-0.26b	-0.39b	29.4a
T4: Delayed trunk shoot removal	-0.38a	-0.47a	-0.42a	-0.63a	25.5a

For each parameter, different letters in the same column indicate significant differences between treatments ( $p \leq 0.05$ )

### Physicochemical and productive parameters

Regarding grape physicochemical parameters, the T2 treatment presented higher soluble solids ( $^{\circ}$ Brix) than the T1 and T4 treatments (with 100% AF); this was expected due to the smaller surface available for the preparation of photosynthates in the T2 treatment. However, the T3 treatment (with 67% final effective leaf area) showed no differences in this variable with respect to the control (Table 4). Moreover, no significant differences were found in pH and titratable acidity among the treatments. In respect to berry productiveness, no statistical differences were observed in the weight of the berries when comparing all the treatments. Furthermore, the T3 treatment presented a greater diameter of the berries than the T1 and T4 treatments (Table 5). Based on this, when the atmospheric demand is high, the plants not only extract water from the soil, but also use the water from the berries, which translates into a change in the diameter of the berries and can be irreversible (Creasy & Lombard, 1993). This supports that fact that the grapevines with a low canopy/root ratio are able to respond better to the evaporative demand of the environment. Pastore *et al.* (2013) reported that pre-bloom defoliation improved sugar and anthocyanin synthesis, whereas defoliation at véraison had a detrimental effect in terms of less anthocyanin accumulation and a higher incidence of sunburn damage. In addition, Keller *et al.* (2006) concluded that sugar accumulation in grape berries by apoplastic phloem unloading can reduce xylem water influx into ripening berries. Moreover, it has been reported that xylem flow in ripening berries, but not berry size, remained responsive to root or shoot pressurisation (Keller *et al.*, 2015). A mass balance analysis of ripening berries sampled in the field suggested that phloem water inflow may exceed growth and transpiration water demands (Keller *et al.*, 2015). These considerations indicate that the

decrease in xylem inflow at the onset of ripening may be a consequence of the sink-driven increase in phloem inflow (Keller *et al.*, 2015).

### Incidence and severity of berry shrinkage

A lower percentage of grapevines affected by berry shrinkage was observed after T2 and T3 treatments. In addition, the same treatments presented a lower percentage of damaged clusters for the categories of mild to severe and moderate to severe damage, as expected for the trial (Table 6). These results agree with those found by Huguet (1985), who measured lower dehydration of fruit after the defoliation of apple and citrus trees. Delayed trunk shoot removal (T4) did not reduce the leaf area of the shoots, and the canopy/root ratio was not altered. Moreover, the T4 treatment did not show statistical differences in stem water potential, berry weight, berry diameter, incidence and severity of berry shrinkage compared to the rest of the treatments. Carlomagno *et al.* (2018) reported that the xylemic backflow is active at pre-véraison but not post-véraison in 'Syrah' grapevines. In addition, these authors showed that there is a 'plant/berry' and 'berry/plant' water communication pre-véraison, whereas this seems to cease progressively post-véraison. Moreover, water movement from the berry back to the parent vine via the xylem (backflow) may be an important component of berry weight loss in 'Shiraz', particularly if the phloem ceases functioning at high osmotic potentials near maximum weight (Tyerman *et al.*, 2004). Based on the aforementioned, it would be expected that a more immature berry is less prone to premature dehydration, since its cell membranes have a lower level of damage. As a result of this, the lower incidence and severity of berry shrinkage in the T2 treatment (with a lower concentration of soluble solids) is not only attributable to the effect of the alteration of canopy/

TABLE 4

Soluble solids content ( $^{\circ}$ Brix), pH and titratable acidity (g/L equivalent of sulfuric acid). Harvest evaluation (2003-05-07), Los Niches-Curicó.

Treatments	Soluble solids ( $^{\circ}$ Brix)	pH	Titratable acidity (g/L sulphuric acid)
T1: Control	24.73a	3.51a	4.66a
T2: Defoliation at 50% of T1	23.07b	3.55a	4.74a
T3: Defoliation at 75% of T1	24.23ab	3.52a	4.56a
T4: Delayed trunk shoot removal	25.47a	3.52a	4.43a

For each parameter, different letters in the same column indicate significant differences between treatments ( $p \leq 0.05$ )

TABLE 5

Berry weight (g) and diameter (mm), from the last evaluation at harvest (2003-05-07), Los Niches-Curicó.

Treatments	Weight of berries (g)	Diameter of berries (mm)
T1: Control	1.44a	10.62a
T2: Defoliation at 50% of T1	1.46a	11.18ab
T2: Defoliation at 75% of T1	1.53a	11.72b
T4: Delayed trunk shoot removal	1.38a	11.07a

For each parameter, different letters in the same column indicate significant differences between treatments ( $p \leq 0.05$ )

TABLE 6

Percentage of affected plants (with at least one bunch with moderate and one with severe dehydration) and bunches affected with light damage to severe, or moderate to severe damage. Harvest evaluation (2003-05-07), Los Niches-Curicó.

Treatment	% Affected vines	% Affected bunches (light to severe)	% Affected bunches (moderate to severe)
T1: Control	62.5ab	52.4a	24.0ab
T2: Defoliation at 50% of T1	22.9c	16.6b	5.2b
T2: Defoliation at 75% of T1	31.3bc	21.2b	3.9b
T4: Delayed trunk shoot removal	83.3a	65.0a	33.9a

For each parameter, different letters in the same column indicate significant differences between treatments ( $p \leq 0.05$ )

root ratio, but also to a different physiological condition that means they are not comparable with the rest of the treatments. However, comparing T3 with respect to the T1 and T4 treatments, it was clear that, despite the fact that the grape berries had similar maturity, there was a considerable difference with reference to the incidence and severity of berry shrinkage, which could be attributable to the changes in canopy/root ratio.

The presence of phytoplasma was detected in the samples collected in the two different seasons, which further suggests that this pathogen may be one of the causal agents of the disorder (Matus *et al.*, 2008). It therefore is possible that the presence of these phytoplasmas have an effect on the canopy/root ratio, leading to changes in the aforementioned relationship. Therefore, when bringing the research into connection with the practice, changes in the canopy/root ratio in a commercial vineyard could be both economically and technically unfeasible. Defoliation without previous tests could affect the normal ripening of grapevines, thereby affecting the technological maturity of grape berries. It therefore would be more practicable to try to change the canopy/root ratio by stimulating the development of roots or reducing grapevine vigour in 'Merlot' vineyards. The correction measures will vary depending on the cause of the imbalance between the foliage and root development.

Among the steps that have succeeded in mitigating or solving the berry shrinkage phenomenon in 'Merlot' vineyards it is possible to mention: a) the use of cover crops in order to reduce excessive spring water in the soil as soon as possible after the winter rains, b) the establishment of ridges to increase the volume of soil explored by the roots in the presence of physical impediments, c) the application of organic amendments to stimulate the growth of rootlets in thin or very heavy soils, d) the management of root pests and diseases, e) avoiding over-fertilisation with nitrogen in order to prevent excessive vigour in the spring, and f) careful planning of irrigation with the aim to avoid large spring growth and periods of stress between véraison and harvest, among others. Despite the aforementioned, the study of changes in the canopy/root ratio must be carried out in more seasons to evaluate the real impact of this form of viticultural management. However, these preliminary results can be useful for 'Merlot' winegrowers in order to mitigate the phenomenon of berry shrinkage.

## CONCLUSIONS

By decreasing the canopy/root ratio, the incidence and severity of berry shrinkage is decreased. Despite the results obtained, more seasons should be studied to evaluate the effects of changes in canopy/root ratio on the incidence and severity of this phenomenon. From a production point of view, the defoliation established in this trial to try to alter the canopy/root ratio is not economically and technically applicable in the vineyard, mainly due to the high economical cost, the lack of practical feasibility and its possible negative effect on grape berry maturation. However, the canopy/root ratio could be altered by other methods, such as the stimulation of root development and reducing vine vigour. It is particularly important to find a canopy/root ratio that allows the fruit to mature in an appropriate way according to the productive objectives of the wine cellar, while at the same time allowing a reduction in berry shrinkage to levels that are acceptable to the producers.

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