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ORIGINAL RESEARCH ARTICLE

Partial double-pruning after bloom delays bunch rot epidemics in *Vitis vinifera* L. cvs. Riesling and Pinot gris

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ABSTRACT

Bunch rot caused by *Botrytis* cinerea is a major fungal disease in grapevines. Under humid climatic conditions, bunch rot development on grapes cannot be completely suppressed and bunch rot control strategies mainly aim to delay the epidemic. In the present study, we investigated the potential of the innovative cultural practice "partial double-pruning after bloom (PDP)" to delay the bunch rot epidemic on Pinot gris and Riesling cultivars over five consecutive seasons (2016-2020) in Remich/Luxembourg. Control vines were pruned at winter to one 10-node fruiting cane per vine, while in PDP, two 10-node fruiting canes per vine were kept; one of the two canes was removed at BBCH 73 (2-3 weeks after bloom).

In all the 10 cultivar*year combinations, the bunch rot disease severity at the final assessment date (shortly before harvest) was lower in PDP than in the control. This reduction was significant ($P \leq 0.05$) in 7 of the 10 cultivar*year combinations. PDP significantly delayed the date when 5 % disease severity was reached; in data pooled over the five years this delay ranged between 10.3 (Pinot gris) and 8.3 days (Riesling). The proportion of non-marketable fruit was significantly reduced by 41 % (Pinot gris) and 53 % (Riesling). Total yield per plant was reduced by 10 % (Pinot gris) and 19 % (Riesling), with a significant increase in total soluble solids at harvest in the case of Riesling. An additional evaluation in the year 2020 revealed reduced cluster compactness in PDP for both cultivars.

PDP turned out to be an innovative, efficient, reliable and relatively cost-efficient cultural practice to delay the bunch rot epidemic in grapes. It can be integrated as one module into the best practice strategy to control bunch rot and contributes to pesticide reduction in viticulture.

KEYWORDS: *Botrytis cinerea*, bunch rot epidemic, cluster structure, cultural practices, pesticide reduction

INTRODUCTION

Bunch rot (also referred to as *Botrytis* bunch rot or grey mould) caused by *Botrytis cinerea* Pers.: Fr. (teleomorph: *Botryotinia fuckeliana* (de Bary) Whetzel) is one of the major fungal diseases of grapevines (*Vitis vinifera* L.), causing severe economic damage in many grape growing regions worldwide (Wilcox *et al*., 2015, Kassemeyer and Berkelmann-Löhnertz, 2009). The disease compromises both grape yield and wine quality in terms of off-flavours, difficulties in clarification, unstable colour, oxidative damage and premature ageing (Ribéreau-Gayon, 1983; Smart and Robinson, 1991; Wilcox *et al*., 2015). Clusters of the traditional *V. vinifera* L. cultivars Pinot gris and White Riesling (from now on referred to as Riesling) are relatively susceptible to Botrytis bunch rot. Pinot gris is classified with 5 and Riesling with 4 out of 9 points in the category bunch rot susceptibility in the cultivar description list of the German Federal Office of Plant Varieties (Bundessortenamt, 2015).

Under the climatic conditions of Central Europe, the disease occurs every year while the onset of the epidemic varies between years (Molitor *et al*., 2020a).

Consequently, bunch rot control strategies mainly focus on delaying the epidemic as much as possible to allow for a sufficiently long ripening period to reach adequate fruit maturity for the production of high-quality wines (Molitor *et al*., 2015a). Besides direct control via routine applications of fungicides (Shtienberg, 2007) with known activity against *B. cinerea* (botryticides), indirect cultural practices are gaining more and more attention in practical viticulture due to their high efficiency in controlling bunch rot and thus reduce pesticide use in viticulture. Examples of cultural practices that have been described as efficient in delaying bunch rot epidemics include leaf removal in the cluster zone (Poni *et al*., 2006; English *et al*., 1989; Zoecklein *et al*., 1992; Evers *et al*., 2010; Molitor *et al*., 2011a; Herrera *et al*., 2016; Vander Weide *et al*., 2021), cluster division (Molitor *et al*., 2012a; Schultz *et al*., 2003), late primary shoot topping (Molitor *et al*., 2015a), artificial shading (Basile *et al*., 2015) and flower debris removal (Wolf *et al*., 1997; Molitor *et al*., 2015b).

Complex bunch rot control strategies include several indirect measures and, potentially, chemical complements (Molitor *et al*., 2018) to reduce the risk of premature harvest enforced by decreasing grape health status (Molitor *et al*., 2012a).

The degree of cluster compactness has been demonstrated to be the key factor in a predisposition toward bunch rot on several occasions (Molitor *et al*., 2011a; Molitor *et al*., 2012b; Molitor *et al*., 2012a; Hed *et al*., 2009; Tello and Ibanez, 2017; Intrigliolo *et al*., 2014).

It is determined by rachis length and level of branching, number and berry size. The latter two factors depend on (besides genetic factors) the flowering process, as well as the subsequent stages of cell division and cell expansion during berry growth (Tello and Ibanez, 2017). Consequently, cultural practices impacting the cluster structure often focus on decreasing berry number, berry size and/or increasing rachis length (Tello and Ibanez, 2017). This is frequently induced by the modulation of the source–sink balance (Poni *et al*., 2006; Tello and Ibanez, 2014; Keller, 2015). Clusters with smaller and/or fewer berries can be obtained by lowering source-sink ratios during cluster development, by adopting cultural practices such as leaf removal (Poni *et al*., 2006), or by increasing crop and bud loads and, thus, vine capacity (Keller, 2015). The amount of reserve carbohydrates that are allocated to individual shoots at the beginning of the season is a function of the shoot number per vine (Keller, 2015). High shoot loads consequently lead to reduced shoot vigour and leaf area per shoot (Kliewer and Dokoozlian, 2005). Flowers developing on vines with high shoot loads are consequently affected by a limited supply of reserve carbohydrates, as well as by a low supply of recent assimilates due to low leaf areas per shoot. Thus, under C limitation, clusters show lower weights with fewer and/or smaller berries than in the case of sufficient C supply. In addition, flower numbers per shoot in the subsequent year may be reduced under the C limitation (Bennett *et al*., 2002 and flower numbers per shoot in the subsequent year may be reduced (May, 2004).

Following these theoretical considerations, we hypothesise that increasing the bud load by retaining two canes instead of one cane per plant until approximately 2 to 3 weeks after bloom should reduce cluster compactness and delay the bunch rot epidemic.

To the best of our knowledge, this innovative technique, from now on referred to as ‘partial double-pruning after bloom” (PDP), has not been presented or tested in the scientific literature so far.

The present investigation on the *Vitis vinifera* cultivars Pinot gris and Riesling aimed to (i) test theoretical considerations about the effects of PDP on the cluster structure and the annual bunch rot epidemic, and (ii) derive recommendations for applications of PDP in viticulture.

Annual meteorological conditions strongly fluctuate between years in Central Europe thereby affecting bunch structure (Tello and Ibanez, 2017) and the onset of bunch rot epidemics (Molitor *et al*., 2020a). Consequently, a multi-annual analysis based on data obtained over five consecutive years (2016–2020) was carried out to test the effects of PDP under various environmental conditions.

MATERIALS AND METHODS

1. Vineyard site and experimental design

Field trials were conducted in the experimental vineyards of the Institut Viti-vinicole in Remich, Luxembourg (lat. 49.54°N; long. 6.35°E) between 2016 and 2020 on *Vitis vinifera* L. cultivars Pinot gris (clone Remich 6) and Riesling (clone Remich 10). Both cultivars were planted in 1994,

grafted onto SO4 rootstock, and trained to a vertical shoot positioning system (VSP). The vineyard faced south with an inclination of 10 %. The rows were oriented in a north-south direction. The soil between the rows was permanently cover cropped and no irrigation took place. The space per plant was 2.4 m^2 (2 m between rows, 1.2 m between vines). Fungicide applications against *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni and *Erysiphe necator* Schwein, following an organic plant protection strategy, were carried out in all seasons at intervals of 10 to 12 days. No products with known activity against *Botrytis cinerea* were applied.

Experiments were realised using randomised complete block designs with four replicate plots with eight vines per plot. Each block consisted of two treatments. The position of the two treatments in each block was randomly distributed.

Treatments were the same in all years and were defined as it follows:

A) Control: vines pruned in winter to one 10-node fruiting cane per vine;

B) Partial double-pruning after bloom (PDP): vines pruned in winter to two 10-node fruiting canes per vine; one of the two canes were removed at BBCH 73 (2–3 weeks after bloom)

In both treatments, one replacement spur per plant with 1 to 2 nodes remained. In treatment A, the cane was tied downhill, while in treatment B, one cane was bound downhill and the second cane uphill. The uphill-bound cane was removed completely at BBCH 73 with a clean cut at the insertion on the older wood. Dates and phenological plant growth stages at the date of second cane removal in the PDP treatment are shown for both cultivars in Table 1.

2. Meteorological and phenological data

Meteorological data were recorded during the period of examination by a weather station of the national agricultural administration ASTA (Administration des services techniques de l'agriculture) located in direct proximity (distance < 100 m) of the experimental vineyard. Air temperatures were measured at 2 m and precipitation at 1 m above the ground. The phenological stage of full flowering (BBCH 65), according to Lorenz *et al*. (1995), was recorded based on observations of six plants/organs per cultivar.

3. Assessment of the cluster morphology

Potential differences in the cluster structure were assessed in both cultivars using the cluster density index according to the protocol by Ipach *et al*. (2005) as previously described (Evers *et al*., 2010). One hundred clusters were assessed per plot at BBCH 79 (2016: 09 August; 2017: 26 July; 2018: 23 July; 2019: 06 August; 2020: 04 August) following the protocol by Ipach *et al*. (2005).

Based on the observation that the bunch rot epidemic was delayed in the PDP treatment in the initial years of the present study, we hypothesised that this effect might be caused by changes in berry numbers per cluster or berry sizes. Consequently, in 2020, an additional assessment was carried out: Prior to harvest, ten clusters were randomly selected per plot (n = 4 * 10) in each of the two cultivars and for both treatments in 2020. Rachis lengths and cluster masses were determined. Clusters were manually destemmed and the number of berries per cluster counted. Very small, still hard berries (shot berries) were not considered. The average mass of individual berries was calculated by dividing the cluster mass by the number of berries per cluster. As an indicator of cluster compactness (Tello and Ibanez, 2014), the cluster mass per cm of cluster length was calculated.

4. Assessment of *B. cinerea* disease progress

The *B. cinerea* disease progress was followed in weekly to bi-weekly intervals between veraison (BBCH 81) and harvest (BBCH 89) by examining 100 clusters per plot (50 clusters from both sides of the canopy) selected randomly at each assessment date. Disease severity was assessed according to the EPPO guideline PP1/17 classifying visually observed disease severity in seven classes (0 %; 1-5 %; 6-10 %; 11-25 %; 26-50 %; 51-75 %; 76-100 %).

To describe the temporal progress, disease severity in both treatments was plotted over time (expressed as day of the year (DOY)). Disease progress curves were fitted to these data according to the sigmoidal equation (1) as previously described (Molitor *et al*., 2015a).

$$y = \frac{100}{1 + e^{-((x - x_0)/b)}} \quad (1)$$

where y is the disease severity, x corresponds to the assessment date expressed as the day of the year (DOY), x_0 is the inflection point of the curve (disease severity of 50 % reached) and b is the slope factor of the curve at the inflection point.

Solving this equation for x provides the time point at which a specific disease severity value was reached. To quantify differences between the treatments in the temporal position of the annual epidemic, the $x_{5\,\%}$-values (DOY corresponding to a disease severity of 5 %) were used according to Beresford *et al*. (2006).

TABLE 1. Date and DOY (day of year) of double-pruning after bloom (identical in both cultivars) and phenological stages according to BBCH in the two cultivars Riesling (Rie) and Pinot gris (Pg).

2016				2017				2018				2019				2020			
Date	DOY	Rie	Pg	Date	DOY	Rie	Pg	Date	DOY	Rie	Pg	Date	DOY	Rie	Pg	Date	DOY	Rie	Pg
12.07.	194	73	73	26.06.	177	73-75	73-75	18.06.	169	73	73	03.07.	184	73	73-75	29.06.	181	73	73-75

5. Harvest parameters

In all years, grapes were harvested separately per plot. Harvest dates were 6 October 2016 (Pinot), 20 October 2016 (Riesling), 27 September 2017, 25 September 2018, 1 October 2019 (Pinot), 7 October 2019 (Riesling) and 8 October 2020. All grapes from each plot were classified into a marketable (no bunch rot) and non-marketable (botrytised parts of clusters) fraction based on a visual assessment of the harvest team. The average yield per plant of both fractions, average total yield per plant and proportion of the non-marketable fraction in the total yield were calculated. A set of 100 healthy berries per plot (50 on each side of the row) were randomly sampled just prior to harvest. Every sample was pressed, the juice was centrifuged and total soluble solids concentration was measured by FT-IR (FOSS NIRSystems, Laurel, MD, USA).

6. Data analyses and statistics

Data sets were generally assessed on a per plot basis (n = 4).

Differences between treatments in the same year/assessment date as well as within the same cultivar were assessed based on the mean comparison ($P < 0.05$) using the software R version 4.0.2. Normality and homogeneity of the data were assessed using Shapiro–Wilk and Fisher–Snedecor tests, respectively. When the data followed a Gaussian distribution and had homogenous variances, a comparison of means was made using Student's *t*-test; when data followed a heterogenous Gaussian distribution but had variances, a comparison of means was done using a Welch *t*-test that is robust against heterogeneous variances and when the distributions were not normal, a Wilcoxon–Mann–Whitney test (U-test) was used.

To detect consistent effects over all five years, annual mean values of the (i) density index, (ii) marketable yield per plant, (iii) non-marketable yield per plant, (iv) total yield per plant, (v) proportion of the non-marketable yield on the total yield and (vi) total soluble solids at harvest were normalised by dividing the value of the treatment by the value of the control treatment. Furthermore, the annual deviation (in days) from the control were calculated per each treatment based on the day of the year, reaching 5 % disease severity. Normalised values of both treatments were compared pairwise ($P \leq 0.05$) by an independent-sample Welch test (unequal variance t-test).

RESULTS

1. Key meteorological data and dates of BBCH 65

Key meteorological data for the years 2016 to 2020 are reported in Table 2.

The average temperatures of the growing seasons (April–October) were rather similar in 2016, 2017, 2019 and 2020, ranging from 15.5 °C to 16.2 °C. In 2018, an average growing season temperature of 17.0 °C was recorded (Table 2). The lowest precipitation sum within the growing season was observed in 2018 (295 mm) and the highest precipitation sum was recorded in 2016 (462 mm) (Table 2). The phenological stage of full flowering (BBCH 65) was reached in Riesling between June 5 (2018) and June 26 (2016) and between June 3 (2020) and June 24 (2016) in Pinot gris (Table 2).

2. Cluster architecture

Cluster density in Pinot gris was significantly higher in 2017 in the PDP treatment than in the control (Table 3). In the other years, no significant differences between the treatments were recorded (Table 3). The average normalised density index in PDP was 1.01 and did not differ from the control (Table 3). In Riesling, significantly lower cluster density was recorded in the PDP treatment compared to the control in 2016 and 2020 (Table 3). The average normalised density index in the PDP treatment was 0.92, while the reduction was not statistically significant according to the Welch *t*-test ($P = 0.08$) (Table 3).

In Pinot gris, the analyses of cluster structure parameters in 2020 did not show significant effects of the treatments on cluster length, weight, number of berries per cluster and berry weight, while the cluster weight per cm of cluster length was significantly reduced in PDP (Table 4). In Riesling, PDP significantly reduced the cluster weight, berry weight and cluster weight per cm of cluster length in 2020. The cluster length and number of berries per cluster were not significantly affected (Table 4).

TABLE 2. Key annual and growing season (April – October) meteorological data as well as dates of full flowering (BBCH 65) assessed in Riesling and Pinot gris grapevines from 2016 to 2020.

	Growing Season				BBCH 65			
					Riesling		Pinot gris	
Year	Mean temperature (°C)	Precipitation sum (mm)	Mean temperature (°C)	Precipitation sum (mm)	Date	Day of the year	Date	Day of the year
2016	10.9	760	15.6	462	26.06.	178	24.06.	176
2017	11.0	725	15.5	413	10.06.	161	10.06.	161
2018	11.8	716	17.0	295	05.06.	156	04.06.	155
2019	11.4	764	15.8	397	21.06.	172	20.06.	171
2020	11.9	774	16.2	380	09.06.	161	03.06.	155
Average	11.4	748	16.0	389	14.06.	165	12.06.	163

TABLE 3. Cluster density index according to Ipach *et al.* (2005) and normalised density index. Significantly different values (n = 4, Sig. ≤ 0.05) are marked in bold font.

		2016	2017	2018	2019	2020	Average normalised density index
Pinot gris	Control	2.7	3.5	3.2	4.2	3.6	1.00
	PDP	2.8	3.7	3.0	4.32	3.5	1.01
	Sig.	0.245	0.023	0.062	0.724	0.114	0.74
Riesling	Control	3.6	3.6	2.8	3.7	3.5	1.00
	PDP	3.3	3.6	2.7	3.6	2.8	0.92
	Sig.	0.012	0.486	0.380	0.697	0.003	0.08

3. Bunch rot progress

In 4 out of the 5 years of the +study (2017, 2018, 2019 and 2020), disease severity in Pinot gris was significantly lower in PDP treatment than in control. In Riesling, the disease severity at the final assessment date was lower in the PDP treatment for all five years, while differences were significant in 2016, 2017 and 2018

The delay of the epidemic due to PDP was 5.1 to 13.5 days in Pinot gris (average delay: 10.3 days) and 0.2 to 13.2 days in Riesling (average delay: 8.3 days) (Supplementary Table 2; Figure 2).

4. Harvest parameters

Marketable yield (healthy grapes) was significantly reduced by PDP in 2 out of the 10 cultivar*year combinations (Pinot gris 2019; Riesling 2020). The average normalised marketable yield in the PDP treatment was 0.95 in Pinot gris (5 % yield reduction) and 0.9 in Riesling (10 % yield reduction), with no significant differences compared to the control (Supplementary Table 3; Figure 3).

The non-marketable yield (rotten grapes) was significantly reduced by PDP in 5 out of the 10 cultivar*year combinations (Pinot gris 2018 and 2019; Riesling 2016, 2018 and 2020). The average normalised non-marketable yield in the PDP treatment was lower than control corresponding to 0.53 in Pinot gris (47 % reduction) and 0.41 in Riesling (59 % reduction) (Supplementary Table 3; Figure 3).

Total yield was significantly reduced by PDP in 2 out of the 10 cultivar*year combinations (Pinot gris 2019; Riesling 2020). The average normalised total yield in PDP was 0.90 in Pinot gris (10 % total yield reduction) and 0.81 in Riesling (19 % total yield reduction) with no significant differences compared to the control (Supplementary Table 3; Figure 3).

The proportion of the non-marketable yield was significantly reduced by PDP in 2 out of the 10 cultivar*year combinations (Riesling 2016 and 2018). The average normalised proportion of non-marketable yield in the treatment PDP was 0.59 in Pinot gris (41 % reduction) and 0.47 in Riesling (53 % reduction), with significant differences compared to the control in both cultivars (Supplementary Table 3; Figure 3).

Total soluble solids at harvest were not reduced by PDP. The average normalised total soluble solids in the PDP treatment were 1.01 in Pinot gris (1 % increase in total soluble solids) and 1.02 in Riesling (2 % increase in total soluble solids). For the Riesling, the increase was statistically significant compared to the control (Supplementary Table 3; Figure 3).

DISCUSSION

1. Effects on bunch rot epidemics

In the present study, PDP was demonstrated to have impressive effects on the development of bunch rot epidemics. In all the 10 cultivar*year combinations, the

TABLE 4. Variation of cluster length, cluster weight, number of berries per cluster, berry weight and cluster compactness in Pinot gris and Riesling grapevines subjected to PDP compared to the control. Data were assessed on 09/08/2020. Significantly different value (n = 4, Sig. ≤ 0.05) are marked in bold font.

		Cluster length (cm)	Cluster weight (g)	Berries per cluster (n)	Berry weight (g)	Cluster compactness (g/cm)
Pinot gris	Control	14.0	193.4	166.5	1.20	13.5
	PDP	14.5	174.1	144.9	1.22	11.9
	Sig.	0.374	0.152	0.065	0.657	0.025
Riesling	Control	11.9	178.4	138.6	1.29	14.8
	PDP	12.1	125.5	120.1	1.04	10.3
	Sig.	0.566	< 0.0001	0.059	0.015	< 0.0001

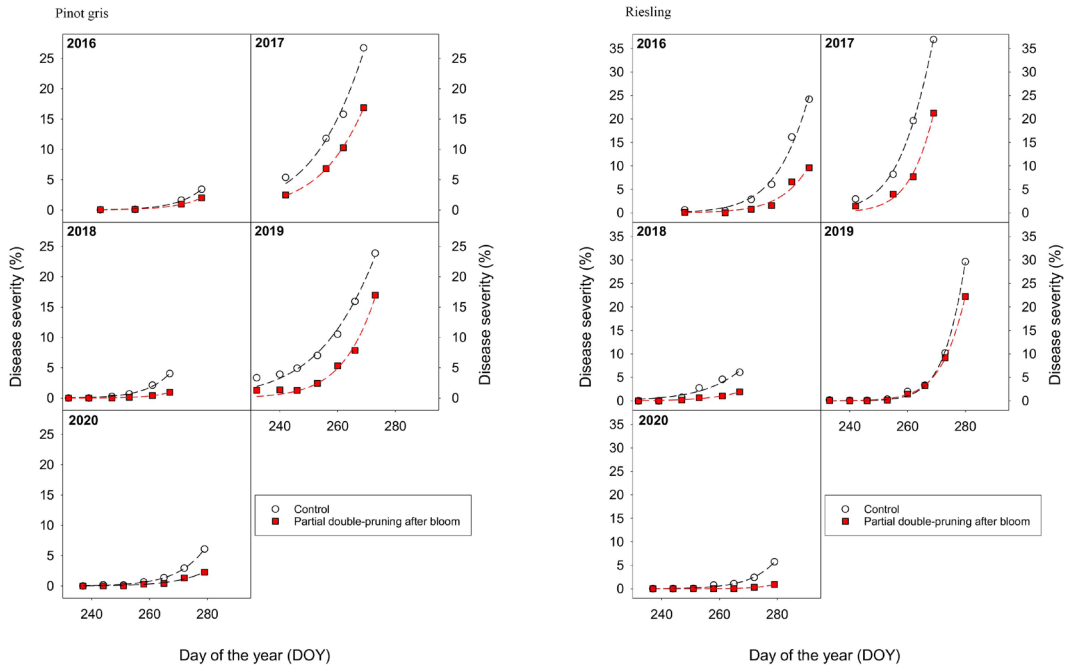


FIGURE 1. Progress of the disease severity of B. cinerea in the different treatments between 2016 and 2020 as functions of the day of the year in Pinot gris (at the top) and Riesling (below). Plot symbols represent the observed disease severity, with the lines showing the calculated progress according to the sigmoidal equation type $y = 100 / (1 + e^{-((x-x0)/b)})$. Values of different treatments of the same date and the same cultivar marked with "*" differ significantly (Sig. < = 0.05).

disease severity at the final assessment date was lower in PDP than in the control; this effect was significant in 7 out of the 10 cultivar*year combinations (Figure 1). PDP delayed the bunch rot epidemic (measured as DOY reaching 5 % disease severity) significantly in both the cultivars and the five years (Figure 2, Supplementary Table 2). The average delay of the epidemic was 10.3 days in Pinot gris and 8.3 days in Riesling (Figure 2; Supplementary Table 2), indicating a high treatment efficiency. In addition, the proportion of non-marketable yield in PDP was reduced by 41 % (Pinot gris) or even 53 % (Riesling) as compared to the control (Figure 3; Supplementary Table 3).

The observed delay of the epidemic is of special interest for relatively cool climate grape growing regions, where the date of harvest is frequently determined by crop health status rather than by optimum grape maturity (Molitor *et al.*, 2012a). Here, the delay of the epidemic creates a temporal as well as a maturation buffer before the grapes reach a disease severity threshold that forces the grower to harvest and thus increases the chances of reaching the status of full grape maturity. Since wines from late-harvested grapes are often perceived as being of higher quality (Spring, 2004), PDP may contribute to increasing the potential wine quality.

In the present study, a reduction of the total yield in PDP compared to the control was observed (on average: –10 % in Pinot gris; –19 % in Riesling), but the effect was not statistically significant. However, this yield reduction as a consequence of PDP is approximately equivalent to yield reductions observed as a consequence of other crop cultural measures reducing cluster compactness (Evers *et al.*, 2010; Molitor *et al.*, 2011a; Molitor *et al.*, 2011b; Molitor *et al.*, 2012a; Molitor *et al.*, 2015a; Molitor *et al.*, 2017). The reduction of the marketable yield caused by PDP (5 % in Pinot gris and 10 % in Riesling), however, is distinctively lower than the reduction of the total yield due to the reduced proportion of clusters or cluster parts affected by rot. This lower proportion of the negative fraction in PDP is supposed to reduce the time needed for negative selection processes during harvest.

The efficiency of PDP against bunch rot infections is likely related to (i) a lower cluster compactness or (ii) a devigorating effect on single shoot growth by the increased bud load, which might improve the canopy microclimate after the removal of the second cane. Both effects potentially have a strong impact on the onset and development of bunch rot epidemics. This might alleviate effects caused by compact bunches and dense canopies such as: (i) increased risk of berry cracking due to high pressure in the interior parts of the cluster (Smart and Robinson, 1991), (ii) low sun exposure of clusters and low air circulation in the cluster zone leading to microclimatic conditions favouring the development of fungal pathogens (Zoecklein *et al.*, 1992), (iii) rapid spread of fungal mycelium from berry to berry (Hed *et al.*, 2009), and (iv) reduced spray penetration and coverage of inner fruit by fungicides (Hed *et al.*, 2009; Molitor *et al.*, 2015a).

Although the effects of lower shoot growth and less compact bunches on the development of botrytis epidemics are difficult

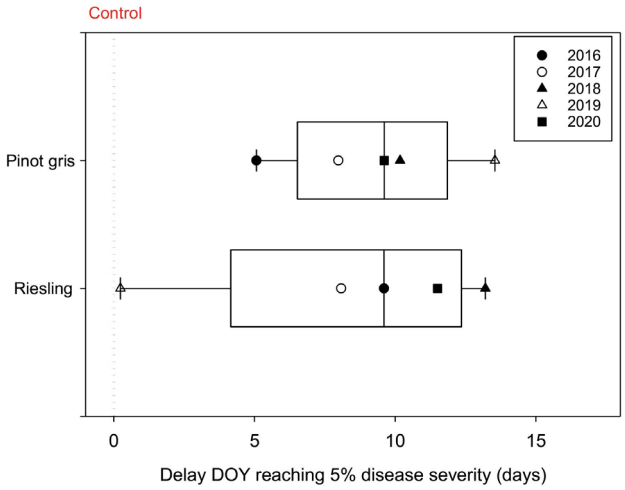


FIGURE 2. Box plots of the delay of the DOY reaching 5 % disease severity in the treatment "partial double-pruning after bloom" (PDP) compared to the control in 2016 to 2020 in the Pinot gris and Riesling cultivars.

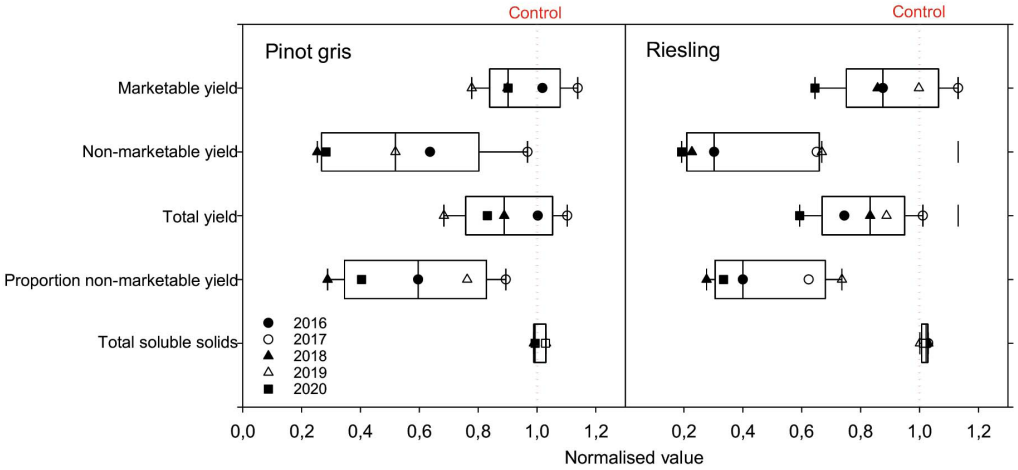


FIGURE 3. Box plots of the normalised values (value of the parameter in the treatment "partial double-pruning after bloom" in a specific year in one cultivar/average value of the control in this year in this cultivar) of the marketable yield, the non-marketable yield, the total yield, the proportion of the non-marketable yield on the total yield and the total soluble solids at harvest from 2016 to 2020 in the cultivars Pinot gris and Riesling.

to separate, we assume that it is rather the improved cluster structure than the microclimate that determines the effectivity of PDP. In this study, we have only gathered detailed data on cluster structure during the 2020 growing season. These data show that berry number per cluster and cluster weight were reduced under PDP irrespective of cultivar, but that there were cultivar x treatment interactions for berry size, which was not reduced in P. gris, but significantly reduced in Riesling. The latter finding was consistent with lower cluster density index values observed under PDP only in Riesling. This indicates that Pinot gris can compensate for perturbations in source/sink relations with an increase in berry size for a longer time than Riesling, in line with other source/sink manipulation experiments from the same vineyard (Molitor *et al*., 2012a).

The extent of yield reduction under PDP seemed to be correlated with its efficiency against botrytis infections, giving an indication that, indeed, the modifications of cluster structure (i.e., berry number and size) determine the efficiency of PDP, and not a more general devigorating effect caused by the increase in vine capacity via the pruning level. If a high number of organs compete for reserves, as in the case of PDP relative to the control, then the shoot length and leaf area development decrease as the amount of reserve carbohydrates allocated to an individual shoot decrease (Kliewer and Dokoozlian, 2005). This limits the C supply to the flowers, thus decreasing cell division rates, flower size, fruit set (Keller *et al*., 2010) and seed number, leading to reduced berry size and numbers (Keller, 2015). Cell division and expansion are still dependent on source/sink relationships after berry set, and thus right up the removal of the second cane in PDP. Such source limitations have been shown to successfully reduce berry size (Hed and Centinari, 2018; Kotseridis *et al*., 2012) and may play a role in defining cluster morphology under PDP.

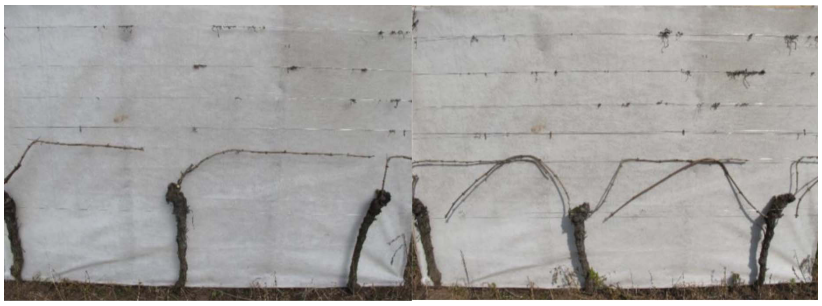


FIGURE 4. Status of the treatment 'control' (left) and 'Partial double-pruning after bloom' (right) after winter pruning.

2. Practical considerations

Previous studies investigated the potential to delay the bunch rot epidemic using direct or indirect measures. While botryticide application (fenhexamid) was reported to delay the epidemic by 0.1 to 3.8 days (Molitor *et al*., 2018), the application of bioregulators (prohexadione-Ca; gibberellic acid) by 2.5 (Molitor *et al*., 2011b) or 0.3 days (Evers *et al*., 2010), flower debris removal by 3.7 days (Molitor *et al*., 2015b), cluster-zone leaf removal by 7.3 days (Molitor *et al*., 2011b), late first shoot topping by 4.3 days (Molitor *et al*., 2015a) and cluster division by up to 28.2 days (Molitor *et al*., 2012a), the observed delay of the time taken to reach 5 % disease severity between 8.3 and 10.3 days due to PDP is remarkable. Consequently, PDP might be incorporated as a strong brick in a complex bunch rot control strategy, including different indirect or direct control measures. Consequently, the decision of which and how many measures are integrated into the complex strategy needs to be decided upon depending on the local and annual disease pressure, the specific degree of bunch rot susceptibility of a cultivar and the oenological target (Molitor *et al*., 2017).

As part of the complex bunch rot control strategy, PDP could provide an additional example for the successful integration of crop cultural measures into Integrated Pest Management.

Via its non-chemical reduction of grape sensitivity to bunch rot, it contributes to the minimisation of pesticide use and its potentially negative effects on the environment.

The time demand for the removal of the second fruiting cane (including the new shoot growth) in PDP was approximately 10 hours per ha (data not shown). Compared to the time demand of other cultural practices, such as manual cluster-zone leaf removal or cluster division (75–100 h/ha (Schultz *et al*., 2003)) or the costs for the application of botryticides or bioregulators, PDP represents a relatively cost-effective operation with high efficiency

In practice, the workload for removal of the second fruiting cane might be lower in cultivars with a lower adhesion power of the tendrils because here, young shoots are fixed to the trellis less firmly.

Furthermore, we observed that fixing the second cane (to be removed later) as a pendulum cane (German: Pendelbogen; Figure 4) rather than wrapping it around the wire might facilitate the removal operations after flowering.

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REFERENCES

Basile, B., Caccavello, G., Giaccone, M., & Forlani, M. (2015). Effects of early shading and defoliation on bunch compactness, yield components, and berry composition of Aglianico grapevines under warm climate conditions. *America Journal of Enology and Viticulture,* 66**,** 234-243. https://doi.org/10.5344/ajev.2014.14066

Bennett, J. S., Jarvis, P., & Trought, M. C. (2002). The importance of over-wintering carbohydrates on inflorescence development and yield potential. Australian and New Zealand Grapegrower and Winemaker, 456, 70-72.

Beresford, R. M., Evans, K. J., Wood, P. N., & Mundy, D. C. (2006). Disease assessment and epidemic monitoring methodology for bunch rot (*Botrytis cinerea)* in grapevines. *New Zealand Plant Protection,* 59**,** 355-360. https://doi.org/10.30843/nzpp.2006.59.4594

Bundessortenamt (2015). Beschreibende Sortenliste Reben 2015. Hannover.

English, J. T., Thomas, C. S., Marois, J. J., & Gubler, W. D. (1989). Microclimates of grapevine canopies associated with leaf removal and control of Botrytis bunch rot. *Phytopathology,* 79**,** 395-401. https://doi.org/10.1094/Phyto-79-395

Evers, D., Molitor, D., Rothmeier, M., Behr, M., Fischer, S., & Hoffmann, L. (2010). Efficiency of different strategies for the control of grey mold on grapes including gibberellic acid (Gibb3), leaf removal and/or botryticide treatments. *Journal International des Sciences de la Vigne et du Vin,* 44**,** 151-159. https://doi.org/10.20870/oeno-one.2010.44.3.1469

Hed, B., & Centinari, M. (2018). Hand and mechanical fruit-zone leaf removal at prebloom and fruit-set was more effective in reducing crop yield than reducing bunch rot in 'Riesling' grapevines. *HortTechnology,* 28**,** 296-303. https://doi.org/10.21273/HORTTECH03965-18

Hed, B., Ngugi, H. K., & Travis, J. W. (2009). Relationship between cluster compactness and bunch rot in Vignoles grapes. *Plant Disease,* 93**,** 1195-1201. https://doi.org/10.1094/PDIS-93-11-1195

Herrera, J. C., Sabbatini, P., & Peterlunger, E. (2016). Impact of leaf removal after berry set on fruit composition and bunch rot in 'Sauvignon blanc'. *Vitis,* 55**,** 64.

Intrigliolo, D. S., Llacer, E., Revert, J., Esteve, M. D., Climent, M. D., Palau, D., & Gomez, I. (2014). Early defoliation reduces cluster compactness and improves grape composition in Mandó, an autochthonous cultivar of *Vitis vinifera* from southeastern Spain. *Scientia Horticulturae,* 167**,** 71-75. https://doi.org/10.1016/j.scienta.2013.12.036

Ipach, R., Huber, B., Hoffmann, H., & Baus, O. (2005). *Richtlinie zur Prüfung von Wachstumsregulatoren zur Auflockerung der Traubenstruktur und zur Vermeidung von Fäulnis an Trauben. Outline for an EPPO-guideline*.

Kassemeyer, H.-H., & Berkelmann-Löhnertz, B. (2009). Fungi of grapes. *In:* König, H., Unden, G. & Fröhlich, J. (eds.) *Biology of microorganisms on grapes, in must and in wine.* Berlin, Heidelberg: Springer-Verlag.

Keller, M. (2015). *The science of grapevines. Anatomy and physiology. 2nd edition,* London, Elsevier Academic Press.

Keller, M., Tarara, J. M., & Mills, L. J. (2010). Spring temperatures alter reproductive development in grapevines. *Australian Journal of Grape and Wine Research,* 16**,** 445-454. https://doi.org/10.1111/j.1755-0238.2010.00105.x

Kliewer, W. M., & Dokoozlian, N. K. (2005). Leaf area/crop weight ratios of grapevines: Influence on fruit composition and wine quality. *American Journal of Enology and Viticulture,* 56**,** 170-181.

Kotseridis, Y., Georgiadou, A., Tikos, P., Kallithraka, S., & Koundouras, S. (2012). Effects of severity of post-flowering leaf removal on berry growth and composition of three red *Vitis vinifera* L. cultivars grown under semiarid conditions. *Journal of Agricultural and Food Chemistry,* 60**,** https://doi.org/10.1021/jf300605j

Lorenz, D. H., Eichhorn, K. W., Bleiholder, H., Klose, R., Meier, U., & Weber, E. (1995). Phenological growth stages of the grapevine, *Vitis vinifera* L. ssp. *vinifera*. Codes and descriptions according to the extended BBCH scale. *Australian Journal of Grape and Wine Research,* 1**,** 100-103. https://doi.org/10.1111/j.1755-0238.1995.tb00085.x

May, P. (2004). Flowering and Fruitset in Grapevines, Lythrum Press, Adelaide.

Molitor, D., Baron, N., Sauerwein, T., André, C. M., Kicherer, A., Döring, J., Stoll, M., Beyer, M., Hoffmann, L., & Evers, D. (2015a). Postponing first shoot topping reduces grape cluster compactness and delays bunch rot epidemic. *American Journal of Enology and Viticulture,* 66**,** 164-176. https://doi.org/10.5344/ajev.2014.14052

Molitor, D., Baus, O., Didry, Y., Junk, J., Hoffmann, L., & Beyer, M. (2020a). BotRisk: simulating the annual bunch rot risk on grapevines (*Vitis vinifer*a L. cv. Riesling) based on meteorological data. *International Journal of Biometeorology,* 64, 1571-1582. https://doi.org/10.1007/s00484-020-01938-5

Molitor, D., Behr, M., Fischer, S., Hoffmann, L., & Evers, D. (2011a). Timing of cluster-zone leaf removal and its impact on canopy morphology, cluster structure and bunch rot susceptibility of grapes. *Journal International des Sciences de la Vigne et du Vin,* 45**,** 149-159. https://doi.org/10.20870/oeno-one.2011.45.3.1495

Molitor, D., Behr, M., Hoffmann, L., & Evers, D. (2012a). Impact of grape cluster division on cluster morphology and bunch rot epidemic. *American Journal of Enology and Viticulture,* 63**,** 508-514. https://doi.org/10.5344/ajev.2012.12041

Molitor, D., Behr, M., Hoffmann, L., & Evers, D. (2012b). Research note: Benefits and drawbacks of pre-bloom applications of gibberellic acid (GA3) for stem elongation in Sauvignon blanc. *South African Journal of Enology and Viticulture,* 33**,** 198-202. https://doi.org/10.21548/33-2-1119

Molitor, D., Hoffmann, L., & Beyer, M. (2015b). Flower debris removal delays grape bunch rot epidemic. *American Journal of Enology and Viticulture,* **66,** 548-553. https://doi.org/10.5344/ajev.2015.15019

Molitor, D., Hoffmann, L., & Beyer, M. (2017). Overall efficacies of combined measures for controlling grape bunch rot can be estimated by multiplicative consideration of individual effects. *Oeno One,* 51**,** 387-393. https://doi.org/10.20870/oeno-one.2017.51.4.1894

Molitor, D., Rothmeier, M., Behr, M., Fischer, S., Hoffmann, L., & Evers, D. (2011b). Crop cultural and chemical methods to control grey mould on grapes. *Vitis,* 50**,** 81-87.

Molitor, D., Schultz, M., Friedrich, B., Viret, O., Hoffmann, L., & Beyer, M. (2018). Efficacy of fenhexamid treatments against *Botrytis cinera* in grapevine as affected by time of application and meteorological conditions. *Crop Protection,* 110**,** 1-13. https://doi.org/10.1016/j.cropro.2018.03.007

Poni, S., Casalini, L., Bernizzoni, F., Civardi, S., & Intrieri, C. (2006). Effects of early defoliation on shoot photosynthesis, yield components, and grape composition. *American Journal of Enology and Viticulture,* 57**,** 397-407.

Ribéreau-Gayon, P. (1983). Alterations of wine quality caused by Botrytis damages. *Vignevini,* 10**,** 48-52.

Schultz, H. R., Kohler, D., & Fox, R. (2003). Eine Erfolg versprechende Ausdünnungsvariante: Trauben teilen. *Das Deutsche Weinmagazin, 22-25.*

Smart, R., & Robinson, M. (1991). *Sunlight into wine. A handbook for winegrape canopy management,* Adelaide SA, Australia, Winetitles.

Spring, J. L. (2004). Influence de la date de vendange sur la qualité des vins de Garanoir. *Revue Suisse de Viticulture Arboriculture Horticulture,* 36**,** 361-365.

Tello, J., & Ibanez, J. (2014). Evaluation of indexes for the quantitative and objective estimation of grapevine bunch compactness. *Vitis,* 53**,** 9-16.

Tello, J. & Ibanez, J. (2017). What do we know about grapevine bunch compactness? A state-of-the-art review. *Australian Journal of Grape and Wine Research,* 24, 6-23. https://doi.org/10.1111/ajgw.12310

Vander Weide, J., Gottschalk, C., Schultze, S. R., Nasrollahiazar, E., Poni, S., & Sabbatini, P. (2021). Impacts of pre-bloom leaf removal on wine grape production and quality parameters: a systematic review and meta-analysis. *Frontiers in Plant Science,* 11**,** 621585. DOI: https://doi.org/10.3389/fpls.2020.621585

Wilcox, W. F., Gubler, W. D., & Uyamoto, J. K. (2015). *Compendium of grape diseases, disorders, and pests. Second edition,* St. Paul Minnesota, APS Press.

Wolf, T. K., Baudoin, A. B. A. M., & Martinez-Ochoa, N. (1997). Effect of floral debris removal from fruit clusters on botrytis bunch rot of Chardonnay grapes. *Vitis,* 36**,** 27-33.

Zoecklein, B. W., Wolf, T. K., Duncan, N. W., Judge, J. M., & Cook, M. K. (1992). Effects of fruit zone leaf removal on yield, fruit composition, and fruit rot incidence of Chardonnay and White Riesling (*Vitis vinifera* L) grapes. *American Journal of Enology and Viticulture,* 43**,** 139-148.