TIMING OF CLUSTER-ZONE LEAF REMOVAL AND ITS IMPACT ON CANOPY MORPHOLOGY, CLUSTER STRUCTURE AND BUNCH ROT SUSCEPTIBILITY OF GRAPES

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Abstract

Aims: Leaf removal in the cluster-zone has proved to be an efficient option to reduce grey mould infestation. The aim of the present work was to determine the optimal timing of this measure in practical viticulture.

Methods and results: Manual leaf removal in the cluster-zone was carried out at six time points between pre-bloom (BBCH 57) and veraison (BBCH 81) on the white varieties Sauvignon blanc (2009), Auxerrois (2009), Pinot gris (2010) and Riesling (2010). Leaf removal conducted between bloom and berries-touching led to a decreased number of interior clusters as well as a reduced cluster compactness and disease severity of *B. cinerea*. Pre-bloom and late (at veraison) leaf removal turned out to be less efficient.

Conclusion: Leaf removal between bloom and berries-touching proved to be very efficient to control bunch rot due to (i) the disaggregation of the cluster structure and (ii) the improved microclimatic conditions and can be recommended in integrated as well as organic viticulture.

Significance and impact of the study: The outcome of the present study constitutes an important tool for improving grape health status and, in consequence, wine quality under cool climate winegrowing conditions.

Key words: *Botrytis cinerea*, canopy morphology, cluster structure, leaf removal, *Vitis vinifera*

Résumé

Objectifs : L'effeuillage de la zone des grappes est une méthode pour réduire les attaques de pourriture grise. L'objectif du présent travail était de déterminer le stade phénologique optimal pour réaliser cette opération.

Méthodes et résultats : L'effeuillage de la zone des grappes a été réalisé manuellement à six moments entre les stades boutons floraux séparés (BBCH 57) et véraison (BBCH 81) sur Sauvignon blanc (2009), Auxerrois (2009), Pinot gris (2010) et Riesling (2010). Un effeuillage réalisé entre la floraison et la fermeture de la grappe conduit à un nombre moindre de grappes intérieures ainsi qu'à une compacité réduite de la grappe et des attaques plus faibles de *B. cinerea*. Les effeuillages aux stades boutons floraux séparés et véraison se révèlent moins efficaces.

Conclusion: Un effeuillage réalisé entre la floraison et la fermeture de la grappe est très efficace pour lutter contre la pourriture grise en raison de (i) la décompaction de la structure de la grappe et (ii) l'amélioration des conditions microclimatiques, et peut de ce fait être recommandé en viticulture intégrée et en viticulture biologique.

Signification et impact de cette étude : Les conclusions de la présente étude représentent un outil important pour améliorer la qualité sanitaire de la grappe et donc la qualité du vin dans les vignobles en zone tempérée.

Mots-clés : *Botrytis cinerea*, morphologie de la canopée, structure de la grappe, effeuillage, *Vitis vinifera*

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INTRODUCTION

Grey mould or bunch rot caused by *Botrytis cinerea* can reduce grape yield as well as quality (Ribéreau-Gayon, 1983) and causes economic damages worldwide. Indeed, besides the direct loss of yield, attack of *B. cinerea* can cause off-flavours, unstable colour, oxidative damages, premature aging and difficulties in clarification (Ribéreau-Gayon, 1983; Smart and Robinson, 1991). Other fungi and bacteria can readily invade grey mould-infected clusters, which further reinforces the occurrence of off-flavours (Smart and Robinson, 1991). Especially if both fungal pathogens *B. cinerea* and *Penicillium expansum* occur simultaneously, earthy-mouldy off-flavours can be the result (La Guerche *et al*., 2005).

In general, the development of fungal pathogens is favoured in poorly exposed and dense clusters. As described by Zoecklein *et al*. (1992) and English *et al*. (1989), clusters in the interior parts of the canopy grow under reduced light quality and quantity, low wind speed and, in consequence, higher humidity conditions than well exposed clusters. These prolonged cluster wetness durations favour successful conidia germination and infections by *B. cinerea* (Smart and Robinson, 1991).

Besides this, the development of *B. cinerea* and other fungal pathogens often starts in the interior parts of dense clusters. Thus, treatments that induce a disaggregation of the cluster structure lead to an increased airflow and a better sun exposure of the interior parts. This results in a reduced interior humidity and, in consequence, in less favourable conditions for the establishment of fungal pathogens (Zoecklein *et al*., 1992). Hence, the tightest clusters generally show the most severe bunch rot infestation (Evers *et al*., 2010; Vail and Marois, 1991). Moreover, a loose structure diminishes the appearance of berry burst, which is due to high pressure inside the cluster induced by berries touching each other (Smart and Robinson, 1991). Burst berries are easy to colonize by fungal pathogens and represent a starting point for massive fungal attack.

In the past, the practical strategies to control *B. cinerea* were mainly focused on the use of organic-synthetic botryticides. Meanwhile, in Integrated Pest Management, crop cultural methods have gained increasing attention. In organic viticulture, these crop cultural practices represent the major option for pathogen control as organic-synthetic fungicides are not allowed. Leaf removal in the cluster-zone, in particular, has shown its potential to reduce bunch rot in several trials under different climatic conditions (Intrieri *et al*., 2008; Ollat and Gaudillère, 1998; Percival *et al*., 1994; Poni and Intrieri, 2001; Tardaguila *et al*., 2008 and 2010; Zoecklein *et al*., 1992). Furthermore, positive effects on wine quality due to a higher assimilation and increased skin-to-pulp ratios were observed (Poni *et al*., 2008). However, for an optimal timing of leaf removal in practical viticulture, further knowledge about the timing of this measure is necessary. Hence, the aim of the present study was to investigate the impact of the timing of leaf removal on (i) canopy morphology, (ii) cluster structure and (iii) bunch rot susceptibility.

MATERIALS AND METHODS

1. Vineyard site and experimental design

The study was carried out in the experimental vineyards of the Institut Viti-Vinicole in Remich, Luxembourg (49.54 N, 6.35 E) on the white *Vitis vinifera* L. varieties Sauvignon blanc (S. blanc) (2009), Auxerrois (2009), Pinot gris (2010) and Riesling (2010). Vines were planted in 2000 (S. blanc), 1984 (Auxerrois) and 1994 (Pinot gris and Riesling). All vineyards were

Table 1 - Dates of leaf removal as a function of the BBCH stage.

		Date of leaf removal			
		S. blanc	Auxerrois	Pinot gris	Riesling
untreated control					
57	leaf removal at BBCH 57 (inflorescences fully developed)	04.06.09	04.06.09	16.06.10	16.06.10
63	leaf removal at BBCH 63 (30% of flowerhoods fallen)	18.06.09	16.06.09	25.06.10	25.06.10
68	leaf removal at BBCH 68 (80% of flowerhoods fallen)	22.06.09	18.06.09	28.06.10	28.06.10
73	leaf removal at BBCH 73 (berries groat-sized)	01.07.09	01.07.09	02.07.10	02.07.10
77	leaf removal at BBCH 77 (berries beginning to touch)	16.07.09	16.07.09	16.07.10	16.07.10
81	leaf removal at BBCH 81 (beginning of ripening)	11.08.09	11.08.09	17.08.10	24.08.10

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vertical shoot positioning trained and the rootstock was SO4 in all varieties.

The field experiments were conducted using a randomized block design with four replicates of eight vines per variant. Variants were the same in all four experimental fields and defined as follows: control (no leaf removal), 57 (leaf removal at BBCH 57 (Lorenz *et al*., 1995)), 63 (leaf removal at BBCH 63), 68 (leaf removal at BBCH 68), 73 (leaf removal at BBCH 73), 77 (leaf removal at BBCH 77) and 81 (leaf removal at BBCH 81). Precise dates are summarized in Table 1.

Leaf removal consisted in manually removing primary (approximately 2 per shoot) and lateral leaves in the cluster-zone on the north or east sides of each row as well as the interior leaves.

Other canopy management measures were conducted in the same manner on all variants. To protect the vineyards against powdery and downy mildew, background applications against *Plasmopara viticola* and *Erysiphe necator* were carried out during the whole season. No background coverage against *B. cinerea* took place.

2. Determination of canopy morphology in the cluster-zone (point quadrat analysis)

The impact of the different leaf removal timings on the canopy structure in the cluster-zone was assessed using point quadrat analysis according to Smart and Robinson (1991) in every plot of S. blanc (2009), Pinot gris (2010) and Riesling (2010) . To this end, a rod was inserted into the cluster-zone every 10 cm from the south or west side of the row on the height of the upper fruiting wire. All contacts of the rod were recorded distinguishing between leaves (L), clusters (C) and gaps (G) on 48 insertion points per plot. The percentage of interior clusters (PIC) was calculated according to Meyers and Vanden Heuvel (2008). Assessments were conducted three times between flowering and veraison (S. blanc: BBCH 73 (03.07.2009), BBCH 79 (21.07.2009), BBCH 81 (12.08.2009); Pinot gris: BBCH 73 (06.07.2010), BBCH 79 (29.07.2010), BBCH 81 (17.08.2010); Riesling: BBCH 73 (06.07.2010), BBCH 79 (29.07.2010), BBCH 81 (24.08.2010)).

3. Determination of cluster structure

To study the influence of the timing of leaf removal on cluster structure, the « density index » was determined according to the protocol of Ipach *et al.* (2005) as described before (Evers *et al*., 2010; Molitor *et al*., 2011). The density index classifies the cluster structure between 1 (very flexible) and 5 (very compact). In each plot, 100 clusters (50 on each side of the row) were evaluated at the phenological growth stage BBCH 79 (S. blanc and Auxerrois: 11.08.2009; Pinot gris and Riesling: 11.08.2010). Values are the means of the observations on 100 clusters per plot, averaged for the four replicates.

In addition, ten clusters were collected in each plot of S. blanc after veraison (01.09.2009). The clusters were sampled at the second position of the ultimate or penultimate inserted shoot on the cane. The length, the weight (with and without stem) and the number of berries were recorded for each cluster. The average berry weight was calculated by dividing the cluster weight without stem by the number of berries. As an additional indicator of cluster density, the weight per cm stem length was computed by dividing the cluster weight by the cluster length.

4. Determination of *B. cinerea* infestation level

Disease incidence and severity were assessed close to harvest by examining 100 randomly selected clusters per plot (50 on each side of the row) according to the EPPO guideline PP1/17.

5. Determination of maturity parameters and yield

In the trials of 2009 (S. blanc and Auxerrois), close to harvest, 25 berries were collected on both sides of the row in each plot and thereafter pooled for every variant to achieve a sufficient sampling volume. In 2010 (Pinot gris and Riesling), 80 berries (40 on each side of the row) were sampled per plot and analyzed plot-wise.

After pressing, the juice was centrifuged. The sugar level and the total acidity were measured by FT-IR.

Each plot was harvested separately. The total yield per plant was calculated by dividing the yield recorded per plot by the number of plants.

6. Statistical analysis

Data were analyzed by one-way ANOVA. For multiple comparison procedures between means, Tukey tests ($\alpha = 5\%$) were performed using SPSS 16.0 for Windows.

RESULTS

1. Cluster exposure

At the first assessment date (BBCH 73), the percentage of interior clusters (PIC) was significantly reduced in comparison to the control in all variants in which leaf removal took place between BBCH 57 and 73 (Table 2).

At the assessment date BBCH 79, the PIC was significantly lower in comparison to the control in any variant in which leaf removal took place between BBCH 63 and 77. In Pinot gris and Riesling, leaf removal at BBCH 57 also led to a significantly lower PIC compared

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Table 2 - Percentage of interior clusters (PIC) according to point quadrat analysis at three developmental stages in S. blanc (2009), Pinot gris (2010) and Riesling (2010) as a function of the leaf removal BBCH stage. Variants not marked with the same letter differ significantly according to Tukey test (α = 5%).

	S. blanc			Pinot gris			Riesling		
	BBCH 73	BBCH 79	BBCH 81	BBCH 73	BBCH 79	BBCH 81	BBCH 73	BBCH 79	BBCH 81
Control	73.4 a	72.5 a	74.8 a	61.9 a	65.8 a	67.2 a	67.0 a	74.7 a	71.2 a
57	40.6 b	46.8 ab	67.9 a	18.2 b	29.1 b	31.4 b	22.0 b	29.0 b	27.2 b
63	11.3 bc	15.5 c	29.2 b	12.7 b	16.6 b	12.6 c	4.5 b	27.7 b	20.2 bc
68	11.3 bc	18.5 bc	23.5 b	5.2 b	12.5 b	14.0 bc	8.6 b	25.2 b	17.9 bc
73	2.5 c	3.6 c	13.3 bc	3.9 b	13.0 b	5.7 c	4.3 b	18.8 b	5.5 bc
77	78.3 a	6.5 c	15.1 bc	60.2 a	8.0 b	7.2 c	61.2 a	9.6 b	3.4 c
81	72.3 a	73.1 a	0.0 c	54.8 a	65.3 a	9.3 c	67.0 a	72.9 a	5.4 bc

Table 3 - Average density index assessed at the developmental stage BBCH 79 (11.08.2009 or 11.08.2010) as a function of the leaf removal BBCH stage. Variants not marked with the same letter differ significantly according to Tukey test (α = 5%).

	S. blanc		Auxerrois		Pinot gris		Riesling	
Control	3.55	a	3.56	a	3.95	a	3.33	a
57	3.42	ab	3.23	ab	3.60	b	3.20	a
63	2.99	c	3.24	ab	3.62	b	3.08	a
68	3.21	bc	3.15	b	3.65	ab	3.10	a
73	3.12	bc	3.18	b	3.65	ab	2.88	a
77	3.19	bc	3.18	b	3.80	ab	3.16	a
81	3.43	ab	3.41	ab	3.93	a	3.36	a

to the control, while in S. blanc the reduction was not significant.

At the final assessment date (BBCH 81), the PIC was significantly decreased compared to the control by any leaf removal that took place between BBCH 63 and 81 (in all three varieties) and at BBCH 57 (in Pinot gris and Riesling) (Table 2).

2. Cluster structure

Leaf removal led to a significant reduction of the density index in comparison to the control when conducted between BBCH 63 and 77 (Sauvignon blanc), between BBCH 68 and 77 (Auxerrois) or at BBCH 57 and 63 (Pinot gris). Riesling had the lowest control value of all varieties and no significant reduction of the density index was observed among the variants. With the exception of the late leaf removal at BBCH 81, all other treatments led in all varieties to a reduction of the density index compared to the untreated control, in tendency (Figure 1, Table 3).

In Sauvignon blanc, a significant reduction (in comparison to the control) of the cluster weight as well as of the number of berries per cluster was observed when leaf removal took place at BBCH 63 or 68. However, no significant impact of any leaf removal on the average berry weight was detected. Leaf removal conducted at BBCH 63, 68 or 73 led to a significant reduction of the cluster weight per cm stem length. Overall, the lowest values for all parameters describing the cluster morphology (density index, cluster weight, berries per cluster, berry weight, and cluster weight per cm stem length) were determined when leaf removal took place at BBCH 63 (Tables 3 and 4). The average cluster weight per cm stem length assessed on 01.09.2009 strongly correlated with the density index ($R^2 = 0.9121$) determined on 11.08.2009.

3. Disease severity *B. cinerea*

The infestation level in the untreated control was comparable in all varieties (disease severity in the untreated control ranged from 18.6 (Auxerrois) to 23.7% (Riesling)) (Table 5). When leaf removal was conducted between BBCH 63 and 77, the disease severity was significantly lower in S. blanc, Auxerrois and Riesling in comparison to the untreated control, whereas in Pinot gris no significant differences were found at all. In addition, leaf removal at BBCH 81 significantly reduced the disease severity in Auxerrois and Riesling. Early leaf removal at BBCH

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Table 4 - Average cluster weight, berries per cluster, berry weight and weight per cm stem length assessed on 01.09.2009 as a function of the leaf removal BBCH stage. Variety: S. blanc. Variants not marked with the same letter differ significantly according to Tukey test (α = 5%).

	cluster weight (g)		berries / cluster		berry weight (g)		weight / stem length (g/cm)	
Control	119.6	a	86.4	a	1.33	a	9.5	a
57	111.5	ab	79.5	ab	1.35	a	8.6	ab
63	85.5	c	67.3	b	1.19	a	6.8	b
68	93.5	bc	68.5	b	1.28	a	7.3	b
73	95.0	abc	74.9	ab	1.20	a	7.6	b
77	101.6	abc	78.7	ab	1.22	a	7.9	ab
81	107.2	abc	75.3	ab	1.33	a	8.5	ab

Table 5 - Disease severity of *B. cinerea* and efficiency of the variants compared to the untreated control (S. blanc: 28.09.2009, Auxerrois: 28.09.2009, Pinot gris: 30.09.2010, Riesling: 06.10.2010) as a function of the leaf removal BBCH stage. Variants not marked with the same letter differ significantly according to Tukey test (α = 5%).

	S. blanc		Auxerrois		Pinot gris		Riesling	
	severity (%)	efficiency (%)	severity (%)	efficiency (%)	severity (%)	efficiency (%)	severity (%)	efficiency (%)
Control	22.9 a		18.6 a		21.8 a		23.7 a	
57	18.2 abc	20.6	13.4 ab	28.2	14.0 a	35.8	22.5 a	5.1
63	9.8 bc	57.3	9.4 bcd	49.5	13.0 a	40.4	13.8 b	41.6
68	11.7 bc	49.1	6.6 cd	64.7	13.7 a	37.2	12.3 b	48.0
73	10.1 bc	55.7	4.8 d	74.5	13.2 a	39.7	12.3 b	48.0
77	8.3 c	63.7	5.9 cd	68.6	13.0 a	40.6	12.6 b	47.0
81	19.2 ab	16.2	11.3 bc	39.3	18.2 a	16.7	13.8 b	41.6

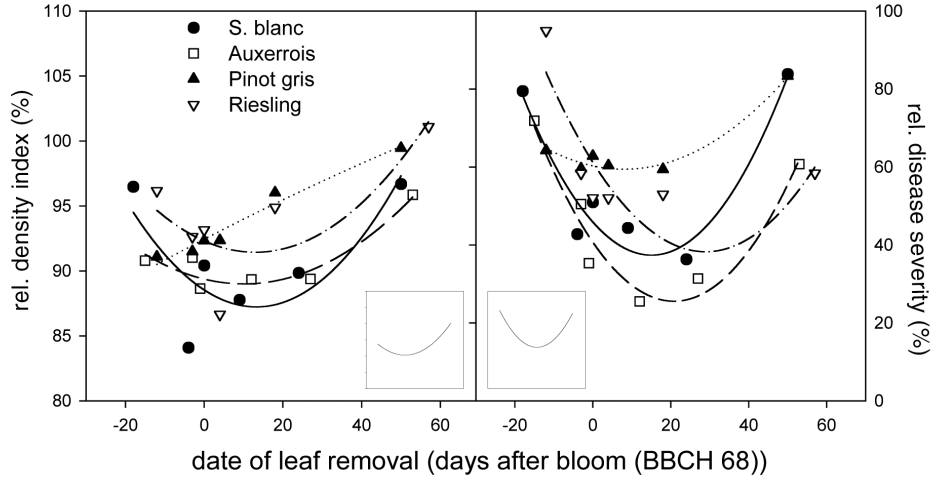


Figure 1 - Relative density index (left) and disease severity (right) compared to the untreated control (set at 100 %) as functions of the date of leaf removal (days after bloom (BBCH 68)). Curves are fitted to the average values of each variant for the four varieties according to the equation $y = y_0 + ax + bx^2$. The inserted graphs represent the process of the different parameters over all varieties.

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57 did not lead to a significant reduction of disease severity in any variety. The average efficiency of the variants compared to the control ranged from 22.4 (leaf removal at BBCH 57) to 55.0% (leaf removal at BBCH 77) (Table 5).

The disease severity at harvest was correlated with the percentage of interior clusters (PIC). The strongest correlation to disease severity at harvest was found in S. blanc and Pinot gris at BBCH 79 ($R^2 = 0.93$ and 0.88, respectively) and in Riesling at BBCH 81 ($R^2 = 0.70$) (Figure 2).

4. Harvest parameters

No statisticall differences in sugar level were observed in any variety between the control and the different leaf removal treatments, whereas in Pinot gris and Riesling the late leaf removal at BBCH 81 led to a significantly lower sugar level than the treatments 57, 63, 68, 73 (Pinot gris) and 57, 68 and 73 (Riesling) (Table 6). In three of the four varieties, the lowest sugar level was measured in the variant BBCH 81 and in all varieties the highest level was measured either in the variant BBCH 68 or 73.

As for the total acidity, the differences between the variants were low in all varieties.

No statistically significant differences in the total yield per plant were found in any variety. On average, the highest yield was measured in the control and the lowest yield was measured in the variant BBCH 68 (Table 7).

DISCUSSION

1. Effects on cluster exposure

The percentage of interior clusters (PIC) is a parameter to describe the cluster exposure in the canopy. Generally, the lower the PIC value is, the better the clusters are exposed to both sun and wind. Increased airflow and better sun exposure reduce the length of wetness periods and, in consequence, lead to less favourable conditions for the germination and infection process of fungal pathogens (Zoecklein *et al*. 1992). Smart and Robinson (1991) defined that PIC values in an optimal canopy should be lower than 40%. In the present trials, this threshold was maintained at both early assessment dates by any variants of Pinot gris and Riesling with a leaf removal between BBCH 57 and 73. However, the late leaf removal (BBCH 81) clearly failed to meet this threshold at both early assessment dates. This means that even though clusters defoliated at veraison were well exposed afterwards, they spent most of their development under unfavourable microclimatic conditions.

In all varieties, a slight re-closure of the canopy was observed in all variants defoliated at BBCH 57. This effect

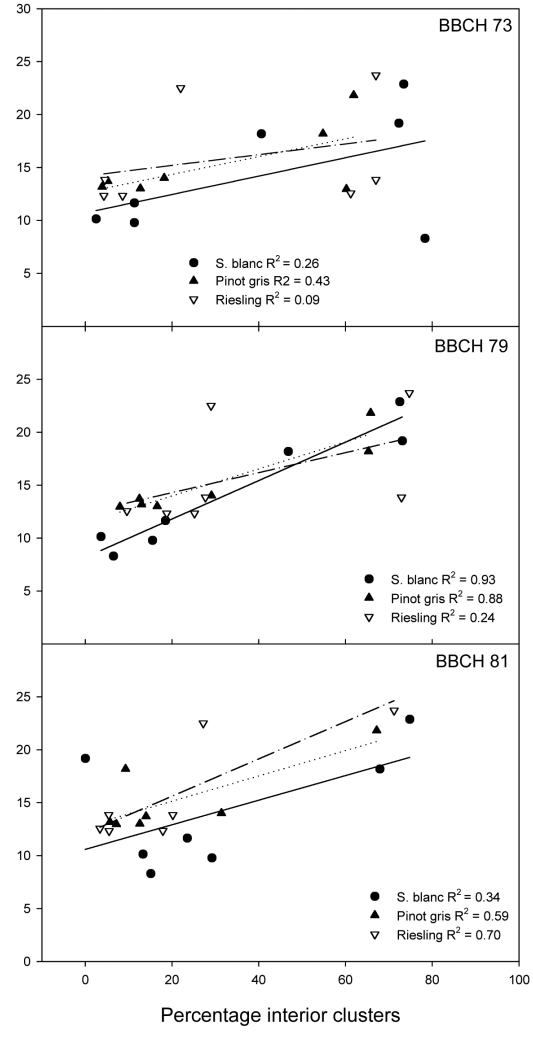


Figure 2 - Correlation between the disease severity of *Botrytis cinerea* at the end of the season and the percentage of interior clusters at the developmental stages BBCH 73, 79 and 81 in the varieties S. blanc (2009), Pinot gris (2010) and Riesling (2010). The spots represent the observed disease severities, the lines the calculated correlations between the percentage interior clusters and the disease severity at harvest according to the equation $y = y_0 + ax$.

was most clear in S. blanc, which is known for its extensive lateral shoot growth. Thus, early leaf removal (pre-bloom) improved the wind and light exposure in a less distinctive way than the leaf removal treatments after flowering. Even though microclimatic conditions were unfavourable in the first weeks of berry development, after leaf removal the PIC values in the variant BBCH 77 were in the optimum range defined by Smart and Robinson (1991). Maigre (2004), Zoecklein *et al*. (1992) as well as Tardaguila *et al*. (2010) confirmed the positive effects of leaf removal on the sunlight penetration and on the microclimate in the cluster-zone when it takes place in this period.

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Table 6 - Maturity parameters (sugar level (°Oechsle) and total acidity (g/l)) at harvest as a function of the leaf removal BBCH stage. Variants of Pinot gris and Riesling not marked with the same letter differ significantly according to Tukey test (α = 5%).

	S. blanc		Auxerrois		Pinot gris		Riesling	
	°Oe	g/l	°Oe	g/l	°Oe	g/l	°Oe	g/l
Control	90.7	8.0	83.0	5.1	71.8 abc	9.2 ab	76.6 ab	13.5 a
57	91.3	8.7	83.0	5.6	72.5 bc	9.2 ab	78.1 b	13.3 ab
63	93.0	8.3	83.7	5.3	74.3 c	8.9 b	77.2 ab	12.8 b
68	94.3	8.1	83.3	5.8	74.9 c	8.6 b	77.8 b	13.3 ab
73	93.7	7.8	84.3	5.5	73.1 bc	9.0 ab	78.3 b	13.2 ab
77	92.0	7.8	83.0	5.2	69.6 ab	9.1 ab	76.5 ab	13.1 ab
81	91.0	7.7	81.3	5.7	68.3 a	9.6 a	74.9 a	13.0 ab

2. Loosening of the cluster structure

In the present study, all leaf removal conducted before veraison led in all varieties to a reduction of the density index, though not always statistically significant. In S. blanc and Auxerrois, leaf removal between BBCH 63 and 77 or BCCH 68 and 77, respectively, led to a significant reduction of the density index, whereas in Pinot gris this was the case for the earlier treatments at BBCH 57 and 63. Riesling had the lowest control density index of all varieties. Here, a reduction of the density index was observed especially in the variants with leaf removal between BBCH 63 and 73, even though the differences were not significant compared to the control.

Defoliation of the cluster-zone conducted before flowering led to a disaggregation of the cluster structure in all varieties. In Pinot gris, early leaf removal at BBCH 57 had even the biggest loosening effect of all variants. The reduction of bunch compactness through pre-bloom leaf removal confirmed the observations previously made by Intrieri *et al.* (2008).

However, in three of the four varieties the bloom and early postbloom leaf removal (BBCH 63 – 77) led to a more severe disaggregation than the pre-bloom treatment. No statistically significant differences of the density index were observed between variants BBCH 63 to BBCH 77 in any of the four tested varieties.

Interestingly, a significant reduction of the density index caused by the rather late leaf removal at BBCH 77 was still detectable in S. blanc and Auxerrois. This means that the reduction of leaf area at a relatively long temporal distance to flowering (around 30 days) had a positive and relatively consistent effect on cluster structure. Still, leaf removal should be conducted clearly before veraison when aiming at a loosening of the cluster structure. Indeed, late leaf removal at BBCH 81 proved to have no loosening effect and leaf removal at veraison did not significantly reduce the density index in comparison with the untreated control.

The reason for a reduction of grape cluster density due to leaf removal is most likely mainly due to an increased abortion of inflorescences or young berries as well as to a smaller berry size. As described by Caspari *et al.* (1998), the carbohydrates produced in basal leaves are of especially high importance for the developing clusters. These basal leaves are eliminated through a cluster-zone leaf removal and this deficit leads to reduced berry number and size in defoliated vines (Intrieri *et al.*, 2008; Ollat and Gaudillère, 1998; Poni *et al.*, 2006). Accordingly, the number of berries in S. blanc was reduced through leaf removal. The reduction was most important when leaf removal took place during the flowering process. Most likely, this is the result of a lack in the supply of carbohydrates, which are the major determinants of fruit set in grapes (Caspari *et al.*, 1998). Vasconcelos *et al.* (2009) described that the degree of abscission is related to the sugar concentration in the inflorescence shortly after anthesis. The present results confirm this statement as the lowest berry numbers per cluster in S. blanc were observed when leaf removal took place during flowering (BBCH 63 and 68). We assume that in this variety the partial re-closure of the cluster-zone due to lateral shoot growth in the early (two weeks before bloom) variant BBCH 57 led to a less severe assimilate deficit and, in consequence, to a reduced loosening of the cluster structure. Moreover, a reduction of the berry size as described by Intrieri *et al.* (2008) for Sangiovese as well as by Ollat and Gaudillère (1998) for Cabernet-Sauvignon can be confirmed in tendency for S. blanc.

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Table 7 - Average total yield per plant as a function of the leaf removal BBCH stage. Variants not marked with the same letter differ significantly according to Tukey test ($\alpha = 5\%$).

	S. blanc		Auxerrois		Pinot gris		Riesling	
	kg		kg		kg		kg	
Control	1.99	a	2.20	a	4.09	a	2.09	a
57	1.45	a	1.73	a	3.73	a	2.28	a
63	1.21	a	1.87	a	3.78	a	2.32	a
68	1.21	a	1.62	a	3.58	a	2.41	a
73	1.39	a	1.74	a	3.66	a	2.13	a
77	1.48	a	1.64	a	4.03	a	2.50	a
81	1.70	a	1.72	a	4.29	a	2.28	a

3. Reduction of grey mould infestation

Leaf removal in the cluster-zone led to a reduction in *B. cinerea* disease severity by 22.4 to 55.0%, on average of all four varieties. The best results were observed when leaf removal took place between the start of flowering and berries-touching. We suppose that the excellent performance of this measure is the consequence of two major factors induced by the leaf removal: (i) the reduced cluster compactness and (ii) the better sun and wind exposure. The efficiency of all leaf removals conducted between the start of flowering (BBCH 63) and the start of berries-touching (BBCH 77) was within a comparable range (from 47.2 to 55.0%, respectively). This means that the period in which leaf removal can be conducted in practical viticulture to reduce grey mould infestation at harvest is relatively long (around 30 days depending on the variety and the weather conditions). However, it has to be checked if there are differences concerning the susceptibility to sunburn depending on the date or degree of leaf removal. In 2009 and 2010, no extreme radiation events inducing severe sunburn occurred, but observations in practical viticulture indicate that leaf removal conducted between pea-size berries and veraison could lead to a more severe infestation level due to sunburn compared to the non-defoliated control.

Most likely, leaf removal in the period between flowering and berries-touching provides – in addition to the positive effects on cluster structure and exposure – a better absorption of fungicides (well exposed clusters, opened canopies). The adsorption of botryticides is highly improved in defoliated cluster-zones (Zoecklein *et al.*, 1992), especially in the grapes in the centre of the canopy, where the penetration is normally low (Smart and Robinson, 1991). Furthermore, Fox (2006) describes a hardening effect on young berries if they are well exposed due to leaf removal between flowering and groat-size. The author argues that the reasons for this might be a modified structure of the epidermis cells and an increased accumulation of phenolic substances. However, these two aspects were not investigated in the present study.

In the present trials, manual leaf removal in the period between flowering and the beginning of berries-touching provided an efficiency level of around 50% without any input of chemical substances into the environment. Thus, grey mould prevention based on cultural practices (leaf removal) represents an excellent example for the successful integration of crop cultural methods in Integrated Pest Management. Furthermore, leaf removal may constitute the most powerful tool against bunch rot in organic viticulture, where botryticides as well as plant growth regulators are not allowed. It is noteworthy that the success of leaf removal shortly after bloom proved to be more efficient than the single use of a botryticide (active ingredient: fenhexamid) or a plant growth regulator (active ingredient: prohexadione-Ca) (Molitor *et al.* 2011). The efficiency of leaf removal after bloom could, however, be enhanced by combining this measure with the application of a plant growth regulator or a botryticide (Molitor *et al.* in press).

Although in the present trials leaf removal between BBCH 63 and 77 led in all varieties to both a loosening of the cluster structure and a better sun and wind exposure, these effects were only partially present in the early (BBCH 57) and late (BBCH 81) leaf removal variants. Due to this, the positive effects of leaf removal conducted pre-bloom or at veraison on the grape health status were not as distinctive as the effects of leaf removal conducted in the period in between. However, some differences were observed among varieties.

On average of all four varieties, early leaf removal (pre-bloom) proved to be the treatment with the lowest efficiency. The reasons for this are the average lower disaggregation of the cluster structure and the lower sun and wind exposure due to the re-closure of the cluster-zone by lateral leaves. However, in the case of Pinot gris, early leaf removal reached a comparable efficiency level than the post-bloom treatments. This can be explained by

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the strong loosening effect of the pre-bloom defoliation observed in this variety in the year 2010.

The efficiency of late leaf removal at veraison was, on average, comparable to the efficiency of pre-bloom leaf removal but clearly less efficient than the treatments between flowering and berries-touching. Leaf removal at veraison had no impact on cluster structure and the young grapes grew under unfavorable microclimatic conditions throughout most of their development. Although the grapes of the late defoliated variants were very well exposed during their ripening period, the influence on the health status of berries was low in S. blanc and Pinot gris. This fact and the observation that the disease severity at harvest in these varieties is more strongly correlated with the percentage of interior clusters at berries-touching than at veraison leads to the hypothesis that the infestation level at harvest in those varieties was strongly predetermined by the microclimatic conditions during the period of berries-touching. We assume this could be due to latent infections, which took place during this period in poorly exposed clusters. The phenomenon of nonsymptomatic, quiescent *B. cinerea* infection taking place at early stages of berry development and becoming visible after veraison has been described by different authors such as Cadle-Davidson (2008), Pezet *et al.* (2003) and Keller *et al*. (2003). In S. blanc and Pinot gris, the level of exposure at berries-touching probably had an important influence on the quantity of *B. cinerea* infections in this period. The situation in Riesling under the conditions of the year 2010 was different, as late leaf removal at harvest reached an efficiency level comparable to the best variants (leaf removal between flowering and berries-touching). Moreover, the control density index in Riesling was the lowest of all control variants. This leads to the assumption that in this variety in this year the differences in grey mould infestation were mainly caused by differences in the sun and wind exposure of the berries and less by differences in the cluster structure. Furthermore, it might be assumed that in the case of Riesling the majority of the infection events of the year 2010 occurred after veraison, that is when the clusters were well exposed. This assumption seems to be confirmed by the fact that for Riesling, the correlation between the disease severity at harvest and the percentage of interior clusters at BBCH 81 was higher than the correlation between the disease severity at harvest and the percentage of interior clusters at BBCH 79.

4. Influence on harvest parameters

The total yield per plant was, in tendency, reduced by leaf removal due to the decreased assimilating leaf area. The reduction in yield was most severe when leaf removal took place in the period between just pre-bloom and berries-touching. Intrieri *et al*. (2008), Poni *et al*. (2006; 2008) and Tardaguila *et al*. (2010) also found a reduction in yield due to leaf removal in the cluster-zone, whereas no significant yield reductions were observed in the trials of Tardaguila *et al*. (2008), Zoecklein *et al*. (1992) and Bledsoe *et al*. (1988). Percival *et al*. (1994) even observed an increased yield as a consequence of leaf removal. It is most likely that the impact on yield is depending on the intensity of leaf removal, the variety, the weather conditions, and the general yield level of the vineyard. In contrast to the other three varieties, in Riesling the lowest yield was observed in the control. This might be explained by the highest grey mould infestation level in this variant and concentration processes due to extremely dry weather conditions in the days before the experimental harvest.

On average of all four varieties, the early leaf removal treatments led to slightly higher sugar levels than in the control, whereas the late leaf removal at veraison reduced the sugar level. Thus, it is obvious that the reduction of the assimilation area due to leaf removal in the period between pre-bloom and berries-touching had no negative influence on sugar accumulation during the ripening period. The loss of leaf area was probably compensated by an increased growth of lateral shoots in the upper parts of the canopy as reported by Poni *et al*. (2009) as well as of Ollat and Gaudillère (1998).

The fact that leaf removal at veraison induced, on average, the lowest sugar level of all variants might be explained by a reduction of assimilating leaf area in the ripening period. The results of Tardaguila *et al*. (2008) further indicate that late leaf removal at veraison is less efficient with respect to the final wine composition and quality than leaf removal at fruit set.

Concerning the impact of leaf removal on total acidity, there was no consistent trend. A general total acidity reduction in variants with well exposed clusters during the ripening period, as described by Bergqvist *et al*. (2001), was not observed in the present study.

CONCLUSIONS

Under the climatic conditions of the Luxembourgish winegrowing region in the years 2009 and 2010, manual leaf removal in the cluster-zone carried out between bloom and berries-touching proved to be very efficient to control bunch rot caused by *B. cinerea* in the varieties S. blanc, Auxerrois, Pinot gris and Riesling. The main reasons for this are (i) the disaggregation of the cluster structure due to limitation of assimilate supply and (ii) the improved microclimatic conditions due to better sun and wind exposure. The period in which leaf removal provided a significant reduction in bunch rot infestation on a consistent efficacy level was relatively long (around 30 days). Leaf removal in the cluster-zone in this period can be recommended in any bunch rot protection strategy

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in integrated as well as in organic viticulture. It provides excellent options to maximize wine quality in two ways, in terms of a reduction of fungal contamination of the crop and/or an improvement of grape maturity through a potential prolongation of the ripening period without any input of chemical substances into the environment.

Pre-bloom and late (conducted at veraison) leaf removal turned out to be less efficient.

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