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Multi-annual comparisons demonstrate differences in the bunch rot susceptibility of nine *Vitis vinifera* L. 'Riesling' clones

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Summary

Botrytis bunch rot is a major fungal disease of grapevines, and causes severe economic damage worldwide. Under humid climatic conditions, the development of bunch rot on grapes cannot be suppressed completely. Selection of planting material with lower bunch rot susceptibility represents one of the most efficient long-term tools in the complex bunch rot minimisation strategy. The present investigation conducted over four consecutive years (2013-2016) under the environmental conditions of the Moselle valley aimed at (i) detecting consistent differences in the bunch rot susceptibility within a group of nine commercially available *Vitis vinifera* L. 'White Riesling' clones, (ii) investigating potential underlying causes and (iii) deriving recommendations for 'Riesling' clone selection in practical viticulture. Disease severity and grape maturity (total soluble solids) progress could be well simulated by sigmoidal curves ($R^2 > 0.89$; $P < 0.038$). On average of all four years, the dates when 5 % bunch rot disease severity were reached differed significantly by 9 days between the clone with the earliest epidemic (Trier 34) and the clone with the latest epidemic (Heinz 65). Multi-annual results enabled a classification of the nine clones according to (i) their relative bunch rot susceptibility as well as (ii) their relative precocity. Based on this, practical recommendations concerning a targeted clone selection as an integral long-term tool (i) in Integrated Pest Management contributing to pesticide reduction in viticulture as well as (ii) in the viticultural climate change adaptation strategy were derived.

K e y w o r d s : *Botrytis cinerea*, bunch rot, climate change adaptation, 'Riesling' clones, pesticide reduction, planting material, *Vitis vinifera*.

Introduction

Botrytis bunch rot (also referred to as bunch rot or grey mould) caused by *Botrytis cinerea* Pers.: Fr. (teleomorph: *Botryotinia fuckeliana* (de Bary) Whetzel) is a major fungal disease of grapevine (*Vitis vinifera* L.) responsible for severe economic damage worldwide (Kassemeyer and Berkelmann-Löhnertz 2009, Wilcox *et al.* 2015). Clusters of the *V. vinifera* L. 'White Riesling' (from now on referred to as 'Riesling') are highly susceptible to bunch rot, and under Central European climatic conditions, the disease occurs virtually every season in this cultivar (Molitor *et al.* 2016). In the Moselle and neighbouring grape growing regions where 'Riesling' is the most widely planted cultivar, this not only threatens grape yield, but also wine quality in terms of off-flavors, unstable color, oxidative damages, premature aging and difficulties in clarification (Ribéreau-Gayon 1983, Smart and Robinson 1991, Wilcox *et al.* 2015).

In many cases the development of bunch rot on grapes cannot be suppressed completely. Control strategies primarily aim at delaying the epidemic as much as possible to allow for a sufficiently long maturation period for the production of high quality wines (Molitor *et al.* 2015a). Bunch rot control strategies are usually based on a combination of long-term, mid-term, and short-term (in season) measures. While traditional strategies primarily relied on routine applications of fungicides (Shtienberg 2007) with known activity against *B. cinerea* (botryticides), short-term (in-season) non-chemical crop cultural measures (such as pre-flowering or early post-flowering leaf removal in the cluster zone (Poni *et al.* 2006, 2008, Molitor *et al.* 2011a, Sternad-Lemut *et al.* 2015), cluster division (Molitor *et al.* 2012), late first shoot topping (Molitor *et al.* 2015b), artificial shading (Basile *et al.* 2015), or flower debris removal (Molitor *et al.* 2015a, Jaspers *et al.* 2016) have

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recently gained more attention in practical viticulture due to their demonstrated efficiency and their potential to reduce pesticide use. In-season crop cultural measures often aim at partially mitigating phenotypic weaknesses of the cultivar grown (such as highly compact cluster structures or generally high genetically determined susceptibility towards bunch rot) and usually result in additional labour and costs.

The identification and selection of genetic traits that reduce susceptibility to bunch rots represent an additional and increasingly important part of bunch rot control strategies, as these traits might enable a reduction (i) of fungicide use as well as (ii) of repetitive ongoing costs associated with manual and mechanical control measures.

As consequence of natural mutations and the by propagation of plants carrying them, frequently more than one clonal line exists in the same grape cultivar. This intra-varietal variability is leading to clones of the same cultivars with genotypic and phenotypic traits that slightly differ from the traits of the common mother plant (De Lorenzis *et al.* 2017). Such clonal variations in existing *V. vinifera* cultivars may (besides other genotypic and phenotypic consequences) confer differential resistance to bunch rot. For instance, significant differences in the cluster compactness and, in consequence, in the bunch rot susceptibility were demonstrated for clones of the *V. vinifera* cultivars 'Chardonnay' (Vail *et al.* 1998) and 'Garnacha Tinta' (Grimplet *et al.* 2017). A targeted selection of such planting material (*i.e.* clones with looser grape clusters) might enable a reduction of pesticides used in order to control bunch rot.

Besides bunch compactness, the cuticle and its epicuticular waxes were described as important berry skin features with regard to the susceptibility of berries to bunch rot (Commenil *et al.* 1997, Gabler *et al.* 2003, Becker and Knoche 2012a and b, Herzog *et al.* 2015). Especially at maturity, cracks in the berry skin can develop (Commenil *et al.* 1997, Becker and Knoche 2012a and b), representing preferential entry sites for the penetration of the hyphae of *B. cinerea*. The formation of cracks is related to the thickness of the cuticle (Commenil *et al.* 1997). Recently, a simple-to-handle "I-Sensor" enabling objective, reliable and fast phenotyping of the thickness and permeability of berry cuticles based on electrical impedance was developed to evaluate the susceptibility of grape berries towards bunch rot (Herzog *et al.* 2015). The cuticle and the wax layer are located between two electrically conducting surfaces: NaCl solution where berries positioned as well as the epidermal cell layer of berries. The impedance as its own is the sum of the real resistor (permeability of the cuticle, the wax layer and air) and the imaginary resistor (thickness of the cuticle, the wax layer and air). Herzog *et al.* (2015) used this novel phenotypic trait as indicator for resistance of grapevine berries towards bunch rot disease.

Under the environmental conditions of the Moselle valley, meteorological conditions are strongly (i) fluctuating among years and (ii) determining the annual bunch rot epidemic (Molitor *et al.* 2016). As a consequence, the relative bunch rot susceptibly of different 'Riesling' clones might vary depending on the annual meteorological conditions. Hence, for drawing conclusions on the bunch rot susceptibility of different clones of general validity, analyses of multi-annual data are required. So far, to the best of our knowledge, multi-annual results on the bunch rot susceptibility of different 'Riesling' clones under Central European conditions are lacking in the scientific literature.

Consequently, the present investigation conducted in four consecutive years (2013-2016) under the environmental conditions of the Moselle valley aimed at (i) detecting consistent differences in the bunch rot susceptibility in a set of nine commercially available and commonly cultivated 'Riesling' clones, (ii) investigating potential underlying causes and (iii) deriving recommendations for 'Riesling' clone selection in practical viticulture.

Material and Methods

Vineyard site and experimental design: The investigations described herein were carried out between the years 2013 and 2016 in the experimental vineyard of the DLR Mosel in Zeltingen-Rachtig, Germany (49.95 N, 7.03 E), located in the valley of the river Moselle. The vineyard is south-west exposed with an inclination of 48 to 56 % and an elevation of 200 to 250 meters above sea level. The soil type is shallow weathering slate. Grapevines grafted onto Börner rootstocks were planted in the year 2006 and trained to a vertical shoot positioning system (VSP) with two horizontal canes per vine (approximately six buds per cane = 12 buds per plant). The planting density was 2.4 m^2 (2 m distance between rows, 1.2 m distance between vines), and the trial was arranged as a randomized block design (4 blocks) with four replicates of approximately 30 vines per plot. Tested 'Riesling' clones (abbreviations in parentheses) were as follows: Colmar 49 (Col 49), Trier 34 (Tr 34), Heinz 65 (Hei 65), Heinz 108 (Hei 108), Bernkastel 68 (Bks 68), Neustadt 90 (N 90), 239 Geisenheim (239 Gm), Weis 17 and Heilbronn 83 (Hn 83).

All plots were managed in the same manner throughout all years. Regular background fungicide applications (10 to 12 d intervals) *via* sprayer against *Plasmopara viticola* and *Erysiphe necator* were carried out in all seasons. The following active ingredients were applied: ametoctradin, azoxystrobin, cyflufenamid, cymoxanil, difenoconazol, dimethomorph, dithianone, folpet, iprovalicarb, mancozeb, metiram, metrafenone, myclobutanil, penconazol, phosphorous acid, quinoxyfen, sulphur, and zoxamide.

Meteorological and phenological data: Meteorological data were recorded directly in the experimental vineyard by a weather station of the DLR. Air temperatures and precipitation sums were measured at 2 m and 1 m above the ground, respectively.

Phenological plant growth stages according to the BBCH scale as defined by Lorenz *et al.* (1995) were recorded in a 'Riesling' vineyard in a distance of approximately 2.8 km from the vineyard of observation. Key meteorological and phenological data are given in Supplementary Tab. 1.

Assessment of canopy morphology (Point Quadrat Analysis): The cluster-zone canopy structure was assessed in all four years of investigation

Table 1

B. cinerea disease severities in the different clones at the different assessment dates in the years 2013 to 2016. Clones in the same year at the same assessment date marked with the same letter did not differ significantly (according to Tukey's multiple comparison procedure ($P = 0.05$)). If no significant differences were observed, no notations were indicated

		Disease severity																							
Year	Date	Col 49		Tr 34		Hei 108		Hei 65		Bks 68		N 90		239 Gm		Weis 17		Hn 83		Average	
2013	10/09	0.0		0.0		0.0		0.0		0.1		0.1		0.0		0.0		0.1		0.0	
2013	20/09	1.3		1.5		2.6		1.0		1.4		1.8		1.8		1.2		1.8		1.6	
2013	27/09	9.1		7.2		9.8		6.6		6.9		10.3		11.2		5.6		10.8		8.6	
2013	04/10	16.2		17.5		19.0		12.2		14.4		17.2		19.4		10.8		16.1		15.9	
2013	11/10	44.3	b	44.7	b	45.8	b	30.1	ab	31.5	ab	43.1	b	44.8	b	25.9	a	43.0	b	39.2	
2013	18/10	71.1	ab	71.4	abc	73.2	bc	66.9	abc	60.0	a	72.4	abc	77.8	c	63.7	ab	75.9	bc	70.3	
2014	29/08	0.2	a	0.6	a	0.4	a	0.8	a	1.1	a	2.5	b	0.6	a	0.8	a	0.9	a	0.9	
2014	09/09	1.4		2.4		1.5		1.9		1.7		3.7		2.5		2.3		1.9		2.1	
2014	19/09	4.2		7.7		3.5		3.5		4.3		6.2		5.7		5.7		3.6		4.9	
2014	26/09	7.6		10.5		8.0		7.2		4.8		9.5		7.8		7.9		7.1		7.8	
2014	03/10	21.9		20.2		19.8		15.6		18.0		25.4		23.7		20.5		18.5		20.4	
2014	10/10	33.8	ab	32.9	ab	26.0	ab	22.1	a	24.3	ab	36.6	b	34.2	ab	30.4	ab	24.9	ab	29.5	
2015	04/09	0.0		0.1		0.1		0.1		0.1		0.4		0.1		0.1		0.1		0.1	
2015	15/09	2.2	a	7.0	b	2.8	a	1.7	a	1.5	a	2.1	a	3.5	ab	3.3	ab	2.3	a	2.9	
2015	25/09	6.6		10.5		5.1		4.0		4.3		8.0		8.3		8.6		7.6		7.0	
2015	02/10	12.2	abc	17.9	c	7.8	a	7.2	a	7.9	a	10.2	ab	16.1	bc	10.2	ab	11.4	abc	11.2	
2015	09/10	15.5	abc	23.9	c	8.7	a	10.4	ab	11.4	ab	17.6	abc	18.5	bc	16.0	abc	12.4	ab	14.9	
2016	26/08	0.0		0.1		0.1		0.0		0.1		0.0		0.1		0.1		0.1		0.1	
2016	07/09	0.0		0.2		0.1		0.0		0.1		0.0		0.0		0.1		0.1		0.1	
2016	16/09	0.1		0.1		0.1		0.0		0.1		0.0		0.1		0.1		0.2		0.1	
2016	26/09	2.3		3.0		2.1		1.2		0.8		1.0		1.2		2.2		1.5		1.7	
2016	03/10	4.9		6.5		5.6		2.6		2.9		2.7		3.5		4.3		3.7		4.1	
2016	10/10	10.1		12.1		8.9		4.5		4.5		4.8		5.7		7.7		6.3		7.2	
2016	17/10	17.2	bc	20.3	c	15.4	bc	7.1	a	7.4	a	9.4	ab	9.5	ab	13.3	abc	10.2	ab	12.2	

for each clone using a Point Quadrat Analysis according to Smart and Robinson (1991) at BBCH 79 (2013: August 20; 2014: August 8; 2015: August 7; 2016: August 11). Here, a rod was inserted into the cluster-zone every 10 cm from the east side of the row on the height of the upper fruiting wire. Contacts of the rod were recorded distinguishing between leaves (L), clusters (C) and gaps (G) on 48 insertion points per plot. The percentages of interior clusters (PIC) were calculated using a spreadsheet template provided by Jim Meyers (Meyers and Vanden Heuvel 2008).

Assessment of the cluster morphology: To investigate potential clonal differences in the cluster structure, the cluster density index according to the protocol by Ipach *et al.* (2005) was assessed as previously described (Evers *et al.* 2010). Fifty clusters per plot were assessed at BBCH 79 (2013: August 20; 2014: August 8; 2015: August 7; 2016: August 11).

Determination of berry firmness (2013-2016) and impedance (2016): Prior to harvest (2013: October 7; 2014: September 24; 2015: September 29; 2016: October 4), six grape clusters were randomly harvested in each plot. Out of these clusters, 50 berries with pedicels were separated for further investigation using fine scissors. Berry firmness was assessed using a FirmTech FT7 Fruit Firmness Tester (BioWorks, Wamego, KS, USA). This device measures the force ($g \cdot mm^{-1}$) required to deform the berry by 1 mm (Hampson *et al.* 2014).

In 2016, the impedance of 50 separated, visually intact berries (with pedicels) per plot was determined in addition to the other berry measurements. Sampling took place on Oc-

tober 4, 2016 as described above. Impedance measurements were conducted at room temperature using the "I-sensor" as developed by Herzog *et al.* (2015). Relative impedance Z_{rel} was calculated according to Herzog *et al.* (2015).

Yield: In the years 2013, 2015 and 2016 grapes were harvested separately per clone. In 2014, no yield determination took place. Generally, all grapes of each clone were pooled each year. In consequence, yield data consist of one value per clone and year.

Assessment of *B. cinerea* disease progress: *B. cinerea* disease progress was followed at intervals of seven to twelve days between veraison and harvest by examining 50 randomly selected clusters per plot. Disease severity was assessed according to the EPPO guideline PP1/17 classifying visually observed disease severities in seven classes (0 %; 1-5 %; 6-10 %; 11-25 %; 26-50 %; 51-75 %; 76-100 %). Average disease severities were calculated by summing the number of observations per class multiplied by the arithmetic mean of the class interval and dividing this sum by the total number of observations (n = 50) (Molitor *et al.* 2015a).

To describe the temporal progress of the disease severity, the average values were plotted versus the assessment date (expressed as day of the year (DOY)). Disease progress curves were fitted to these data according to the sigmoidal equation (1) as described before (Molitor *et al.* 2015b).

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

where y is the disease severity, x corresponds to the assessment date expressed as day of the year (DOY), x_0 is the inflection point of the curve (disease severity of 50 % reached) and b the slope factor of the curve in the inflection point. Solving this equation for x provides the time point at which a specific disease severity value was reached. To quantify clonal differences in the temporal position of the annual epidemic, the $x_{5\%}$-values (DOY reaching a disease severity of 5 %) were used following Beresford *et al.* (2006) and Evers *et al.* (2010).

Maturation progress: The maturation progress was followed at intervals of seven to 12 d between veraison and harvest (same dates as for bunch rot assessments) by collecting 30-40 randomly selected berries (clusters from different positions on the shoot; berries of different positions in the cluster) per plot (avoiding berries with visible bunch rot symptoms). Total soluble solids were determined from the extracted juice (mixed sample of all berries per plot) using a refractometer (RHB-32ATC, Huake Instruments Co. Ltd, Lirenfuzone, China).

Since berry sugar accumulation after veraison follows a sigmoidal pattern (Coombe 1992), sigmoidal maturity progress curves were fitted to observation data according to equation (2).

$$y = \frac{a}{1 + e^{-((x - x_0)/b)}} \quad (2)$$

where y are the total soluble solids, x corresponds to the sampling date expressed as day of the year (DOY), x_0 is the inflection point, a the maximum of the curve and b the slope factor of the curve in the inflection point.

Solving this equation for x provides the time point at which a specific total soluble solids value was reached. In present investigations, the calculated DOYs reaching 16.47 brix (= 70 °Oechsle (Oe)) were selected for comparing the precocity of the different clones.

In addition, disease progress curves were plotted against the grape maturation progress (expressed as total soluble solids) according to equation (1). In this case, y is the disease severity, x corresponds to the total soluble solids, x_0 is the inflection point of the curve (disease severity of 50 % reached) and b the slope factor of the curve in the inflection point.

Calculated total soluble solids (brix) at the moment of reaching 5% disease severity were compared between the different clones.

Data analyses and statistics: Data sets consisting of average values per plot (four replicate plots per treatment) were (after testing Gaussian distribution and homogeneity of variance) analysed for the effect of the clone by one-way ANOVAs using SPSS Statistics 19 (IBM, Chicago, IL, USA). In case null-hypotheses were rejected ($P \leq 0.05$), pair-wise multiple comparisons according to Tukey were performed for clone effects.

To detect consistent effects over all four years, annual average (i) PIC, (ii) density index and (iii) berry firmness values were normalized in terms of division by annual average values of all nine clones. Furthermore, the annual deviations from the averages of all nine clones of (i) the $x_{5\%}$-values (ii) the date reaching 16.47 brix total soluble solids and (iii) the total soluble solids at the moment of reaching 5 % disease severity in the case of the different clones were calculated.

Normalized values of PIC, density index and berry firmness as well as annual deviations of the $x_{5\%}$-values, the date reaching 16.47 brix total soluble solids and the total soluble solids at the moment of reaching 5 % disease severity were compared pairwise ($P \leq 0.05$) following independent-samples Kruskal-Wallis test (SPSS 19).

Results

Key meteorological data and phenological growth stages: Average temperatures within the growing seasons (April-October) were rather similar and ranged from 15.2 °C in 2013 to 15.8 °C in 2014. The lowest cumulative precipitation within the growing season was observed in 2015 (360 mm) and the highest cumulative precipitation was observed in 2013 (616 mm). The phenological stage beginning of flowering (BBCH 61), used here as a comparison for annual plant phenology, was reached between day of the year 157 (2014) and 172 (2013) (Supplementary Tab. 1).

Percentage interior clusters: Average PIC values ranged from 44.4 (2016) to 70.3 (2013). No significant differences in the PIC values of the different clones were observed in any year. Average normalised PIC values ranged from 0.94 in 239 Gm to 1.05 in Weis 17 and Col 49. Also, no statistically significant differences with regard to average normalised PIC values between clones were observed according to a Kruskal-Wallis test for independent samples (Suppl. Tab. 2).

Cluster architecture: Average density index values ranged from 2.89 (2013) to 3.42 (2014). The clone Hn 83 showed significantly lower cluster compactness (i) than the clones Tr 34, Hei 108, Hei 65, Bks 68 and Weis 17 in 2013, (ii) than Col 49 in 2015 and (iii) than Tr 34 in 2016. Bks 68 was in 2015 of significantly lower cluster compactness than Col 49. Average normalised density index values ranged from 0.91 in Hn 83 and to 1.04 in Tr 34 as well as in Weis 17 with no statistically significant differences between clones according to an independent-sample Kruskal-Wallis test (Suppl. Tab. 3).

Berry firmness and impedance of the berry cuticle: Average berry firmness values ranged from 136.8 g·mm^{-1} (2016) to 160.7 g·mm^{-1} (2014). In 2013, the berry firmness was significantly lower in Tr 34 than in N 90 and Weis 17. The berry firmness in Hn 83 was in 2014 lower than in any other clone and in 2015 lower than in Bks 68. Average normalized berry firmness values ranged from 0.97 in Hn 83 to 1.02 in Weis 17 with no statistically significant differences between clones (Suppl. Tab. 4).

In 2016, 'Riesling' clones Bks 68 revealed the lowest Z_{rel} values (402) while highest Z_{rel} values were recorded in case of the clone Hei 65 (464). No significant differences were observed in the Z_{rel} values between the nine clones (Suppl. Tab. 5).

Yield: On average of all nine clones, the yield was 6.1 (2013), 5.0 (2015) and 5.5 tons ha^{-1} (2016). Average normalised yields were lowest in N 90 (0.80) and highest in Bks 68 (1.22) (Suppl. Tab. 6).

Bunch rot and maturity progress: *B. cinerea* disease severities on average of all nine clones reached at the final assessment date were 70.3 % (2013), 29.5 % (2014), 14.9 % (2015) and 12.2 % (2016) (Tab. 1). At the final assessment date, the following significant differences between clones were observed:

- In 2013, the diseases severity in Bks 68 was significantly lower than in Hei 108, 239 Gm and Hn 83.
- In 2014, a significantly lower disease severity was observed in Hei 65 compared to N 90.
- In 2015, disease severity in Hei 108 was significantly lower than in Tr 34 and 239 Gm.
- In 2016, significantly lower disease severities were recorded in Hei 65 and Bks 68 than in Col 49 and Tr 34 (Tab. 1).

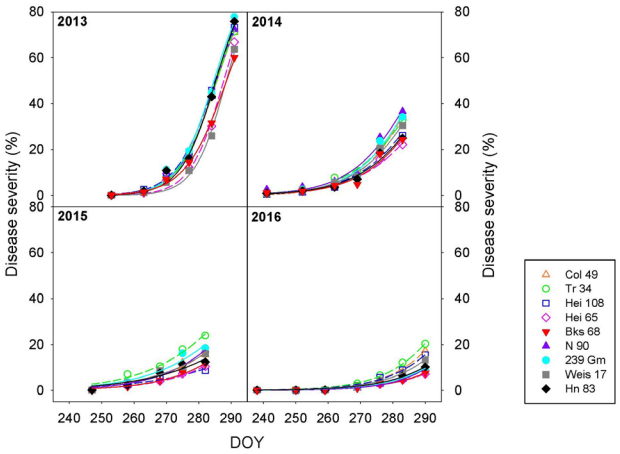

Fig. 1: Progress of the disease severity of *B. cinerea* in the different clones in the years 2013 to 2016 as functions of the assessment date (DOY). Plot symbols represent the observed disease severities, the lines the calculated progress according to the sigmoidal equation type $y = 100 / (1 + e^{-((x-x_0)/b)})$.

Coefficients of determination (R^2) of sigmoidal equations [$y = 100 / (1 + e^{-((x-x_0)/b)})$] ranged from 0.95 to 1 with *P*-values between 0.0001 and 0.0106, demonstrating highly significant correlations (Fig. 1; Suppl. Tab. 7). Calculated dates (DOY) reaching a disease severity of 5 % on average of all nine clones ranged from 261.9 in 2014 to 280.1 in 2016 (Tab. 2). On average of all four seasons, the highest positive deviation between the DOY reaching 5 % disease severity in a specific clone and the average of all nine clones was 4.1 d in Hei 65 and the absolutely highest negative deviation was -4.8 d in Tr 34 (Tab. 2). Concerning these deviations, the two clones (Hei 65 and Tr 34) differed significantly according to an independent-sample Kruskal-Wallis test (Tab. 2).

Total soluble solids at the final assessment dates were on average of all nine clones 18.8 (2013), 16.9 (2014), 21.1 (2015) and 19.4 brix (Tab. 3). Significant differences in the total soluble solids at the final assessment date were observed in the following cases:

- In 2013, the total soluble solids in N 90 were significantly lower than in Col 49, Hei 108 and 239 Gm.
- In 2014, significantly lower total soluble solids were observed in Hei 65 compared to Col 49.
- In 2016, significantly lower total soluble solids were

Table 2

Calculated dates (day of the year (DOY)) reaching a bunch rot disease severity of 5 % in the years 2013, 2014, 2015 and 2016 as well as deviations (Δ) between the DOY reaching 5 % disease severity in a clone in a specific year and the average DOY reaching 5 % disease severity of all clones in this year. Average (2013 to 2016) deviations of different clones marked with the same letter did not differ significantly (according to pairwise comparisons following independent-samples Kruskal-Wallis test ($P = 0.05$))

Clone	2013	Δ	2014	Δ	2015	Δ	2016	Δ	Average Δ	
Col 49	268.46	-1.1	262.81	1.0	264.16	-0.1	276.20	-3.9	-1.1	AB
Tr 34	268.44	-1.2	259.38	-2.5	255.12	-9.2	273.72	-6.4	-4.8	A
Hei 108	267.43	-2.2	262.70	0.8	268.83	4.6	276.51	-3.6	-0.1	AB
Hei 65	272.59	3.0	263.48	1.6	271.00	6.7	285.28	5.1	4.1	B
Bks 68	269.73	0.1	263.92	2.1	270.12	5.8	284.90	4.8	3.2	AB
N 90	268.22	-1.4	259.26	-2.6	264.41	0.1	283.38	3.2	-0.2	AB
239 Gm	268.21	-1.4	261.09	-0.8	259.46	-4.8	282.05	1.9	-1.3	AB
Weis 17	274.00	4.4	261.36	-0.5	262.38	-1.9	278.32	-1.8	0.0	AB
Hn 83	269.32	-0.3	262.70	0.8	263.03	-1.2	280.91	0.8	0.0	AB
Average	269.60	0.0	261.86	0.0	264.28	0.0	280.14	0.0	0.0	

Table 3

Total soluble solids (°Brix) in the different clones at the different sampling dates in the years 2013 to 2016. Clones in the same year at the same assessment date marked with the same letter did not differ significantly (according to Tukey's multiple comparison procedure; ($P = 0.05$)). If no significant differences were observed, no notations were indicated

Year	Date	Total soluble solids																
		Col 49		Tr 34		Hei 108		Hei 65		Bks 68		N 90		239 Gm		Weis 17		Hn 83		Average
2013	10/09	13.2	c	12.2	abc	12.6	bc	11.7	abc	11.4	ab	12.2	abc	12.7	bc	11.0	a	12.2	abc	12.1
2013	20/09	14.9	b	14.0	ab	14.8	a	14.4	ab	13.4	a	14.1	ab	14.8	b	13.7	ab	14.7	ab	14.3
2013	27/09	17.3	b	16.4	ab	17.6	b	16.9	ab	15.6	a	16.9	ab	17.1	ab	16.8	ab	16.8	ab	16.8
2013	04/10	18.6	ab	18.4	ab	18.6	ab	18.0	a	17.5	a	18.6	ab	18.6	ab	18.2	ab	19.2	b	18.4
2013	11/10	19.3	c	18.3	abc	18.6	bc	18.1	abc	17.8	ab	17.1	a	19.1	bc	18.4	abc	17.9	ab	18.3
2013	18/10	19.5	c	18.6	abc	19.3	bc	18.8	abc	18.2	ab	17.8	a	19.5	c	18.8	abc	18.4	abc	18.8
2014	29/08	11.7	c	10.4	abc	10.2	abc	9.5	a	9.5	a	10.2	ab	11.2	bc	9.6	a	10.4	abc	10.3
2014	09/09	13.9	c	11.8	ab	11.8	ab	11.2	a	11.5	ab	12.5	abc	13.2	bc	11.6	ab	13.1	abc	12.3
2014	19/09	16.2	b	14.9	ab	13.8	ab	13.5	a	13.5	a	13.6	ab	14.9	ab	13.9	ab	14.8	ab	14.3
2014	26/09	17.5	b	15.7	ab	15.2	ab	14.9	a	14.9	a	15.9	ab	16.4	ab	15.2	ab	16.4	ab	15.8
2014	03/10	18.8	c	16.2	ab	16.5	ab	15.6	a	16.1	a	16.0	a	18.3	bc	15.9	a	17.5	abc	16.8
2014	10/10	18.8	b	16.8	ab	16.9	ab	15.6	a	16.4	ab	16.6	ab	17.0	ab	16.9	ab	17.3	ab	16.9
2015	04/09	13.9	d	13.2	bc	13.4	cd	12.8	ab	12.5	a	13.0	abc	13.5	cd	13.2	bc	13.3	bcd	13.2
2015	15/09	17.6	b	17.6	ab	17.8	ab	17.4	ab	16.7	a	17.3	ab	17.6	ab	18.0	b	18.2	b	17.6
2015	25/09	19.9	b	19.2	ab	19.9	b	19.1	ab	18.9	a	18.8	a	19.5	ab	18.9	a	19.4	ab	19.3
2015	02/10	22.2	b	21.4	ab	21.2	ab	21.0	a	20.5	a	20.8	a	20.9	a	21.2	ab	21.5	ab	21.2
2015	09/10	21.7		20.8		21.3		21.2		20.3		21.0		20.8		21.2		21.4		21.1
2016	26/08	6.7		6.4		6.5		5.8		5.9		5.8		6.3		5.7		6.2		6.1
2016	07/09	14.5	c	11.8	ab	13.6	bc	12.9	abc	11.5	a	12.8	ab	13.6	bc	12.4	ab	13.6	bc	13.0
2016	16/09	16.8	b	15.4	ab	16.2	b	16.0	ab	14.7	a	15.9	ab	15.6	ab	15.8	ab	16.1	ab	15.8
2016	26/09	18.2		16.4		17.8		18.0		16.1		17.6		17.2		18.1		17.2		17.4
2016	03/10	19.2	d	16.6	a	18.9	d	18.7	cd	16.8	ab	18.3	abcd	17.2	abc	18.2	abcd	18.4	bcd	18.0
2016	10/10	19.6	c	17.4	a	19.2	bc	19.0	abc	17.5	ab	19.1	abc	17.9	abc	19.5	c	18.5	abc	18.6
2016	17/10	20.1	c	18.2	ab	20.0	c	20.1	c	18.1	a	19.9	bc	18.8	abc	20.3	c	19.1	abc	19.4

measured in Bks 68 than in Col 49, Hei 108, Hei 65, N 90 and Weis 17 (Tab. 3).

Highly significant correlations were observed when fitting sigmoidal equations of the type $y = a / (1 + e^{-((x-x_0)/b)})$ to describe the maturation progress. The coefficient of determination ranged from 0.89 to 1 with *P*-values between 0.0001 and 0.0377 (Suppl. Tab. 8). Calculated dates (DOY) reaching 16.47 brix on average of all nine clones ranged from 253.1 in 2015 to 276.8 in 2014 (Tab. 4). On average of all four seasons, the highest positive deviation between the DOY reaching 16.47 brix in a specific clone and the average of all nine clones was 5.0 days in Bks 68 and the highest absolute negative deviation was -5.6 d in Col 49 (Tab. 4). Concerning these deviations, the clones Col 49 and Hn 83 differed significantly from Bks 68 (Kruskal-Wallis test) (Tab. 4).

In addition, sigmoidal equations of the type $y = 100 / (1 + e^{-((x-x_0)/b)})$ were used to describe the disease progress as function of maturation progress (total soluble solids in brix). Here, the coefficient of determination ranged from 0.35 to 1.00 with *P*-values between 0.0001 and 0.2203. In 34 out of 36 "year x clone - combinations", disease progress was significantly correlated with the maturation progress (Suppl. Figure; Suppl. Tab. 9). Calculated total soluble solids at the moment of reaching a disease severity of 5 % on average of all nine clones ranged between 14.8 in 2014 and 18.7 brix in 2015 (Suppl. Tab. 10). On average of all four seasons, the highest positive deviation between the total soluble solids at the moment reaching 5 % disease severity in a specific clone and the average of all nine clones was 1.0 brix in Col 49 and the absolutely highest negative deviation was -0.9 brix in Tr 34 (Suppl. Tab. 10). Concerning these deviations, no significant differences were observed according to independent-sample Kruskal-Wallis test (Suppl. Tab. 10).

Temporal deviations of the day of the year (DOY) reaching 5 % disease severity in the different clones (compared

Table 4

Calculated dates (day of the year (DOY)) reaching 16.47 °Brix (= must density of 1070 $g \cdot L^{-1}$ = 70 °Oe) in the years 2013, 2014, 2015 and 2016 as well as deviations (Δ) between the DOY reaching 16.47 °Brix in a clone in a specific year and the average DOY reaching 16.57 °Brix of all clones in this year. Average (2013 to 2016) deviations of different clones marked with the same letter did not differ significantly (according to pairwise comparisons following independent-samples Kruskal-Wallis test ($P = 0.05$))

Clone	2013	Δ	2014	Δ	2015	Δ	2016	Δ	Average Δ	
Col 49	267.01	-2.3	263.27	-13.6	251.66	-1.4	256.31	-5.2	-5.6	A
Tr 34	270.74	1.4	277.75	0.9	252.90	-0.2	267.35	5.8	2.0	AB
Hei 108	266.73	-2.6	278.16	1.3	251.87	-1.2	259.03	-2.5	-1.2	AB
Hei 65	269.49	0.2	290.63	13.8	254.38	1.3	259.85	-1.7	3.4	AB
Bks 68	274.35	5.0	281.53	4.7	255.94	2.9	269.00	7.5	5.0	B
N 90	269.75	0.4	270.13	-6.7	254.38	1.3	260.67	-0.8	-1.5	AB
239 Gm	267.77	-1.6	279.84	3.0	252.26	-0.8	260.94	-0.6	0.0	AB
Weis 17	270.34	1.0	279.24	2.4	252.64	-0.4	261.09	-0.4	0.6	AB
Hn 83	267.77	-1.6	271.11	-5.7	251.62	-1.5	259.40	-2.1	-2.7	A
Average	269.33	0.0	276.85	0.0	253.07	0.0	261.52	0.0	0.0	

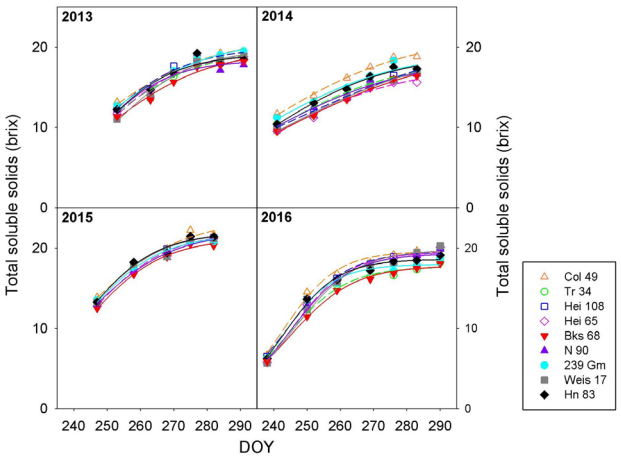

Fig. 2: Progress of the total soluble solids in the different clones in the years 2013 to 2016 as functions of the assessment date (DOY). Plot symbols represent the observed disease severities, the lines the calculated progress according to the sigmoidal equation type $y = a / (1 + e^{-((x-x_0)/b)})$.

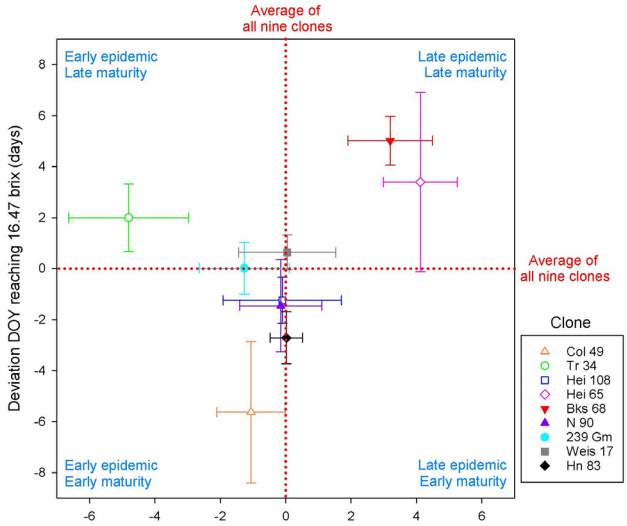

Fig. 3: Temporal deviations of the of the day of the year (DOY) reaching 5 % disease severity in the different clones compared to the average of all nine clones plotted against the temporal deviations of the day of the year (DOY) reaching 16.47 brix in the different clones compared to the average of all nine clones. Error bars = standard errors.

to the average of all nine clones) are plotted against the temporal deviations of the day of the year (DOY) reaching 16.47 brix in the different clones (compared to the average of all nine clones) in Fig. 3.

Discussion

Clonal differences in bunch rot susceptibility and precocity: The present investigation revealed clonal differences in both, bunch rot susceptibility and precocity. Sigmoidal curves of the type $y = a / (1 + e^{-((x-x_0)/b)})$ were chosen to simulate the disease severity progression as well as berry maturation. This form of equation shows an almost exponential growth at the beginning of the epidemic, while the rate of progress decelerates at higher disease severities or total soluble solids. In case of the disease severity, the maximum value of the curve is restricted to 100 %. This restriction to 100 % does not exist in case of the maturity progress curve. Fitted equations were able to describe both the disease as well as maturity progress plotted versus the time ($R^2 > 0.89$). This confirms previous results for *B. cinerea* epidemics on grape clusters (e.g., Molitor *et al.* (2016)) as well as the increase in total soluble solids in the grape berry after veraison (Coombe 1992).

To allow comparisons between the clones across the four seasons, the deviation of the dates for each clone reaching a diseases severity of 5 % and 16.47 brix relative to the average of all clones were calculated. For the date when reaching 5 % disease severity, an average total deviation between the clones Hei 65 and Tr 34 of 8.9 days was observed. The potential delay caused by the clones in the present study was therefore more pronounced than the delay of the epidemic attained by the best treatment in recent trials applying a botryticide in 'Riesling' at different time points (3.5 d) (Molitor *et al.* 2018). This demonstrates the high potential of targeted plant material selection in the complex bunch rot control strategy leading to a potential reduction of pesticides (here: botryticides) in viticulture.

Potential underlying causes: As potential underlying causes for clonal differences, cluster exposure, cluster architecture, berry firmness and impedance of the berry cuticle were examined.

The **percentage of interior clusters** (PIC) as determined via Point Quadrat Analysis according to Smart and Robinson (1991) describes the cluster exposure. Generally, low PIC values indicate a high exposure towards both sun and wind (Molitor *et al.* 2011). Increased airflow and better sun exposure shorten the length of wetness periods and, in consequence, provide less favourable conditions for the germination and infection process of fungal pathogens (Zoecklein *et al.* 1992).

In the present data set, no significant differences in the percentage of interior clusters (PIC) between the different clones were recorded. Furthermore, also no significant correlations between the normalised PIC and the deviation of the date reaching 5 % disease severity (compared to the average of all nine clones) was observed ($r = -0.123$; $P = 0.475$). This indicates that differences in the cluster zone canopy morphology are not the primary underlying cause of the observed clonal differences in the bunch rot susceptibility.

A strong link between the **cluster compactness** and the bunch rot susceptibility has been observed in several studies in different cultivars and different viticultural regions (Hed *et al.* 2009, Molitor *et al.* 2012, Intrigliolo *et al.* 2014, Tello and Ibanez 2014, Molitor *et al.* 2015a). Hence, the density index developed by Ipach *et al.* (2005) has been proposed as an valuable tool to describe the predisposition to bunch rot several weeks prior to the start of the epidemic (Molitor *et al.* 2015a). However, in the present investigation no significant correlation between the normalised density index and the deviation of the date reaching 5 % disease severity (compared to the average of all nine clones) was observed ($r = -0.256$; $P = 0.131$). Interestingly, investigations of Vail *et al.* (1998) on 'Chardonnay' also demonstrated that the general link between cluster compactness and bunch

rot susceptibility does not always hold true within a group of clones. As a consequence of the present investigations, we conclude that the existing differences in the bunch rot susceptibility of the nine 'Riesling' clones tested did not primarily seem to be explained by differences in cluster structure.

Examinations of **berry firmness** did not indicate links with bunch rot susceptibility. The normalised berry firmness was not significantly correlated with the deviation in the date of reaching 5 % disease severity (compared to the average of all nine clones) (r = 0.0147; P = 0.9321). Similarly, the **impedance of the cuticle** (determined only in the year 2016) did not differ significantly between the nine clones and was not significantly correlated with the deviation in the date of reaching 5 % disease severity (r = -0.2492; P = 0.5179). However, the positive correlation between the impedance of the cuticle and the total soluble solids at the moment of reaching 5 % disease severity (r = 0.5358; P = 0.1378) indicates that berries of 'Riesling' clones with higher impedance tended to be more mature when reaching 5 % disease severity. This indication as well as potential additional factors causing the clonal differences in the bunch rots susceptibility merit further investigations in the future.

Practical recommendations for 'Riesling' clone selection: Decisions concerning the cultivation of a specific clone need to be taken based on number of different factors including yield (Suppl. Tab. 6), yield stability, as well as other viticultural and enological traits. In addition, (i) the date at which fruit attains suitable sugar content for winemaking, and (ii) the risk of bunch rots occurring before this point is reached, are two of the key considerations in cool climate viticulture. Based on the calculations for the average deviations in DOY reaching 5 % disease severity and DOY reaching 16.47 brix, the present clones can be classified in the following four categories (Fig. 3):

- early epidemic, late maturity: Tr 34;
- early epidemic, early maturity: Col 49;
- late epidemic, late maturity: Hei 65, Bks 68;
- medium late epidemic, medium late maturity: Hei 108, N 90, 239 Gm, Weis 17, Hn 83.

Unfortunately, none of the nine clones investigated showed a late epidemic and at the same time an early maturity.

Based on the results of the present investigation, clone **Tr 34** does not appear well suited to the environmental conditions of the Moselle valley since it shows an early bunch rot epidemic as well as a late maturity (Fig. 3). Consequently, under the present climatic conditions, there may be pressure to harvest fruit from this clone prior to full maturity which is supposed to result in wines of lower quality (Spring 2004).

The clone **Col 49** showed on average across the four years of investigation the earliest maturity and the highest total soluble solids at the moment of reaching 5 % disease severity. Due to its characteristics, Col 49 can be recommended for

(i) cool (e.g., high latitudes, high elevation, poor exposition) vineyards or regions. Due to its relative precocity, Col 49 increases the chance for full maturity even under relatively cool conditions.
(ii) the production of noble rot wines. Due to its relatively early maturity and relatively early epidemic, Col 49 increases the chance for high total soluble solids and an epidemic under relatively warm conditions (earlier in the season) increasing the speed of drying and concentrating processes (provided beneficial environmental conditions).

The clones **Bks 68** and **Hei 65** were characterized by a late epidemic as well as late maturity. These clones can, in consequence, recommended

(i) for warm (e.g., low latitudes, low elevation, south-exposition) vineyards or regions.
(ii) under future (and most likely warmer (Junk *et al.* 2014, Molitor *et al.* 2014)) conditions. Here, these relatively late ripening clones might build valuable tools in the climate change adaptation strategy.

Due to their later maturation, fruit from both clones usually ripen under cooler conditions. This is generally leading to (i) a reduced pace of the bunch rot epidemic (Molitor *et al.* 2016), (ii) a deceleration of the degradation of organic acids and loss of acidity as well as (iii) a limitation of negative effects of high temperatures on grape and wine aroma content (both contributing to the typicity of cool climate wines) (Duchêne *et al.* 2010). These clones would not be suited to vineyards or regions were the annual heat accumulation represents a limiting factor for full fruit maturity.

Generally, targeted clone selection represents an integral long-term tool (i) in the bunch rot control strategy in Integrated Pest Management contributing to pesticide reduction in viticulture as well as (ii) in the viticultural climate change adaptation strategy.

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