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Relationship between meteorological data, physical-mechanical characteristics of grapes and *Botrytis* bunch rot

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Summary

Botrytis bunch rot (BBR) is the economically third most important disease in cool climate viticulture. In order to avoid or delay spreading of BBR infections until the grapes reach physiological ripeness, different management strategies like early defoliation or specific fungicide applications were developed. The scope of most grapevine breeding programs is the selection of mildew fungus-resistant, climatic adapted grapevines with balanced, healthy yield and outstanding wine quality. Within the long-term breeding process, the application of marker-assisted selection (MAS) is the most efficient way for early selection of desired grapevine seedlings. Since no resistances have yet been described for BBR, grapevines shall be selected for developing fruits with physical-mechanical barriers reducing the risk for BBR infection like loose grape bunch architecture and thick, impermeable berry cuticle.

In the present study first results regarding the investigation of the relationship between physical-mechanical fruit traits (bunch architecture, berry impedance and berry texture), meteorological data and the degree of BBR infection are shown. Varieties and elite breeding lines were phenotyped using high-throughput, objective sensors in 2021 and 2022, two years with contrasting growing conditions (Siebeldingen, Germany). In comparison to 2021, 2022 was characterized by a higher temperature sum *D* (+196°days between veraison and harvest) and huge differences in the precipitation sum (PS; -62 mm up to + 105 mm). In order to categorize BBR resistance/susceptibility, berries from different genotypes showing high variability in their berry characteristics were sampled at maturity and were tested under controlled lab conditions for BBR susceptibility. For some varieties, it could be shown that meteorological conditions affect both, berry traits as well as infection with BBR. In addition to the environment and the training system, physical-mechanical berry traits and the mean berry diameter could be confirmed as promising phenotypic traits for the prediction of BBR resistance. In summary, the consideration of sensor-based physical-mechanical berry traits enables an improved risk prediction for BBR, which is of outstanding importance for the evaluation of breeding material and new varieties growing under different environmental conditions, as well as for phenotyping of mapping populations for QTL analyses and the development of molecular markers. As meteorological conditions were contrasting in 2021 and 2022 and varieties with high phenotypic variability were considered, additional years of investigations are recommended in order to verify the reliability of the detected relationships.

Keywords

grapevine, *Vitis vinifera* ssp. *vinifera*, high-throughput phenotyping, grey mold, meteorological conditions, 3D grape bunch architecture, phenology, SMPH, veraison, PIWI varieties, training system

Introduction

In modern grapevine breeding, marker-assisted selection (MAS) is one of the most effective tools in order to select seedlings shortly after germination regarding powdery and downy mildew resistance loci (Töpfer and Trapp, 2022). Mapping approaches, the development of molecular markers and their application in MAS are highly effective for the mildew resistance traits. In contrast, resistance to *Botrytis* bunch rot (BBR) is a more complex and a typical quantitative trait, mainly affected by physical-mechanical properties of the berry skin as well as the grape bunch architecture (*i.e*. grape bunch compactness) as shown in Fig. 1 (Deytieux-Belleau *et al.*, 2009; Gabler *et al.*, 2003; Herzog *et al.*, 2021; Tello and Ibáñez, 2018; Vail *et al.*, 1998). These traits are composed of several sub-traits, for instance the number of berries and the berry size (Rist *et al.*, 2018), the pedicel length (Richter *et al.*, 2019), the berry skin characteristics and berry cuticle (Herzog *et al.*, 2021). In order to increase the throughput and effectiveness of phenotyping saving time and effort by increasing precision and objectivity of resulting evaluation data, sensor-based methods are the most efficient tools. Regarding the stated physical traits, different methods have been established. The grape berry impedance Z_{REL} for instance was

Submitted/accepted for publication: May 11, 2023/June 26, 2023

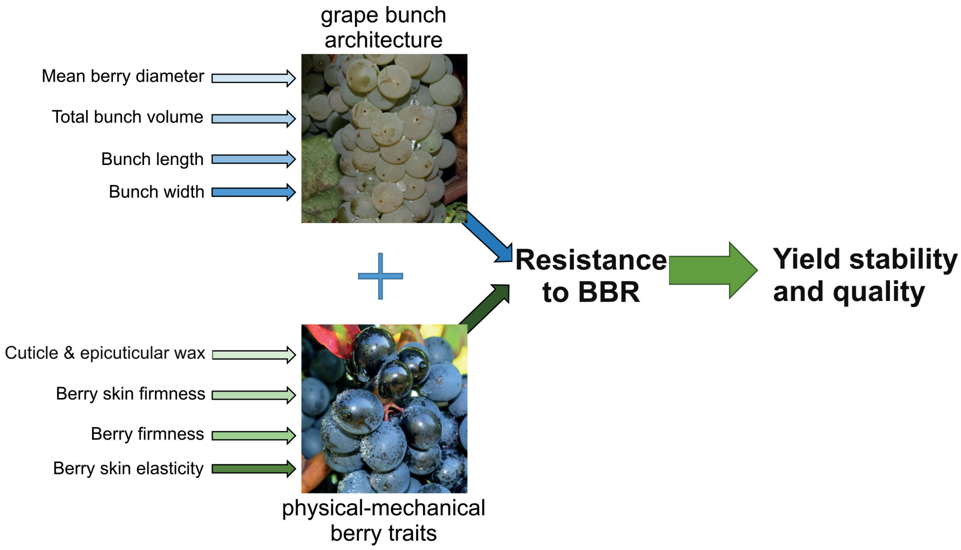

Fig. 1: The grape bunch architecture and physical-mechanical berry traits are known as the most effective grapevine traits in order to reduce the risk for *Botrytis* bunch rot in breeding material. The resistance of grapevine varieties to *Botrytis* bunch rot (BBR) is a prerequisite for yield stability and quality independent of the environment.

shown to describe the behavior of the berry cuticle, *i.e.* the cuticle and epicuticular wax thickness and permeability (Herzog *et al.*, 2015). The measurement can be easily conducted using a specific device named BI-sensor. The grape berry texture measured by a commercial available TX.TA sensor system precisely describes berry skin characteristics like the berry skin firmness (TA_{FORCE}), whole berry firmness (TA_{AREA}) and the berry skin elasticity (TA_{GRAD}) (Carreño *et al.*, 2015). For the grape bunch architecture as the most common trait regarding BBR selection, several sensor-based phenotyping tools have been published (Cubero *et al.*, 2015; Kicherer *et al.*, 2015; Palacios *et al.*, 2019; Rist *et al.*, 2018; Underhill *et al.*, 2020). Hereby the automated, 3D-based pipeline was selected for the present study as one of the most time-efficient tools (Rist *et al.*, 2018; Rist *et al.*, 2022). To determine the importance of each trait for natural field infection with BBR, Herzog *et al.* (2021) used the beforementioned sensor-based physical-mechanical phenotypes to predict BBR. Hereby, the mean berry diameter (MBD), Z_{REL} and berry texture (TA) have shown a very high prediction accuracy and thus, were identified as important physical-mechanical traits influencing BBR resistance (Herzog *et al.*, 2021). So far, meteorological data or different management practices were not considered in the given studies.

It is known that the berry cuticle, epicuticular waxes and the berry texture are affected by heat, sun exposure and water deficit (Dimopoulos *et al.*, 2020; Porro *et al.*, 2010) and that simultaneously, warm temperatures and high air humidity increase the risk for BBR (Broome, 1995; Molitor *et al.*, 2020). In addition to these natural factors, management practices like defoliation and training systems have another lasting influence on grape bunch architecture and thus occurrence of BBR (Gubler, 1987; Mundy *et al.*, 2022; Poni *et al.*, 2006; Würz *et al.*, 2020). Regarding the training systems, semi-minimal-pruned-hedge (SMPH) is described as one system with a huge potential to reduce the risk for BBR due to lower grape bunch compactness (Kraus *et al.*, 2018; Molitor *et al.*, 2019; Schäfer *et al.*, 2021). Effects of meteorological conditions and vineyard management on the physical-mechanical berry properties have rarely been investigated for sensor-based traits like Z_{REL} and berry texture (Kraus *et al.*, 2018; Porro *et al.*, 2010).

In addition, the present study investigates the susceptibility of genotypes towards BBR using a controlled laboratory infection test in order to predict the risk for BBR with standardized data and independent from the natural infection.

The present study aims at three major aspects:

(1) Investigation of the relationship between meteorological conditions, physical-mechanical berry traits and the degree of BBR infection;

(2) Preliminary evaluation the extension of the BBR prediction based on a standardized laboratory test for BBR considering both, berry traits and meteorological data;

(3) Principal component analysis (PCA) and phenotypic expression of different berry traits of BBR resistance in comparison to BBR susceptible genotypes.

Material and Methods

Plant material

Plant material was used from the experimental vineyards of JKI Geilweilerhof located in Siebeldingen, Germany (49°13'07.0"N 8°02'45.0"E). In total 46 grapevine genotypes (37 varieties, 7 elite breeding lines and 2 genebank accessions) were used for phenotyping in the years 2021 and 2022 (Table 1). All plants were grafted on the rootstock SO4 (Selection Oppenheim 4 (VIVC11473)) and trained as Vertical-Shoot-Positioned (VSP) trellis without artificial irrigation as commonly applied in German wine regions. As indicated below, five varieties trained in both, Semi-Minimal-Pruned-Hedge (SMPH) and VSP system. All grapevines were planted with an inter-row distance of 2.0 m and grapevine-spacing of 1.0 m.

Sensor-based phenotyping

For sensor-based phenotyping, berries were sampled twice: in the mid-ripening stage (t_1) and at harvest (t_2). t_1 hereby was the time point when 5-10 randomly measured berries had shown a sugar content of approx. 16°Brix in the field.

Table 1: Plant material that was investigated for the present study. The unique identifier includes metadata about the *VIVC* (*Vitis* International Variety Catalogue) number as well as the year of planting. In addition, the years of phenotyping are indicated.

Grapevine variety/genotype	Unique identifier	Year of planting	Year of phenotyping
ALLEGRO[1]	DEU098_VIVC19997_DEU098-2020-036	2020	2022
AROMERA[1]	DEU098_VIVC16305_DEU098-2018-025	2018	2022
BACCHUS[3]	DEU098_VIVC851_DEU098-2008-043	2008	2021/2022
BARON[1]	DEU098_VIVC20010_DEU098-2018-027	2018	2022
Gf.2000-305-0081[BL]	-	2017	2021/2022
Gf.2001-041-0003[BL]	-	2016	2021/2022
Gf.2001-041-0004[BL]	-	2016	2021/2022
Gf.2004-043-0010[BL]	-	2016	2021/2022
Gf.2004-043-0021[BL]	-	2016	2021/2022
Gf.2004-043-0034[BL]	-	2016	2021/2022
Gf.2010-011-0048[BL]	-	2016	2021/2022
BRONNER[1]	DEU098_VIVC17116_DEU098-2018-029	2018	2022
CABERNET BLANC[1]	DEU098_VIVC22355_DEU098-2018-030	2018	2022
CABERNET CANTOR[1]	DEU098_VIVC20008_DEU098-2020-034	2020	2022
CABERNET CAROL[1]	DEU098_VIVC20006_DEU098-2018-032	2018	2022
CABERNET SAUVIGNON[3]	DEU098_VIVC1929_DEU098-2000-050	2000	2021/2022
CABERTIN[1]	DEU098_VIVC22329_DEU098-2018-030	2018	2022
CALARDIS BLANC[1]	DEU098_VIVC22828_DEU098-2010-083	2010	2021/2022
CALARDIS BLANC[1]	DEU098_VIVC22828_DEU098-2018-036	2018	2022
CALARDIS MUSQUE[1]	DEU098_VIVC4549_DEU098-2010-068	2010	2021/2022
CALARDIS MUSQUE[1]	DEU098_VIVC4549_DEU098-2018-038	2018	2022
CHARDONNAY[3]	DEU098_VIVC2455_DEU098-2008-050	2008	2021/2022
DAKAPO[3]	DEU098_VIVC14728_DEU098-2011-046	2011	2021/2022
DORNFELDER[3]	DEU098_VIVC3659_DEU098-2008-057	2008	2021/2022
FELICIA[1]	DEU098_VIVC3547_DEU098-2018-039	2018	2022
GF.GA-52-42[2]	DEU098-2009-033	2009	2022
JOHANNITER[1]	DEU098_VIVC17127_DEU098-2018-042	2018	2022
MERZLING[1]	DEU098_VIVC4251_DEU098-2018-043	2018	2022
MORIO MUSKAT[3]	DEU098_VIVC7996_DEU098-2008-066	2008	2022
MUSCARIS[1]	DEU098_VIVC22628_DEU098-2018-045	2018	2022
NORTON[3]	DEU098_VIVC3304_DEU098-1990-358	1990	2021/2022
OPTIMA[3]	DEU098_VIVC8791_DEU098-2008-071	2008	2021/2022
ORION[1]	DEU098_VIVC8802_DEU098-2018-046	2018	2022
PHOENIX[1]	DEU098_VIVC9224_DEU098-2018-047	2018	2022
PINOT BLANC[3]	DEU098_VIVC9272_DEU098-2008-072	2008	2021/2022
PINOT NOIR[3]	DEU098_VIVC9279_DEU098-2008-075	2008	2021/2022
PINOTIN[1]	DEU098_VIVC19994_DEU098-2018-048	2018	2022
PRINZIPAL[1]	DEU098_VIVC17124_DEU098-2018-049	2018	2022
PRIOR[1]	DEU098_VIVC19993_DEU098-2020-035	2020	2022
REGENT[1]	DEU098_VIVC4572_DEU098-2008-078	2008	2021/2022
REGENT[1]	DEU098_VIVC4572_DEU098-2018-052	2018	2022
RIESLING[3]	DEU098_VIVC10077_DEU098-2008-085	2008	2021/2022
SAUVIGNAC[1]	DEU098_VIVC22322_DEU098-2019-045	2019	2022
SAUVIGNON BLANC[3]	DEU098_VIVC10790_DEU098-2000-084	2000	2021/2022
SAUVITAGE[1]	DEU098_VIVC24398_DEU098-2018-060	2018	2022
SEIBEL 7511[2]	DEU098_VIVC11249_DEU098-2003-132	2003	2021/2022
SIRIUS[1]	DEU098_VIVC11833_DEU098-2018-055	2018	2022
SOLARIS[1]	DEU098_VIVC20340_DEU098-2018-056	2018	2022
VILLARIS[1]	DEU098_VIVC20347_DEU098-2018-059	2018	2022

Table 1: Continued.

Grapevine variety/genotype	Unique identifier	Year of planting	Year of phenotyping
Comparison of SMPH and VSP			
CHARDONNAY[3]	DEU098_VIVC2455_DEU098-2008-050	2008	2020
FELICIA[1]	DEU098_VIVC3547_ DEU098-2001-123	2001	2020
REGENT[1]	DEU098_VIVC4572_DEU098-2002-024 DEU098_VIVC4572_DEU098-2003-223	2002 2003	2020 2020
RIESLING[3]	DEU098_VIVC10077_DEU098-2008-085	2008	2020
VILLARIS[1]	DEU098_VIVC20347_DEU098-2001-126	2001	2020

[1] PIWI variety
[2] Genetic Repository
[3] traditional grapevine variety
[BL] Elite Breeding Line

At t_1, 20 undamaged berries with intact pedicels were sampled in the field per plant with 3-6 plants per genotype in order to measure the berry impedance and berry texture profile (TA_{FORCE}, TA_{AREA} and TA_{GRAD}) as described by Herzog *et al.* (2021). Relative berry impedance Z_{REL} was calculated regarding the method of Herzog *et al.* (2015). After berry phenotyping, Fourier-Transform-Infrared-Spectroscopy (FTIR) was conducted in order to determine grape must sugar content, pH, tartaric acid and malic acid.

At t_2, three bunches per plant and genotype were sampled in the field (identical plants as at t_1). Grape bunches were carefully transferred to the lab, where 360° scans (dense 3D point clouds) were acquired using a 3D Scanner (Artec® Spider, Artec® 3D, L-1748 Senningerberg, Luxemburg) as described by Rist *et al.* (2018; 2022). Afterwards, point cloud analysis was performed with the '3D Bunch Tool' in order to extract objective phenotypic characteristics of the Mean Berry Diameter (MDB), Total Bunch Volume (TBV), Bunch Width (BW) and Bunch Length (BL) as explained by Rist *et al.* (2018). Based on these traits and the results of Rist *et al.* (2018), the Bunch Density Factor (BDF) was calculated (the higher the value, the higher the grape bunch density) using the following formula:

$$BDF = \frac{TBV}{(BW * BL)}$$

Following the 3D scan, 15 undamaged berries with intact pedicels per grape bunch and genotype were sampled for the BBR infection test. The remaining berries were used for FTIR analysis.

Botrytis bunch rot under controlled lab conditions

For each genotype, 3x 15 berries per genotype (three replicates) were tested regarding BBR susceptibility under controlled lab conditions at t_2 (average must sugar was 21.6°Brix). Therefore, berries of each genotype were distributed on three black grid plates (one plate providing space for 80 berries, *i.e.* 16 genotypes, 5 berries each). Berries were inoculated with on average $1.9*10^4$ *Botrytis* spores ml^{-1} using a spray flask. The plates were placed in boxes sealed with aluminium foil. Wet tissue paper on the bottom of each closed box ensured high air humidity for the BBR infection. As positive control, the first berry of each row was injured with a razor blade cut. BBR incubation condition was set at 23°C in the dark for seven days. Seven days post inoculation (7 dpi) each berry was scored visually regarding the degree of infection (class I – no infection, class III – spots of discoloration without injury or burst of the berry skin, class V – spots of growing mycelia, class 7 – half of the berry with sporulating mycelium, class 9 – whole berry infested with sporulating mycelium).

Metadata and statistics

As metadata for the growing conditions, the date of veraison, *i.e.* the beginning of berry ripening was recorded, temperature sum *D* and precipitation sum were calculated. Therefore, veraison was scored regarding the BBCH scale (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) for each investigated plant (Lorenz *et al.*, 1995). In order to capture environmental data for each year, temperature and precipitation sums were calculated using data recorded by the local weather station (www.dlr.rlp.de) in the individual period between veraison and t_1 as well as t_2. As the precipitation sum is the sum of the daily rainfall within these periods, *D* was calculated as sum of heat unit accumulation (*D*) in degree-days (°d) using the following formulas by Nendel (2010):

$$D = \begin{cases} 0 & \text{for } T_0 \geq T_{max} \\ \left(\frac{Tmax - T0}{2}\right)\left(\frac{Tmax - T0}{Tmax - Tmin}\right) & \text{for } T_{min} < T_0 < T_{max} \\ Tm - T0 & \text{for } T_0 \leq T_{min} \end{cases}$$

where T_{max}, T_m and T_{min} are the daily maximum, mean and minimum temperature 2 m above the ground. As threshold temperature (T_0) for BBR $T_{0\,=}$ 12 °C was assumed based on the study of Broome (1995).

All of the statistical data analyses were conducted using the open source software R, version 3.6.3 (R Core Team 2017) and RStudio (version 1.2.5019). Pearson correlations were calculated using the 'Hmisc' package and rcorr function (Harrell Jr., 2021). For BBR prediction based on BBR lab data, the package 'nnet' was used to calculate multinominal models (Ripley, 2021). Finally, models were tested using the package 'DescTool' and the Pseudo R^2 McFadden ($R^2_{McFadden}$) was calculated (Signorell, 2021). Furthermore, phenotypic as well as meteorological data were used to perform a principal component analysis (PCA) for which the 'FactoMineR' package was implemented (Lê *et al.*, 2008). PCA was conducted for one data set (2021 and 2022) because of the small sample size.

Results

The present study investigates potential relations between the meteorological conditions during berry development, the degree of BBR infection (lab test) as well as the physical-mechanical berry traits Z_{REL}, berry texture and grape bunch architecture traits.

First, the differences between 2022 and 2021 of all investigated traits were calculated (Supplementary Table S1). Therefore, genotypes with two-year phenotyping were analyzed, *i.e.* 19 genotypes including four elite breeding lines. Both years are characterized by very contrary conditions, cold and rainy (2021) as well as outstanding hot and dry (2022), which is clearly visible e.g. for the temperature sum *D* in Supplementary Table S1. The dates of veraison (t_0), t_1 and t_2 were on average 15, 19 and 12 days earlier in 2022. At t_1, the overall temperature sum *D* was 113° days higher and the mean precipitation sum 40 mm lower in 2022 compared to 2021. The different environmental growing conditions in 2022 led to notably increased mean Z_{REL} (>200) and slightly increased TA_{FORCE} and TA_{AREA} as well as decreased TA_{GRAD}. At harvest maturity, the mean temperature sum *D* was approx. 200 °days higher in 2022 but the precipitation sum between veraison (t_0) and harvest (t_2) was roughly equal. Interestingly, the phase of maturity of some varieties was characterized with a decreased amount of rainfall for early varieties (like 'Bacchus' with -36 mm or 'Regent' with – 62 mm) and an increased amount of rain for late varieties like 'Riesling' (+64 mm) or 'Cabernet Sauvignon' (+ 105 mm). Concurrently, the BBR laboratory test resulted in huge differences of berry infection. As visible in Supplementary Table S1, 'Calardis Musqué' showed a clearly reduced BBR infection in 2022 (with reduced amount of precipitation during ripening) while 'Riesling' ripened with increased rainfall around harvest in 2022 and showed a high BBR infection. However, the mean berry diameter (MBD) or the bunch density factor were only slightly increased in 2022 and it seems that meteorological conditions might have an effect to some varieties and their susceptibility to BBR (laboratory test). Varieties as 'Riesling' and 'Sauvignon Blanc' for instance showed very high BBR infection in 2022 compared to 2021 (all variety-specific phenotypic data are given in Supplementary Table S2). 'Riesling' berries ripened with higher precipitation in 2022, which favors BBR infection. 'Sauvignon Blanc' berries ripened with lower amount of rain in 2022 compared to 2021 resulting in 7 °Brix higher sugar content in the grape must. In comparison to BBR resistant varieties like 'Calardis Blanc' [Z_{REL} (2021) = 844 and Z_{REL} (2022) = 1,072], Z_{REL} of both susceptible varieties is relatively low with Z_{REL} (2021) = 618 ('Riesling') and Z_{REL} (2021) = 518 ('Sauvignon Blanc') as well as Z_{REL} (2022) = 838 ('Riesling') and Z_{REL} (2022) = 857 ('Sauvignon Blanc').

Second, Pearson correlations of all traits and environmental growing conditions were investigated as shown in Fig. 2. BBR

(a)

		date of Veraison t_0 [DOY]	date of t_1 [DOY]	*D* [°d] t_1	t_0- PS [mm] t_0-t_1	Z_{REL}	TA_{FORCE} [N]	TA_{AREA} [N*sec]	TA_{GRAD} [N/sec]	Lab BBR*
				t_1						t_2
	Veraison t_0		0.00	0.00	0.00	0.02	n.s.	n.s.	0.01	0.03
	date of t_1	0.87		0.00	0.00	0.00	n.s.	n.s.	0.01	n.s.
t_1	*D* [°d] t_0-t_1	-0.81	-0.57		0.00	0.00	n.s.	n.s.	0.01	n.s.
t_1	PS [mm] t_0-t_1	0.56	0.79	-0.54		0.00	n.s.	0.01	0.05	n.s.
t_1	Z_{REL}	-0.29	-0.46	0.34	-0.65		n.s.	n.s.	n.s.	n.s.
t_1	TA_{FORCE} [N]	0.04	0	0.02	-0.14	0.09		0.00	0.00	n.s.
t_1	TA_{AREA} [N*sec]	-0.15	-0.19	0.22	-0.3	0.1	0.78		n.s.	n.s.
t_1	TA_{GRAD} [N/sec]	0.31	0.29	-0.32	0.23	-0.08	0.52	-0.1		0.01
t_2	t_2 Lab BBR*	-0.28	-0.11	0.25	0.06	-0.09	-0.16	0.08	-0.32	

(b)

		date of Veraison t_0 [DOY]	date of t_1 [DOY]	date of harvest t_2 [DOY]	*D* [°d] t_0-t_2	PS [mm] t_0-t_2	°Brix	MBD [mm]	BDF	Lab BBR*
					t_2					t_2
	Veraison t_0		0.00	0.00	0.00	n.s.	0.00	n.s.	n.s.	0.01
	date of t_1	0.87		0.00	0.00	n.s.	0.00	n.s.	n.s.	n.s.
	harvest t_2	0.63	0.72		0.00	0.00	0.04	0.05	n.s.	n.s.
t_2	*D* [°d] t_0-t_2	-0.91	-0.82	-0.48		n.s.	0.00	n.s.	n.s.	0.01
t_2	PS [mm] t_0-t_2	0.07	0.13	0.71	0.07		n.s.	n.s.	n.s.	0.04
t_2	°Brix	-0.36	-0.49	-0.25	0.35	-0.02		0.03	0.00	0.05
t_2	MBD [mm]	-0.11	-0.04	-0.24	0.03	-0.23	-0.26		0.00	n.s.
t_2	BDF	-0.04	-0.02	-0.15	0.05	-0.09	-0.45	0.52		n.s.
t_2	t_2 Lab BBR*	-0.28	-0.11	-0.03	0.32	0.27	0.25	0.03	0.08	

r -1 0 1

p n.s. 0.01-0.05 0-0.01

Fig. 2: Correlation matrix showing first relationships between phenological, meteorological data and berry traits as well as BBR infection at t_1 (a) and t_2 (b). Pearson correlation was applied, except for BBR infection (*): Spearman correlation rho and corresponding p values are shown. Below the diagonal = values of correlation coefficient; above the diagonal = *p* values. Z_{REL} – relative berry impedance; TA – berry texture analysis; TA_{Force} – berry skin firmness; TA_{Area} – whole berry firmness; TA_{Grad} – berry skin elasticity; *D* – Temperature sum; PS – precipitation sum; MBD – mean berry diameter; BDF – bunch density factor; Lab BBR – laboratory test *Botrytis* bunch rot.

infection in the laboratory (Spearman correlation) of the investigated genotypes is hereby significantly correlated with the date of veraison, temperature sum *D* (t_0-t_2), precipitation sum (t_0-t_2), TA_{GRAD} (berry skin elasticity) and berry maturity (°Brix). As expected, the environmental data like precipitation sums are significantly correlated with the date of veraison, t_1 and t_2. As assumed from the yearly differences (Supplementary Table S1), berry texture TA_{FORCE} and TA_{AREA} are slightly correlated with *D* or precipitation sum during berry ripening. Finally, Z_{REL} is significantly correlated with temperature and precipitation sum. Hereby, the precipitation sum showed the strongest impact on berry impedance with r = -0.65.

The results of the controlled BBR infection test were used as target variable for BBR prediction. As the meteorological data are important risk factors for BBR in the field, the date of t_2 (DOY), temperature sum $D_{t0\text{-}t2}$ as well as precipitation sum $PS_{t0\text{-}t2}$ were used for prediction resulting in $R^2_{McFadden}$ = 0.36. The additional involvement of grape must sugar content (°$Brix_{t2}$) and BDF raised the prediction accuracy to $R^2_{McFadden}$ = 0.68. The prediction using Z_{REL}, TA_{FORCE}, TA_{AREA} and TA_{GRAD} as stated from Herzog *et al.* (2021) reveals $R^2_{McFadden}$ = 0.53. The exchange of TA_{AREA} with the bunch density factor BDF raised $R^2_{McFadden}$ to 0.80 and with the mean berry diameter (MBD) up to $R^2_{McFadden}$ = 0.86. Based on that result, the phenotypic variation within both BBR categories, BBR resistant and BBR susceptible were analyzed (Fig. 3). The underlying data for this analysis can be found in the supplements (Supplementary Table S2).

As visible in Fig. 3, most of the traits differed not significantly in the investigated plant material. But with regard to e.g. molecular marker development, it is essential to determine the importance of the individual traits. Finally, the phenotypic variation between genotypes showing BBR susceptibility or BBR resistance using the standardized test system was investigated using a principal component analysis (Supplementary Fig. S1). Thereby, dimension 1 (Dim1) that represents the first principal component (PC1) explained 27.7 % of the variation and Dim2 that expresses PC2 explained 18.3 %. However, no specific pattern could be detected in the analyzed data set since there is a strong overlap between BBR susceptible and BBR resistant grapevine varieties.

Finally, the impact of the training system on grape and berry traits was investigated (Fig. 4). Except the berry texture, all investigated traits were significantly affected by the training system. In summary, the SMPH system seems to be suitable to reduce the compactness of grape bunches, to reduce the berry size and finally to increase Z_{REL} and thus, the training system could be implemented in the future as another prediction variable into the model.

Discussion

Compared to 2021, the year 2022 was characterized by a higher temperature sum *D* and a lower amount of rainfall between veraison (t_0) and mid ripening stage (t_1), the measurement time point of relative berry impedance (Z_{REL}) and berry texture (TA). The increased mean Z_{REL} indicates that the year and different meteorological conditions had an effect on that trait within the considered plant material. Since Z_{REL} is described as indicator for the berry cuticle thickness and permeability (Herzog *et al.*, 2015), the increased Z_{REL} indicates that berries developed thicker cuticles and thicker epicuticular waxes due to the persistent heat stress. This observation could be traced to the role of the cuticle as transpiration border and the increase of total berry waxes and total amount of triterpenoids as abiotic stress reaction of grapevines (Van der Weide *et al.*, 2022). In addition, the water deficit due to absent precipitation in 2022 might have led to an increased amount of total cuticular waxes, which was described by Dimopoulos *et al.* (2020) as abiotic stress response in grapevines that results in a decreased berry transpiration, too. In 2022, some elite breeding lines showed Z_{REL} >400 compared to 2021 while other varieties have shown only slight differences like 'Pinot Noir' with Z_{REL} >7 or 'Dornfelder' with Z_{REL} >84. In general, higher Z_{REL} values indicate a higher resistance towards BBR. In summary, Z_{REL} might also be suitable as indicator for abiotic stress response. In contrast, it is also described that rainfall affects the leaf epicuticular wax in other fruit crops (Medeiros *et al.*, 2017) resulting in an increased risk for micro cracking in grape berries (Becker and Knoche, 2012) but also, e.g. of apples (Khanal *et al.*, 2021). Concurrently, TA_{FORCE} and TA_{AREA}, *i.e*. the berry skin and whole berry firmness, were only slightly increased and TA_{GRAD}, the berry skin elasticity, showed a small decrease. Porro *et al.* (2010) also described this observation in table grapes with water deficit. Surprisingly, the mean berry size (MBD) and the bunch density factor (BDF) were only affected in some varieties by the hot and dry growing conditions in 2022 after a cold and rainy 2021. For both traits, it is known that irrigation or rainfalls before harvest result in an increased berry size and higher yield (Intrigliolo and Castel, 2008). Concurrently, grapevines show a delayed response to abiotic stress, *i.e*. the berry size and yield in one season are affected not only by the ongoing seasonal conditions but also by the weather conditions of the previous season, when flower buds are initiated (Mosedale *et al.*, 2016; Watt *et al.*, 2008). Because of the diversity of the investigated varieties, valid evaluation of varieties need phenotyping of several years. MBD and Z_{REL} are negatively correlated traits (Fig. 2 and Herzog *et al.*, 2021). An increased Z_{REL} and only a small change of the berry size underline the probability that an increased amount of epicuticular waxes in some varieties like 'Pinot Blanc' or 'Riesling' might be responsible for higher Z_{REL} values. However, in the present study several berry traits showed a small correlation coefficient to each other as well as to meteorological conditions and BBR infection (mostly not significant). Regarding to studies about susceptible varieties that showed the influence of weather conditions on berry skin properties (using analytical methods) and of course, the increasing risk for BBR during berry ripening (Deytieux-Belleau *et al.*, 2009; Gabler *et al.*, 2003). Further data recording and might an extension of berry cuticle and berry texture phenotyping to the time of berry maturity (t2) will provide important information to understand the investigated relationship. As several of the investigated traits are correlated but phenotypic differences between BBR resistant and BBR susceptible samples (Fig. 3) are not significant, PCA was performed in order to reveal phenotypic variation between both. Hereby, BBR-related sensor-based berry traits as well as meteorological data of both years were involved. Based on that

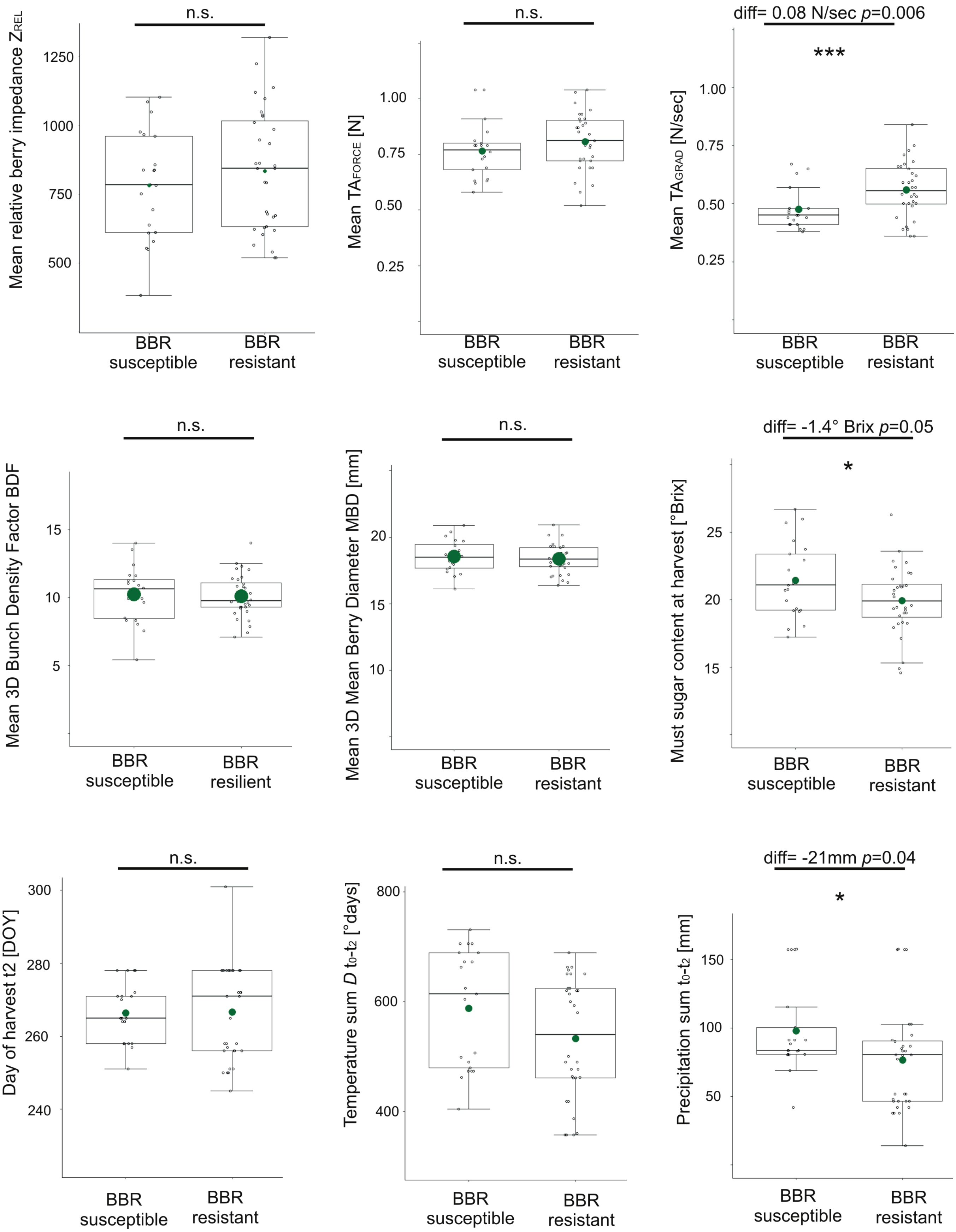

Fig. 3 Boxplot of identified BBR-relevant physical-mechanical berry traits and meteorological data dependent on phenology. N=53 variety samples from two years. Differences were analyzed applying a Tukey-test. BBR – *Botrytis* bunch rot; Z_{REL} – berry impedance; TA – berry texture analysis; TA_{FORCE} – berry skin firmness; TA_{GRAD} – berry skin elasticity; diff – difference.

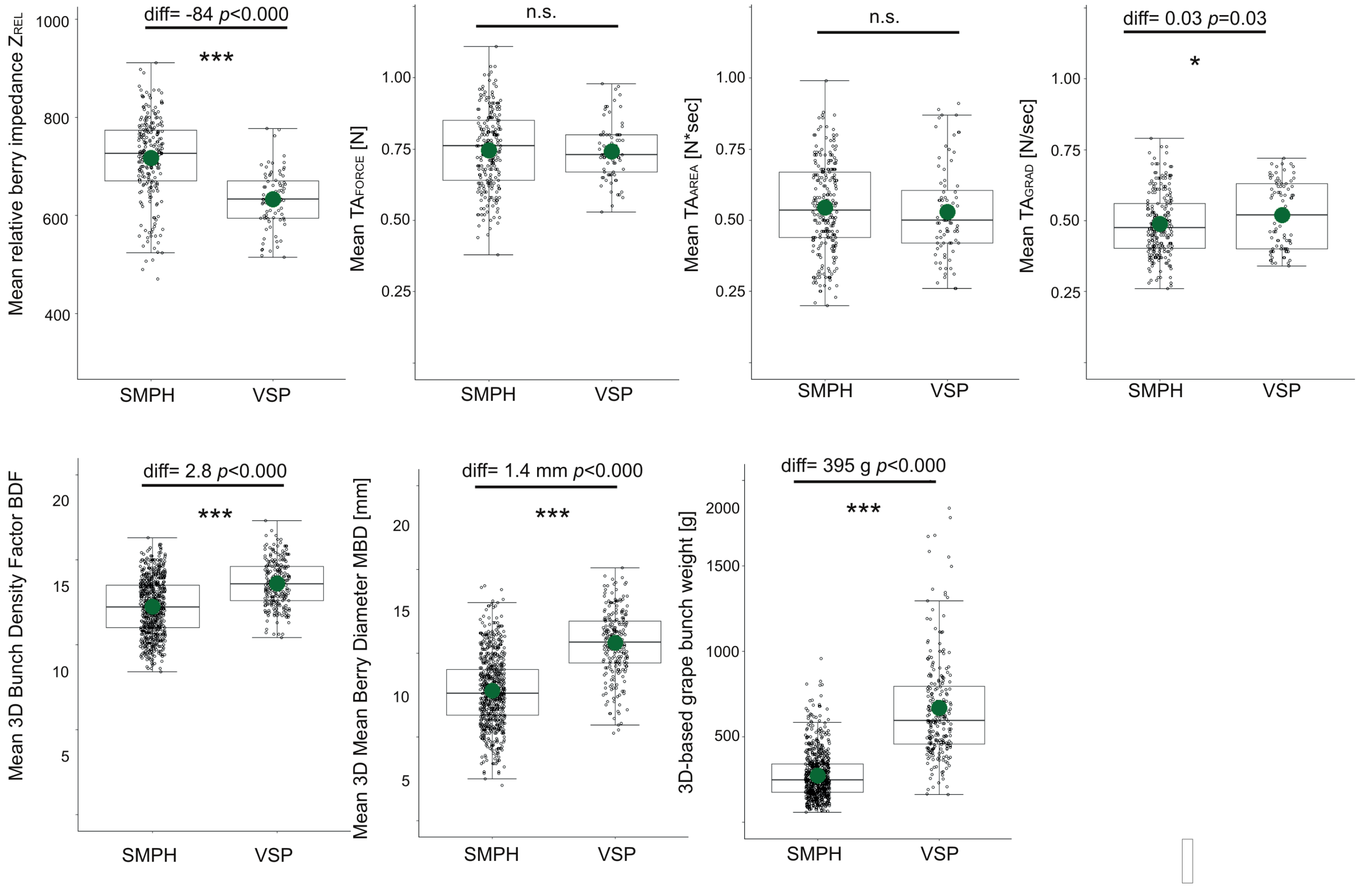

Fig. 4 The training system of SMPH affect grape bunch characteristics and berry impedance Z_{REL} and showed slightly effects on grape berry texture. N=310 plants from 5 varieties per training system, *i.e.* 6,200 berry samples per berry trait and training system and 930 bunches per training system. Differences were analyzed applying a Tukey-test. SMPH – semi-minimal-pruned-hedges, VSP – vertical-shoot-positioned trellis system, TA – berry texture analysis; TA_{Force} – berry skin firmness; TA_{Area} – whole berry firmness; TA_{Grad} – berry skin elasticity; MBD – mean berry diameter; BDF – bunch density factor; diff – difference.

data set, no clear separation between BBR susceptible and resistant grapevine varieties was possible including all the parameters. As the BBR prediction revealed high Pseudo $R^2_{McFadden}$ and as the phenotypic variation is high in both, 2021 and 2022, we assume that the sample size has to be increased (including more individuals and experimental years) in order to distinguish the variation of the phenotypic data. However, Deytieux-Belleau *et al.* (2009) as well as Gabler *et al.* (2003) evaluated different berry skin traits and cuticle features with classical microscopic or analytical methods in order to study their influence on grape bunch resistance towards BBR. Some of these traits are recordable by sensors used in our work and can thus be easily phenotyped in breeding material. Based on that, breeding material should be phenotyped regarding grape bunch architecture (MBD or BDF), berry impedance (Z_{REL}) and berry texture (TA_{FORCE}, TA_{AREA} and TA_{GRAD}) in order to evaluate potential BBR resistance and genotyping markers should be developed for such traits (Herzog *et al.*, 2021). In addition, phenological and meteorological data also need to be monitored as they can influence the risk of BBR infection. In addition, monitoring of airborne spores can support the assessment of the overall risk for BBR infection in vineyards (González-Fernández *et al.*, 2020). Another strategy in order to increase BBR resistance, grapevines can be trained in the SMPH system. This system has an effect on several physical-mechanical traits increasing the general resistance to BBR of a variety: (1) it increases Z_{REL} (Herzog *et al.*, 2015; Herzog *et al.*, 2021) and TA_{GRAD} (Herzog *et al.*, 2021), (2) it decreases the berry size and the grape bunch density (Molitor *et al.*, 2019; Schäfer *et al.*, 2021). However, the total amount of samples should be further increased in order to improve the prediction accuracy and to apply tools like artificial intelligence for trait prediction or to implement such data into grapevine simulation as developed for 'Riesling' (Bahr *et al.*, 2021; Schmidt *et al.*, 2019).

Conclusion

The aim of the present study was to involve two-year meteorological data from one field plot for BBR prediction based on physical-mechanical berry traits assessed by different sensors. The data indicate that especially berry impedance (Z_{REL}) and BBR infection in the lab are possibly affected by the environmental growing conditions of investigated plant material from veraison to harvest. Surprisingly, the environment did not affect the berry texture as well as mean berry diameter (MBD) in all investigated varieties as expected based on other studies. Additionally, berry texture was not influenced by the training system and seems to be more independent from the environment as for instance Z_{REL}. However, sensor-based berry traits could be shown as suitable variables in an extended variety assembly in order to predict variety-specific BBR risk. For future analysis, phenology and corresponding meteorological conditions should be monitored even when artificial BBR tests are planned using field grown berries. Considering sensor-based physical-mechanical berry traits enable an improved risk prediction for BBR, which is outstandingly important for the evaluation of breeding material and new varieties growing in different environments. In addition, the sample size should be increased with additional years and environmental conditions to improve prediction accuracy, to validate transferability of the prediction to other field plots, other vine growing regions and vineyard management or to implement this phenotypic data in grapevine simulations. Beyond that, the effect of canopy properties like leaf area, photosynthetic activity, nutrient availability or soil characteristics are not considered in the study, but represent important parameters, which should be included into the evaluation of breeding material and the degree of infection with BBR.

Conflicts of interest

The authors declare that they do not have any conflicts of interest.

Acknowledgement

The authors thank Moritz Cappel, Ulrike Braun, Claudia Vogel and Teresa Claus for their excellent technical support during FTIR analysis. Further, we would like to thank Patrick Römer, Madelaine Stoll and Hannes Engler for grape sampling and the conduction of the 3D grape scan, and Shuta Kurokawa and Muhammad Idrees Khan supporting the measurement of berry texture and berry impedance. We also gratefully acknowledge Nagarjun Malagol, providing his expertise in statistical anlysis using R. Finally, we gratefully acknowledge the German Federal Ministry of Education and Research [Bundesministerium für Bildung und Forschung (BMBF), Bonn, Germany (NoViSys: FKZ 031A349E)], for the funding of the SMPH experiments.

References

Bahr, C., Schmidt, D., Friedel, M., Kahlen, K., 2021: Leaf removal effects on light absorption in virtual Riesling canopies (*Vitis vinifera*). *in silico* Plants 3 (2), diab027, DOI: 10.1093/insilicoplants/diab027.

Becker, T., Knoche, M., 2012: Water induces microcracks in the grape berry cuticle. Vitis 51 (3), 141-142, DOI: 10.5073/VITIS.2012.51.141-142.

Broome, J., 1995: Development of an infection model for Botrytis bunch rot of grapes based on wetness duration and temperature. Phytopathology 85 (1), 97, DOI: 10.1094/phyto-85-97.

Carreño, I., Cabezas, J.A., Martínez-Mora, C., Arroyo-García, R., Cenis, J.L., Martínez-Zapater, J.M., Carreño, J., Ruiz-García, L., 2015: Quantitative genetic analysis of berry firmness in table grape (*Vitis vinifera* L.). Tree Genetics & Genomes 11 (1), 1-10, DOI: 10.1007/s11295-014-0818-x.

Cubero, S., Diago, M.P., Blasco, J., Tardaguila, J., Prats-Montalbán, J.M., Ibáñez, J., Tello, J., Aleixos, N., 2015: A new method for assessment of bunch compactness using automated image analysis. Australian Journal of Grape and Wine Research 21 (1), 101-109, DOI: 10.1111/ajgw.12118.

Deytieux-Belleau, C., Geny, L., Roudet, J., Mayet, V., Donèche, B., Fermaud, M., 2009: Grape berry skin features related to ontogenic resistance to Botrytis cinerea. European Journal of Plant Pathology 125 (4), 551-563, DOI: 10.1007/s10658-009-9503-6.

Dimopoulos, N., Tindjau, R., Wong, D.C.J., Matzat, T., Haslam, T., Song, C., Gambetta, G.A., Kunst, L., Castellarin, S.D., 2020: Drought stress modulates cuticular wax composition of the grape berry. Journal of Experimental Botany 71 (10), 3126-3141, DOI: 10.1093/jxb/eraa046.

Gabler, F.M., Smilanick, J.L., Mansour, M., Ramming, D.W., Mackey, B.E., 2003: Correlations of morphological, anatomical, and chemical features of grape berries with resistance to *Botrytis cinerea*. Phytopathology 93 (10), 1263-1273, DOI: 10.1094/PHYTO.2003.93.10.1263.

González-Fernández, E., Piña-Rey, A., Fernández-González, M., Aira, M.J., Rodríguez-Rajo, F.J., 2020: Identification and evaluation of the main risk periods of *Botrytis cinerea* infection on grapevine based on phenology, weather conditions and airborne conidia. The Journal of Agricultural Science 158 (1-2), 88-98, DOI: 10.1017/S0021859620000362.

Gubler, W.D., 1987: Control of Botrytis bunch rot of grape with canopy management. Plant Disease 71 (7), 599, DOI: 10.1094/PD-71-0599.

Harrell Jr., F.E., 2021: Package Hmisc – Harrell Miscellaneous, URL: https://cran.r-project.org/web/packages/Hmisc/Hmisc.pdf.

Herzog, K., Schwander, F., Kassemeyer, H.-H., Bieler, E., Dürrenberger, M., Trapp, O., Töpfer, R., 2021: Towards sensor-based phenotyping of physical barriers of grapes to improve resilience to botrytis bunch rot. Frontiers in Plant Science 12, 808365, DOI: 10.3389/fpls.2021.808365.

Herzog, K., Wind, R., Töpfer, R., 2015: Impedance of the grape berry cuticle as a novel phenotypic trait to estimate resistance to *Botrytis cinerea*. Sensors 15 (6), 12498-12512, DOI: 10.3390/s150612498.

Intrigliolo, D.S., Castel, J.R., 2008: Effects of irrigation on the performance of grapevine cv. Tempranillo in Requena, Spain. American Journal of Enology and Viticulture 59 (1), 30-38, DOI: 10.5344/ajev.2008.59.1.30.

Khanal, B.P., Imoro, Y., Chen, Y.H., Straube, J., Knoche, M., 2021: Surface moisture increases microcracking and water vapour permeance of apple fruit skin. Plant Biology 23 (1), 74-82, DOI: 10.1111/plb.13178.

Kicherer, A., Roscher, R., Herzog, K., Förstner, W., Töpfer, R., 2015: Image based evaluation for the detection of cluster parameters in grapevine. Acta Horticulturae (1082), 335-340, DOI: 10.17660/ActaHortic.2015.1082.46.

Kraus, C., Pennington, T., Herzog, K., Hecht, A., Fischer, M., Voegele, R.T., Hoffmann, C., Töpfer, R., Kicherer, A., 2018: Effects of canopy architecture and microclimate on grapevine health in two training systems. Vitis 57 (2), 53-60, DOI: 10.5073/VITIS.2018.57.53-60.

Lê, S., Josse, J., Husson, F., 2008: FactoMineR: An R Package for Multivariate Analysis. Journal of Statistical Software 25 (1), DOI: 10.18637/jss.v025.i01.

Lorenz, D. H., Eichhorn, K.W., Bleiholder, H., Klose, R., Meier, U., Weber, E., 1995: Growth stages of the grapevine: 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 1995 (1), 100-110, DOI: 10.1111/j.1755-0238.1995.tb00085.x.

Medeiros, C.D., Falcão, H.M., Almeida-Cortez, J., Santos, D., Oliveira, A., Santos, M.G., 2017: Leaf epicuticular wax content changes under different rainfall regimes, and its removal affects the leaf chlorophyll content and gas exchanges of *Aspidosperma pyrifolium* in a seasonally dry tropical forest. South African Journal of Botany 111, 267-274, DOI: 10.1016/j.sajb.2017.03.033.

Molitor, D., Baus, O., Didry, Y., Junk, J., Hoffmann, L., Beyer, M., 2020: BotRisk: simulating the annual bunch rot risk on grapevines (*Vitis vinifera* L. cv. Riesling) based on meteorological data. International Journal of Biometeorology 64 (9), 1571-1582, DOI: 10.1007/s00484-020-01938-5.

Molitor, D., Schultz, M., Mannes, R., Pallez-Barthel, M., Hoffmann, L., Beyer, M., 2019: Semi-Minimal Pruned Hedge: A Potential Climate Change Adaptation Strategy in Viticulture. Agronomy 9 (4), 173, DOI: 10.3390/agronomy9040173.

Mosedale, J.R., Abernethy, K.E., Smart, R.E., Wilson, R.J., Maclean, I.M.D., 2016: Climate change impacts and adaptive strategies: lessons from the grapevine. Global Change Biology 22 (11), 3814-3828, DOI: 10.1111/gcb.13406.

Mundy, D.C., Elmer, P., Wood, P., Agnew, R., 2022: A review of cultural practices for Botrytis bunch rot management in New Zealand vineyards. Plants 11 (21), 3004, DOI: 10.3390/plants11213004.

Nendel, C., 2010: Grapevine bud break prediction for cool winter climates. International Journal of Biometeorology 54 (3), 231-241, DOI: 10.1007/s00484-009-0274-8.

Palacios, F., Diago, M.P., Tardaguila, J., 2019: A non-invasive method based on computer vision for grapevine cluster compactness assessment using a mobile sensing platform under field conditions. Sensors 19 (17), 3799, DOI: 10.3390/s19173799.

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 (4), 397-407, DOI: 10.5344/ajev.2006.57.4.397.

Porro, D., Ramponi, M., Tomasi, T., Rolle, L., Poni, S., 2010: Nutritional Implications of water stress in grapevine and modifications of mechanical properties of berries. Acta Horticulturae (868), 73-80, DOI: 10.17660/actahortic.2010.868.5.

Richter, R., Gabriel, D., Rist, F., Töpfer, R., Zyprian, E., 2019: Identification of co-located QTLs and genomic regions affecting grapevine cluster architecture. Theoretical and Applied Genetics 132 (4), 1159-1177, DOI: 10.1007/s00122-018-3269-1.

Rist, F., Herzog, K., Mack, J., Richter, R., Steinhage, V., Töpfer, R., 2018: High-precision phenotyping of grape bunch architecture using fast 3D sensor and automation. Sensors 18 (3), 763, DOI: 10.3390/s18030763.

Rist, F., Schwander, F., Richter, R., Mack, J., Schwandner, A., Hausmann, L., Steinhage, V., Töpfer, R., Herzog, K., 2022: Relieving the phenotyping bottleneck for grape bunch architecture in grapevine breeding research: implementation of a 3D-based phenotyping approach for quantitative trait locus mapping. Horticulturae 8 (10), 907, DOI: 10.3390/horticulturae8100907.

Schäfer, J., Friedel, M., Molitor, D., Stoll, M., 2021: Semi-minimal-pruned hedge (SMPH) as a climate change adaptation strategy: impact of different yield regulation approaches on vegetative and generative development, maturity progress and grape quality in Riesling. Applied Sciences 11 (8), 3304, DOI: 10.3390/app11083304.

Schmidt, D., Bahr, C., Friedel, M., Kahlen, K., 2019: Modelling approach for predicting the impact of changing temperature conditions on grapevine canopy architectures. Agronomy 9 (8), 426, DOI: 10.3390/agronomy9080426.

Signorell, A., 2021: Package 'DescTools': Tools for Descriptive Statistics, URL: https://cran.r-project.org/web/packages/desctools.pdf.

Tello, J., Ibáñez, J., 2018: What do we know about grapevine bunch compactness? A state-of-the-art review. Australian Journal of Grape and Wine Research 24 (1), 6-23, DOI: 10.1111/ajgw.12310.

Töpfer, R., Trapp, O., 2022: A cool climate perspective on grapevine breeding: climate change and sustainability are driving forces for changing varieties in a traditional market. TAG. Theoretical and applied genetics. Theoretical and Applied Genetics 135 (11), 3947-3960, DOI: 10.1007/s00122-022-04077-0.

Underhill, A., Hirsch, C., Clark, M., 2020: Image-based phenotyping identifies quantitative trait loci for cluster compactness in grape. Journal of the American Society for Horticultural Science 145 (6), 363-373, DOI: 10.21273/JASHS04932-20.

Vail, M.E., Wolpert, J.A., Gubler, W.D., Rademacher, M.R., 1998: Effect of cluster tightness on botrytis bunch rot in six Chardonnay clones. Plant Disease 82 (1), 107-109, DOI: 10.1094/PDIS.1998.82.1.107.

Van der Weide, J., Yan, Y., Zandberg, W.F., Castellarin, S.D., 2022: Modulation of grape cuticular wax composition following multiple heatwaves influences grape transpiration.

Environmental and Experimental Botany 202, 105036, DOI: 10.1016/j.envexpbot.2022.105036.

Watt, A.M., Dunn, G.M., May, P.B., Crawford, S.A., Barlow, E., 2008: Development of inflorescence primordia in *Vitis vinifera* L. cv. Chardonnay from hot and cool climates. Australian Journal of Grape and Wine Research 14 (1), 46-53, DOI: 10.1111/j.1755-0238.2008.00006.x.

Würz, D.A., Rufato, L., Bogo, A., Allebrandt, R., Pereira de Bem, B., Marcon Filho, J.L., Brighenti, A.F., Bonin, B.F., 2020: Effects of leaf removal on grape cluster architecture and control of Botrytis bunch rot in Sauvignon Blanc grapevines in Southern Brazil. Crop Protection 131, 105079, DOI: 10.1016/j.cropro.2020.105079