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An experimental design applied to vineyards for identifying spatially and temporally variable crop parameters

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Summary

Harvesting uniform batches of grapes is required to optimize must quality as one prerequisite for premium wine production. The definition of sub-units of vineyards based on within-field variation allows unit-based vineyard management during cultivation and harvest. Essential for such vineyard management is the definition of sub-units that correspond with uniform batches of quality parameters of the fruit (e.g. berry residual sugar, anthocyanin content) at harvest time or with physiological parameters measuring the vine during berry development until ripeness. The definition requires geo-referenced sampling and parameter analysis, usually in combination with interpolation and kriging methods employed to describe spatial vineyard variation.

In an attempt to develop an assay for within-variation in vineyards physiological parameters assessed through chlorophyll fluorescence measurements and leaf temperature were assessed at bloom, veraison and post veraison in a randomized block design in two vineyards of Lower Austria. A statistical model based on a repeated measurement ANOVA was developed and showed suitability for the detection and monitoring of vineyard variability throughout the vegetation period based on the maximum quantum yield of photosystem II (Fv/Fm), the maximum leaf temperature ($maxT_{leaf}$) and malic acid. These parameters allow the prospective classification of sub-units according to the vine's vitality and may be adopted for scientific experimentation and for practical viticulture.

K e y w o r d s : within-vineyard variation, chlorophyll fluorescence, thermal imagery, *Vitis vinifera*, vineyard variability.

A b b r e v i a t i o n s : LAI, Leaf area index; GPS, global positioning systems; GIS, geographic information systems; dGPS, differential global positioning systems; PCD, plant cell density index; NDVI, normalized difference vegetation index; PSII, photosystem II; PAM, pulse amplitude modulation; PEA, plant efficiency analyser; PPC, potential performance classes; Fo, minimum fluorescence; Fm, maximum fluorescence; Fv, variable fluorescence; Fv/Fm, maximum quantum yield of photosystem II; PI, performance index; $minT_{leaf}$, minimum leaf temperature; $maxT_{leaf}$, maximum leaf temperature; avT_{leaf}, average leaf temperature; TSS, total soluble solids; AIC, Akaike Information Criterion.

Introduction

Environmental differences within a vineyard affect grapevine development that imparts within-field variability on the fruit development and crop yield (Tardaguila *et al.* 2011). The main factors affecting the grapevines' growth are soil (e.g. type, composition, water availability) and microclimate (temperature, humidity, radiation), the cultivar and the vineyard management e.g. cover crop selection (Panten *et al.* 2010). Within-field variability has been studied in terms of soil characteristics such as resistivity (Paoli *et al.* 2005), electrical conductivity (Li *et al.* 2008), organic matter, plasticity index and soil type (Bakhsh *et al.* 2000, Tardaguila *et al.* 2011), of vegetative variables such as total shoot length, leaf area index (LAI) and total leaf area (Tardaguila *et al.* 2011, Hall *et al.* 2008), of quality parameters such as phenolics, titratable acidity and pH (Bramley 2005, Cortell *et al.* 2005, Panten and Bramley 2011) and of yield (Bramley and Hamilton 2004, Gonçalves *et al.* 2007, Li *et al.* 2008, Bramley *et al.* 2011). Most of the studies combine parameters constitutive to the vineyard (soil characteristics) or yield and fruit quality traits determined with global positioning systems (GPS) and geographic information systems (GIS). The use of GPS (especially differential GPS, dGPS) has provided reliable and accurate positions of and within the vineyard. Additionally, GIS software has the capability to generate and overlay several spatial data sets (layers) in order to investigate their interaction over space and time (Bakhsh *et al.* 2000).

Many studies on spatial variability in vineyards use indices based on remotely sensed imageries e.g. the plant cell density index (PCD) (Panten and Bramley 2011, Bramley *et al.* 2011) or the normalized difference vegetation index (NDVI) (Hall *et al.* 2008, Hall *et al.* 2011, Mazzetto *et al.* 2010) but few studies have been undertaken to our knowledge which study within-vineyard variability based on physiological parameters throughout the vegetation period (e.g. Zhang *et al.* 2010). Physiological parameters may describe the vitality of plants and link different approaches (e.g. gas exchange, leaf reflectance spectra, thermal imaging, water potential, chlorophyll fluorescence) (Schoedl *et al.* 2012).

In the presented study chlorophyll fluorescence measurements and thermal images of leaves are combined to assess variation in the photosynthetic activity of grapevines

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within one vineyard. Photosynthetic activity can be non-destructively measured; therefore, temporal monitoring of one and the very same plant over the ripening period is useful for a combined spatial and temporal field assessment.

As rapid and non destructive, chlorophyll fluorescence measurements have become a routine method in plant physiology experiments in many species (FLEXAS *et al.* 2002, SOJA and SOJA 2005, LICHTENTHALER *et al.* 2007) and for various experimental questions like water stress effects on *Rosa* x hybrida (CALATAYUD *et al.* 2006) or *Vitis vinifera* (DÜRING 2000), salt stress in *Solanum lycopersicum* (ZRIBI *et al.* 2009), varying water contents in *Sphagnum* moss (VAN GAALEN *et al.* 2007) or spray drift damage in *Gossypium* (HUANG *et al.* 2010). Chlorophyll fluorescence describes radiation emitted by the electron acceptors in the chlorophyll molecules, especially from photosystem II (PSII) and can be detected by different types of fluorimeters either based on the pulse amplitude modulation (PAM) technique or on the continuous excitation technique like the Handy Plant Efficiency Analyser (PEA) Chlorophyll Fluorometer (Hansatech Instruments, Norfolk, England) used in this study. The advantage of the Handy PEA is its ability to measure the fast chlorophyll fluorescence induction kinetics. Thermal imagery visualizes leaf surface temperatures and has been postulated as an indicator of transpiration and stomatal conductance (JONES 1999). Since leaf temperature and stomatal conductance are correlated with stress (STOLL and JONES 2007), thermal imagery can be used to monitor physiological performance of plants.

Assessing of within-vineyard variation requires sophisticated data analysis and advanced data collection. Spatial data analysis and variability map construction of vineyards are based on geostatistical methods providing statistical tools for incorporating the spatial coordinates in data processing, allowing description and modeling of spatial patterns, predicting unsampled locations and assessing the uncertainty attached to these predictions (GOOVAERTS 1998). Pre-treatment data (e.g. soil characteristics, data on global positioning) need to be collected and analysed prior experiments using various data processing methods (including interpolation of measured data, kriging, clustering and normalising of data) (BRAMLEY and HAMILTON 2004, PANTEN *et al.* 2010) sometimes resulting in so called 'potential performance classes (PPCs)'. These performance classes are set into relation with other measurements data e.g. berry weight and the interactions of PPCs with applied treatments are studied (PANTEN *et al.* 2010). Besides PPCs, mono-variable maps (e.g. yield maps) can be generated from these data but often multiple layers of data (e.g. slope – soil – rootstock – scion – etc.) are used to produce spatial maps of fields (SMITH and WHIGHAM 1999), complicating data analysis and result interpretation.

Cluster analysis enables combining values interpolated from the maps into homogenous groups (classes) in relation to the variable chosen (ARNÓ *et al.* 2009). Multivariate k-means clustering has been used to analyze and demonstrate the patterns in yield variation and identified different yield zones (BRAMLEY and LAMB 2003). Moreover, the use of normalized yield maps has been considered in some experiments (BAKHSH *et al.* 2000, BRAMLEY and HAMILTON 2004) to reduce the influence of seasonal yield differences on map interpretation and subsequent zoning of the area. However, the use of remote sensing technologies (e.g. airborne methods) and spatial data analysis methods in determining vineyard variability is expensive, difficult and time-consuming. These methods are not used routinely so far, thus cannot reflect short-time changes in the field due to natural changes. It is therefore necessary to develop an easy to implement method to measure variability in photosynthesis performance and yield over time which can be used to monitor changes during the current vegetation period.

The objective of this work is to develop a simple and fast method for the detection of within-vineyard variation by selected physiological parameters during the berry development and ripening period.

Material and Methods

Experiments were conducted from July until October 2010 in two vineyards planted in 2007: (1) located at Retz, AT (0.76 ha), 'Pinot Noir' (clones 1-84 Gm, 18 Gm) on 5BB (N 48° 46' 36.6" and E 15° 56' 42.7"), facing N-S, row distance 3 m in a randomized block design including 20 blocks; (2) located at Krems, AT (0.63 ha), 'Riesling' (clones A71, 239-17 Gm, 198-44 Gm) on 5BB (N 48° 25' 19.5" and E 15° 37' 11.9"), facing N-S, row distance 2.65 m in a randomized block design including 24 blocks. The measurements took place in twenty blocks in vineyard (1) and twenty blocks in vineyard (2) on three developmental stages of plants (Bloom - BBCH 63, Beginning of berry touch - BBCH 77 and Beginning of berry ripening where berries are already soft but not fully ripe - BBCH 87) and at harvest (BBCH 89) (BBCH Codes according to EICHHORN and LORENZ 1977).

Measurements performed were (1) leaf temperature (ThermaCam B2, FLIR Instrument, Oregon, USA) and (2) chlorophyll fluorescence parameters (Handy- PEA, Hansatech Instruments, England, UK) on six plants per block based on a randomized sample design (Fig. 1). Chlorophyll fluorescence parameters were measured on three leaves per plant (in total 360 measurements), while leaf temperature was determined from one leaf per plant (in total 120 measurements) per measurement date. For chlorophyll fluorescence measurements leaves were dark adapted for 20 to 30 min using the Handy PEA leaf clips to define the measurement area and prevent ambient light leakage ensuring accuracy of the measurement. Total chlorophyll fluorescence parameters measured are listed in Tab. 1.

The emissivity value of 0.95 was used to complete leaf temperature measurements (STOLL *et al.* 2008). Infrared photos were analyzed with the ThermaCam Reporter 8.0 (Flir Instruments, Oregon, US) to illustrate the minimum ($minT_{leaf}$), maximum ($maxT_{leaf}$), and average temperature (avT_{leaf}) employing the polygon function analyzing the whole leaf blade by visual documentation. Control photos were taken to retrieve and compare shape of the leaf during analysis. At harvest, samples of fruit clusters of four plants per block were taken and analysed, resulting in 80 sampled

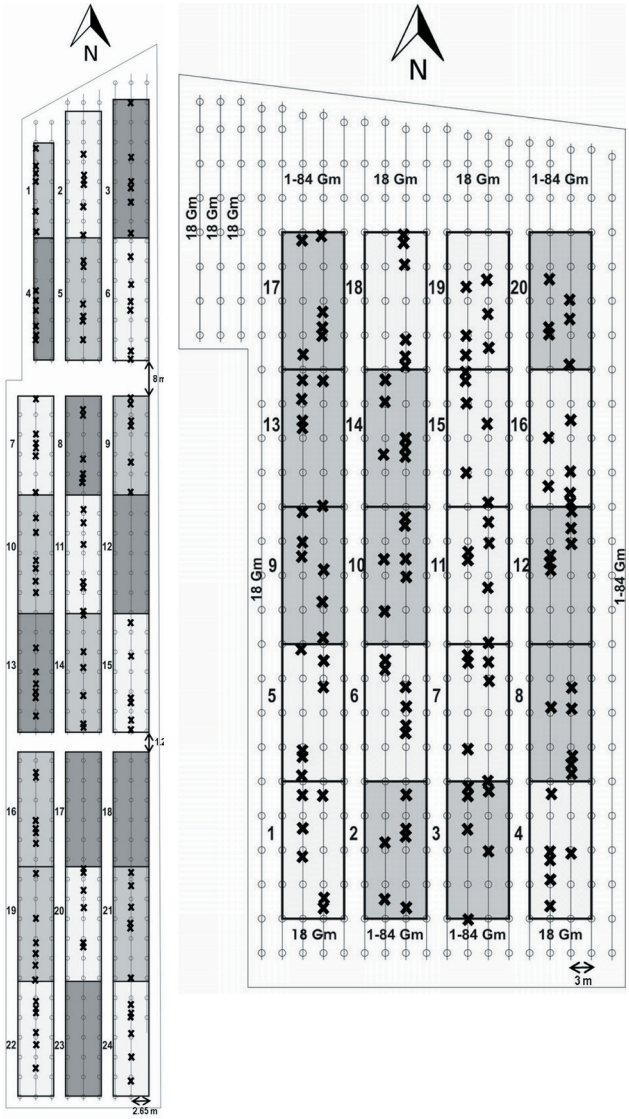

Fig. 1: Schematic map of the vineyard in Krems (left) and Retz (right). x indicate positions of measured plants. Krems vineyard: 'Riesling' clone A7-1 white coloured blocks, clone 198-44 Gm light-grey coloured blocks, clone 239-17 Gm dark-grey coloured blocks. Retz vineyard: 'Pinot Noir' clone 18 Gm light-grey coloured blocks, clone 1-84 Gm dark-grey coloured blocks.

Table 1

Parameters measured with the Handy PEA

Parameter	Calculation	Meaning
Fo	-	Minimum fluorescence
Fm	-	Maximum fluorescence
Fv	Fm-Fo	Variable fluorescence
Fv/Fm	(Fm-Fo)/Fm	Maximum quantum yield of PSII
Fv/Fo	(Fm-Fo)/Fo	
Area		Area above fluorescence induction curve from Fo to Fm (Strasser *et al*. 2000)
PI		Performance index (Strasser *et al*. 2000)

Annex Table 1

Results of the ANOVA for tested parameters

Variable	Location	Type 3 Tests of Fixed Effects				
		Effect	Num DF[1]	Den DF[2]	F Value	Pr > F[3]
avT_{leaf}	Krems	block	19	168	2.71	0.0004
		day	1	272	4877.80	<.0001
		day*block	19	220	1.73	0.0324
	Retz	block	19	175	2.28	0.0027
		day	1	230	2339.87	<.0001
		day*block	19	202	1.20	0.2584
$maxT_{leaf}$	Krems	block	19	165	2.60	0.0006
		day	1	306	1704.16	<.0001
		day*block	19	254	2.01	0.0086
	Retz	block	19	174	1.16	0.2974
		day	1	304	1122.53	<.0001
		day*block	19	271	1.57	0.0629
$minT_{leaf}$	Krems	block	19	168	3.67	<.0001
		day	1	266	4380.73	<.0001
		day*block	19	224	0.84	0.6523
	Retz	block	19	180	0.76	0.7471
		day	1	276	917.73	<.0001
		day*block	19	244	1.75	0.0291
Fm	Krems	block	19	517	2.58	0.0003
		day	1	950	9.24	0.0024
		day*block	19	796	3.47	<.0001
	Retz	block	19	545	2.38	0.0009
		day	1	606	181.12	<.0001
		day*block	19	785	5.51	<.0001
Fo	Krems	block	19	487	0.88	0.6061
		day	1	883	25.51	<.0001
		day*block	19	801	3.43	<.0001
	Retz	block	19	560	1.14	0.3096
		day	1	656	0.68	0.4101
		day*block	19	764	2.71	0.0001
Fv	Krems	block	19	520	2.59	0.0003
		day	1	946	3.11	0.0779
		day*block	19	793	3.82	<.0001
	Retz	block	19	564	2.52	0.0004
		day	1	610	181.88	<.0001
		day*block	19	788	4.67	<.0001
Fv/Fm	Krems	block	19	509	1.75	0.0261
		day	1	940	10.01	0.0016
		day*block	19	795	3.71	<.0001
	Retz	block	19	571	1.89	0.0128
		day	1	663	160.42	<.0001
		day*block	19	779	4.66	<.0001
Fv/Fo	Krems	block	19	477	1.33	0.1577
		day	1	802	0.31	0.5800
		day*block	19	794	3.59	<.0001
	Retz	block	19	567	1.58	0.0556
		day	1	650	99.67	<.0001
Area	Krems	block	19	961	0.35	0.9954
		day	1	961	42.85	<.0001
		day*block	19	961	2.55	0.0003
	Retz	-	-	-	-	-
		day*block	19	777	2.10	0.0041
PI	Krems	block	19	446	0.23	0.9997
		day	1	460	24.87	<.0001
		day*block	19	797	3.67	<.0001
	Retz	block	19	508	0.48	0.9690
		day	1	554	9.24	0.0025
		day*block	19	777	4.20	<.0001

Annex Tab. 1 continued

Variable	Location	Type 3 Tests of Fixed Effects				
		Effect	Num DF[1]	Den DF[2]	F Value	Pr > F[3]
Malic acid	Krems	block	19	59	3.18	0.0003
		pH	1	59	8.62	0.0047
	Retz	block	19	59	12.11	<.0001
		pH	1	59	7.17	0.0096
TSS	Krems	block	19	59	3.44	0.0001
		pH	1	59	21.74	<.0001
	Retz	block	19	59	3.85	<.0001
		pH	1	59	24.75	<.0001
Titratable acidity	Krems	Block	19	59	5.19	<.0001
		pH	1	59	31.07	<.0001
	Retz	block	19	59	14.28	<.0001
		pH	1	59	26.62	<.0001
Tartaric acid	Krems	block	19	59	2.40	0.0055
		pH	1	59	0.52	0.4738
	Retz	block	19	59	3.67	<.0001
		pH	1	59	0.67	0.4174

[1] Num DF: Value of the largest sample -1
[2] Den DF: Value of the smaller sample -1
[3] PR > F: resulting p value

plants per vineyard, to be able to relate temperature and fluorescence parameters to grape quality parameters. The parameters total soluble solids (TSS) (° Brix), titratable acidity ($g \cdot L^{-1}$), pH value, tartaric acid ($g \cdot L^{-1}$) and malic acid ($g \cdot L^{-1}$) were analysed using the WineScan FT 120 (Foss, Hilleroed, Denmark). Calibration was confirmed by external control samples and quantifications of individual compounds were performed using standard reference methods (Eder and Brandes 2004).

Statistical analysis: All statistical analyses were performed using the SAS software, Version 9.1.3 of the SAS System (SAS Institute Inc., Cary, NC, USA). For evaluation of variability in the experimental plots a repeated measurement ANOVA was applied (Littell *et al.* 1996); the developed model is a mixed effects model for repeated measurements.

As three measurements per plant were done for the non-temperature parameters, a slightly different model was set up for that case. Dependencies over time within a plant/leaf were modeled using the autoregressive correlation structure (AR(1)) and the clone effect was modeled using the group statement within the repeated statement. The interaction term time*block reflects differences in development of blocks over time, e.g. differences in the soil that affect the parameter.

Model quality was assessed by visual inspection of the QQ-Plot of the residuals and the predicted mean versus residual-plot. In general, model quality for the tested parameter is given if no dependency between predicted means and raw residuals is visible and if the values in the QQ-plot follow the predicted line well. Further homogeneity between blocks was investigated by a Levene-Test of the residuals by block. For must analyses, a changed model was used. Clone was not included as either the model did not converge in that case or the fit was not better in terms of the Akaike information criterion (AIC). On the other hand, pH was included in the model as it resulted in a better fit.

For selection as variability indicator the co-operation of four criteria is important: (1) detection of differences, (2) no interactions as far as possible, (3) normal distribution and (4) variance homogeneity as far as possible. Variance homogeneity and the assumption of normality are preconditions for applying an ANOVA. If variance homogeneity or normality is not demonstrated, results are possibly contorted leading to either too many or too less significant results.

The hypotheses tested were: (1) the studied photosynthetic parameters are suitable to steadily describe within-vineyard variation throughout the period from bloom to harvest and (2) must parameters determined at harvest prove the occurrence of variability within the vineyard.

Results

Ten photosynthesis parameters and four must parameters have been tested with the statistical model described above (see Annex Tab. 1).

Model quality was assessed by visual inspection of the predicted mean versus residual-plot and the QQ-Plot of the residuals (Fig. 2). Model quality was well according to the stipulated criteria of independency of residuals and predicted values as well as non-relevant departures from the predicted line in the QQ-plot. Instead of one expected cluster of data in Fig. 2 the predicted mean versus residual plot shows two clusters. As a reason an air temperature difference on the third measurement day with an average day temperature of 14.1 °C compared to the first two measurement days (22.4 °C and 24.1 °C, respectively) was identified, influencing $maxT_{leaf}$ (Jones 1999, Jones *et al.* 2002).

The test of fixed effects showed a significant difference between blocks in the parameter $maxT_{leaf}$ with a significance of $p = 0.0006$ in Krems. Analyses of differences of least squares means resulted in blocks 16, 19, 20, 22 and 24 differing from all other blocks (Fig. 3). The described vineyard exhibits a small fall in north-south direction with the differing blocks being located in the south. Since no shading objects are located around and within the vineyard parameters causing the block differences in the maximum temperature could not have been identified, possibly being soil properties, slope etc.

In Retz block 6 differed mostly from other blocks followed by block 12 (Fig. 4). Blocks 5, 15, 18 and 19 did not differ in the parameter $maxT_{leaf}$ from any other block. The predicted mean versus residual-plot shows similar two clusters as in the vineyard in Krems. As before, the lower air temperature on the last measurement caused the clustering of data (data not shown).

The chlorophyll fluorescence parameter Fv/Fm is also suitable for detection of within-vineyard variation using the developed method (data not shown). Fv/Fm is often used as indicator for the plant's photosynthetic performance and as indicator for stress impacts (Mohammed *et al.* 2003, Baker and Rosenqvist 2004). Its competence to describe vineyard variability is firstly described here and

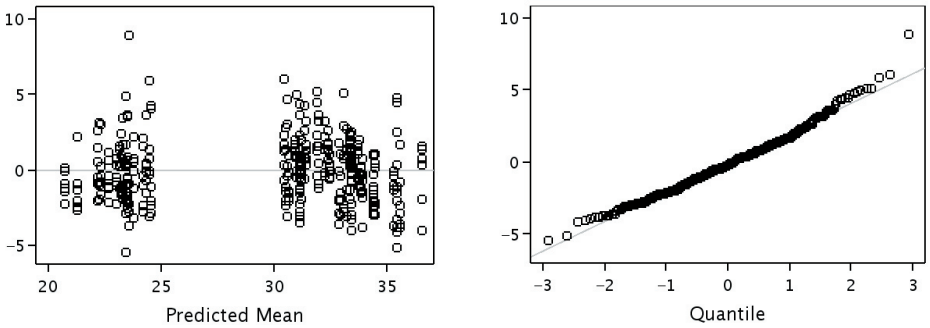

Fig. 2: Predicted mean versus residual-plot (left) and QQ-Plot of the residuals (right) used for assessing the applied model's quality of $maxT_{leaf}$ in Krems vineyard.

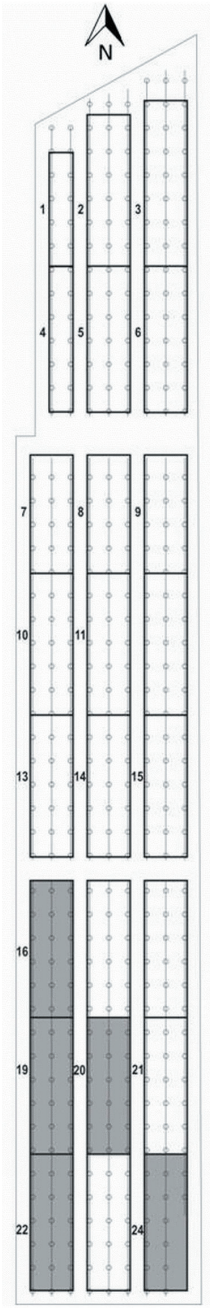

Fig. 3: Schematic block variation map of Krems vineyard using the parameter $maxT_{leaf}$. Different colours indicate significant differences between blocks. Blocks without number have not been investigated.

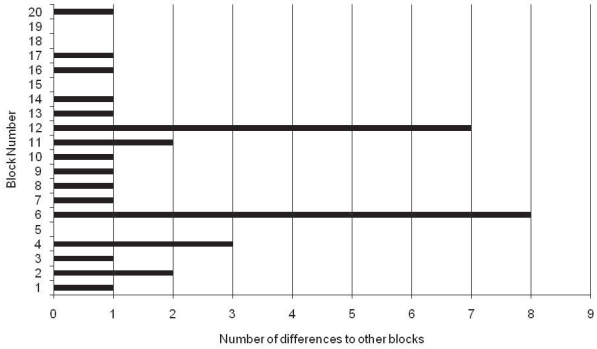

Fig. 4: Number of differences to other blocks per block for $maxT_{leaf}$ in Retz vineyard.

enhances the worthiness of the parameter in all kind of research addressing the grapevines' performance.

Within the must parameters malic acid is the best candidate as identifier for field variability with a significant p value of 0.0003 in Krems and <0.001 in Retz in the test of fixed effects. No dependency of predicted means and raw residuals was detected and the values in the QQ-plot perfectly reproduce the predicted line with both proving the model's applicability on this must parameter (data not shown). The variability of the grapes' content of malic acid over the vineyard in Krems and Retz is shown in Fig. 5. As clearly can be seen the difference from one block to the other is high, indicating high variability over the vineyard. In Krems vineyard block 10 and 21 are the ones which least differ from other blocks whereas block 1, 5 and 20 each differ from 11 other blocks in the vineyard. In Retz vineyard the number of differences to other blocks per block is generally higher with block 1 showing no difference and block 2 differing from three other blocks.

For demonstrating a parameter with bad model quality the performance index PI is presented. As can be seen in the predicted mean versus residual-plot a clear dependency of mean and residuals is given indicating autoregression. Further the values in the QQ-plot display a very different shape than those of the predicted line (Fig. 6). Consequently, all parameters showing these characteristics were eliminated as indicators for variability determined by the proposed model. Additionally to the presented analyses, the Levene test for testing the homogeneity of variances of the vineyard blocks was applied (Table 2).

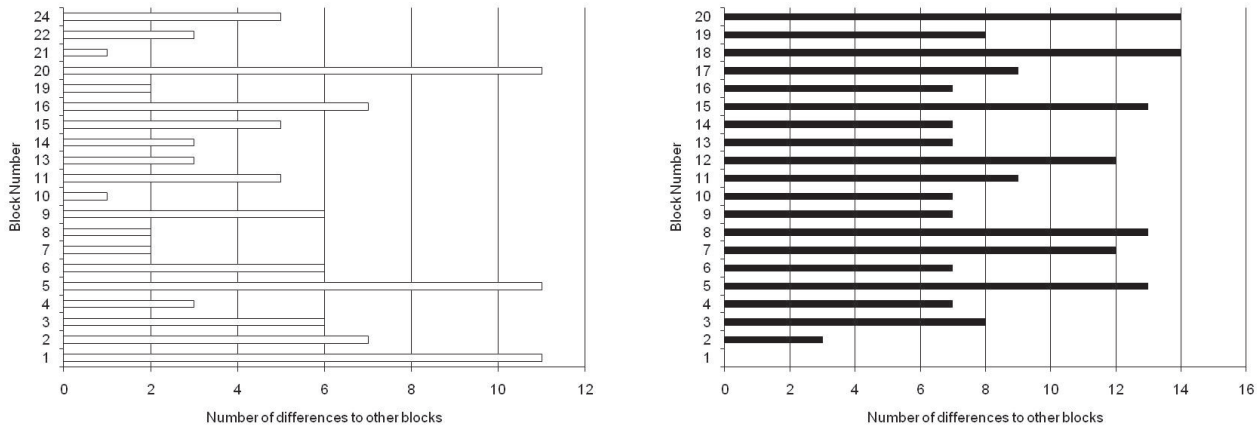

Fig. 5: Number of differences to other blocks per block in Krems vineyard (left) and Retz vineyard (right) for the quality parameter malic acid.

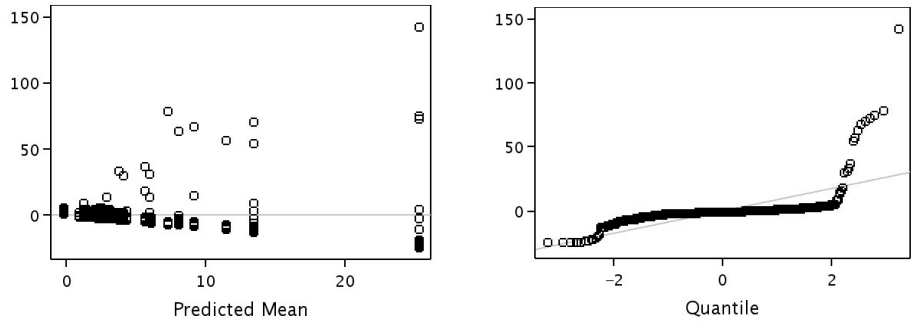

Fig. 6: Predicted mean versus residual-plot (left) and QQ-Plot of the residuals (right) used for assessing the applied model's quality of PI in Krems vineyard.

Table 2

Results of Levene-test for homogeneity of variances in selected parameters

	Parameter	location	p-value
Leaf temperature	$minT_{leaf}$	Krems	<0.0001
		Retz	0.1672
	$maxT_{leaf}$	Krems	0.2944
		Retz	0.0066
Chlorophyll fluorescence	Fv/Fm	Krems	0.3602
		Retz	0.0167
	Fv	Krems	0.0228
		Retz	0.3351
	Fo	Krems	0.7274
		Retz	0.0027
	Fm	Krems	0.3117
		Retz	0.2646
	PI	Krems	0.0014
		Retz	0.1456
Must	tartaric acid	Krems	0.0071
		Retz	0.0548
	malic acid	Krems	0.1147
		Retz	0.1041
	TSS	Krems	0.0554
		Retz	<0.0001

Discussion

The presented approach allows detection of within-vineyard variability based on existing or randomly scheduled vineyard sub-units (blocks) by physiological parameters. By testing the measured parameters with the method and criteria mentioned above, the parameters malic acid, $maxT_{leaf}$ and Fv/Fm are considered to fulfill criteria 3 (normal distribution) with malic acid being the best of these. These three parameters proved to be the best ones to describe the within-vineyard variation in both plots.

Malic acid is known to decrease until harvest indicating physiological ripeness (KLIEWER 1966) and can therefore be used as reference for differential stages of ripeness within vineyards, enabling block-precised harvest management. The physiological parameters $maxT_{leaf}$ and Fv/Fm have often been reported to indicate photosynthetic performances of plants under various conditions in terms of stomatal conductance (e.g. JONES *et al.* 2002) and PSII performance (e.g. ZRIBI *et al.* 2009). Both can be used for monitoring the adequate supply with e.g. water, radiation and nutrients of vines within vineyards; hence, enabling selective management reactions like irrigation of reduced leaf removal.

Among the tested parameters, malic acid and Fm are the only ones demonstrating variance homogeneity of the blocks in both locations. The two parameters $maxT_{leaf}$ and Fv/Fm expose homogeneity of variances of blocks only in Krems.

Significant interactions between measurement day and block were found for all parameters except $maxT_{leaf}$ and avT_{leaf} in Retz and $minT_{leaf}$ in Krems indicating a different development of blocks over time with regard to the respective parameter. As known from other studies (e.g. TROUGHT and BRAMLEY 2011) spatial maps of vineyards

predicting berry quality attributes such as soluble solids or titratable acidity differ in dependence on the measurement date, thus make it reasonable to measure several times a year and complicating the general use of resulting maps for practical viticulture. In the applied approach a development of blocks over time is included by the interaction between measurement day and block. The advantage of the presented approach for both growers and scientists is the possibility to analyze repeated measurements and to test whether the selected block design was reasonable.

To be able to link the physiological parameters for grapevines' performance to fruit quality parameters and hence the predictive strength of physiological parameters several years of field studies need to be undertaken. Consequently, in conjunctions of soil performance classes, vine performance classes or physiological parameters with fruit quality attributes the date of prediction/measurement needs to be considered to get a reliable prediction of quality parameters. In contrast, from a statistical point of view no more years of experiments need to be undertaken because the model itself is established and it does not need any proof of the parameters' predictive strength. Hence the statistical model itself can directly be applied to any vineyard tested.

The model relies on units (blocks) of any plot and was confirmed through the experimental set up (randomized block design) in our experimental vineyards, however the grid can be easily adopted to existing commercial or other experimental fields. The classification of sub-units (blocks) may be executed through visual observation and experience of the growers or experimental set ups of further field trials. This flexible grid allows grower and scientist to decide about the data input and thus data quality through the model. By using the grid long-time known units may be redefined and yet unknown units may be defined within the vineyard at various times, offering the opportunity to adapt applied management strategies. The approach combines physiological parameters used in biological science (e.g. chlorophyll fluorescence) with simple quality indicators (e.g. malic acid) and offers an advanced yet easy way for any academic research in the field. Experimental set ups for biological testings in the field (e.g. plant protection products, canopy management strategies) could be developed by defining "vitality"-units (blocks) prior or simultaneous to experimentation thus minimizing the experimental error generated in the field and detect possible interactions of the vitality state and pre- or post-treatment effects.

The prerequisite for effective use of the approach is an adequately sized sample set per measurement date. This is difficult to achieve using measures and parameters employed in other studies (e.g. Panten *et al.* 2010, Tardaguila *et al.* 2011) working on single plant studies; however it is easily feasible using the equipment applied in our study.

The use of remote sensing technologies reduces time and efforts for generating variability data since no measurements on an individual plant level are applied. Unfavorable are the high costs for technical sensor equipment and the complexity and time needed for data analysis. However, recent studies aiming to develop practical ground sensing solutions to be directly used at farm level have been undertaken (Mazzetto *et al.* 2010).

We propose that our approach may be used at farm level and may be applied to any vineyard site allowing precise vineyard management and sustainable use or resources in this long-living culture. Interaction of experts may be helpful to assist in designing the grid according to the existing sites and satisfy the requirements of appropriate statistical analysis (e.g. sample size). We understand that vitality units may change through years, reflecting stress responses of the vines under differing conditions. A winery applying a vineyard zone management system may thus need a quick and real-time approach to adapt the existing units (zone) for day-to-day routine.

For conclusion, the proposed statistical model is appropriate for selecting parameters within the pool of tested chlorophyll fluorescence and leaf temperature parameters. The parameters $maxT_{leaf}$, Fv/Fm and malic acid were able to differentiate among blocks and thus describe within-vineyard variability over the vegetation period from bloom until harvest or at harvest, respectively. The results presented derive from a one year field trial conducted in two vineyards; perennial validation experiments in several vineyards are necessary to confirm the suggested parameters within the model suggested.

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References

Arnó, J.; Martínez-Casasnovas, J. A.; Ribes-Dasi, M.; Rosell, J. R.; 2009: Review. Precision viticulture. Research topics, challenges and opportunities in site-specific vineyard management. Spanish J. Agric. Res. **7**, 779-790.

Baker, N. R.; Rosenqvist, E.; 2004: Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. J. Exp. Bot. **55**, 1607-1621.

Bakhsh, A.; Colvin, T. S.; Jaynes, D. B.; Kanwar, R. S.; Tim, U. S.; 2000: Using Soil Attributes and GIS for Interpretation of Spatial Variability in Yield. Transact. ASABE **43**, 819-828.

Bramley, R. G. V.; 2005: Understanding variability in winegrape production systems 2. Within vineyard variation in quality over several vintages. Aust. J. Grape Wine Res. **11**, 33-42.

Bramley, R. G. V.; Hamilton, R. P.; 2004: Understanding variability in winegrape production systems. Aust. J. Grape Wine Res. **10**, 32-45.

Bramley, R. G. V.; Lamb, D. W.; 2003: Making sense of vineyard variability in Australia. In: R. Ortega; A. Esser (Eds): IX Congreso Latinoamericano de Viticultura y Enologia, 35-54. Chile.

BRAMLEY, R. G. V.; TROUGHT, M. C. T.; PRAAT, J. P.; 2011: Vineyard variability in Marlborough, New Zealand: characterising variation in vineyard performance and options for the implementation of Precision Viticulture. Aust. J. Grape Wine Res. **17**, 72-78.

CALATAYUD, A.; ROCA, D.; MARTINEZ, P. F.; 2006: Spatial-temporal variations in rose leaves under water stress conditions studied by chlorophyll fluorescence imaging. Plant Physiol. Biochem. **44**, 564-573.

CORTELL, J. M.; HALBLEIB, M.; GALLAGHER, A. V.; RIGHETTI, T. L.; KENNEDY, J. A.; 2005: Influence of Vine Vigor on Grape (*Vitis vinifera* L. cv. Pinot Noir) and Wine Proanthocyanidins. J. Agric. Food Chem. **53**, 5798-5808.

DÜRING, H.; 2000: High light and water stress in grapevines: Photoinhibition and photoprotection. Acta Hort. (ISHS) **493**, 45-54.

EDER, R.; BRANDES, W.; 2004: Weinanalysen im eigenen Betrieb: Grundparameter, Stuttgart, Eugen Ulmer.

EICHHORN, K. W.; LORENZ, D. H.; 1977: Phänologische Entwicklungsstadien der Rebe. Nachrichtenblatt Dt. Pflanzenschutzdienst **29**, 119-120.

FLEXAS, J.; ESCALONA, J. M.; EVAIN, S.; GULIAS, J.; MOYA, I.; OSMOND, C. B.; MEDRANO, H.; 2002: Steady-state chlorophyll fluorescence (Fs) measurements as a tool to follow variations of net CO_2 assimilation and stomatal conductance during water-stress in C3 plants. Physiol. Plant. **114**, 231-240.

GONÇALVES, E.; ST.AUBYN, A.; MARTINS, A.; 2007: Mixed spatial models for data analysis of yield on large grapevine selection field trials. TAG Theor. Appl. Genet. **115**, 653-663.

GOOVAERTS, P.; 1998: Geostatistical tools for characterizing the spatial variability of microbiological and physico-chemical soil properties. Biol. Fertility Soils **27**, 315-334.

HALL, A.; LAMB, D.; HOLZAPFEL, B.; LOUIS, J.; 2011: Within-season temporal variation in correlations between vineyard canopy and winegrape composition and yield. Precision Agri. **12**, 103-117.

HALL, A.; LOUIS, J. P.; LAMB, D. W.; 2008: Low-resolution remotely sensed images of winegrape vineyards map spatial variability in planimetric canopy area instead of leaf area index. Aust. J. Grape Wine Res. **14**, 9-17.

HUANG, Y.; THOMSON, S. J.; ORTIZ, B. V.; REDDY, K. N.; DING, W.; ZABLOTOWICZ, R. M.; BRIGHT, J. R.; 2010: Airborne remote sensing assessment of the damage to cotton caused by spray drift from aerially applied glyphosate through spray deposition measurements. Biosystems Engineering **107**, 212-220.

JONES, H. G.; 1999: Use of thermography for quantitative studies of spatial and temporal variation of stomatal conductance over leaf surfaces. Plant, Cell Environ. **22**, 1043-1055.

JONES, H. G.; STOLL, M.; SANTOS, T.; DE SOUSA, C.; CHAVES, M. M.; GRANT, O. M.; 2002: Use of infrared thermography for monitoring stomatal closure in the field: application to grapevine. J. Exp. Bot. **53**, 2249-2260.

KLIEWER, W. M.; 1966: Sugars and Organic Acids of *Vitis vinifera*. Plant Physiol. **41**, 923-931.

LI, Y.; SHI, Z.; WU, C. F.; LI, H. Y.; LI, F.; 2008: Determination of potential management zones from soil electrical conductivity, yield and crop data. J. Zhejiang University - Science B **9**, 68-76.

LICHTENTHALER, H. K.; AC, A.; MAREK, M. V.; KALINA, J.; URBAN, O.; 2007: Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. Plant Physiol. Biochem. **45**, 577-588.

LITTELL, R. C.; MILLIKEN, G. A.; STROUP, W. W.; WOLFINGER, R. D.; 1996: SAS® System for mixed models. Cary, NC, USA, SAS Institute Inc.

MAZZETTO, F.; CALCANTE, A.; MENA, A.; VERCESI, A.; 2010: Integration of optical and analogue sensors for monitoring canopy health and vigour in precision viticulture. Precision Agric. **11**, 636-649.

MOHAMMED, G. H.; ZARCO-TEJADA, P. J.; MILLER, J. R.; 2003: Applications of chlorophyll fluorescence in forestry and ecophysiology. In: J. R. DEELL; P. M. A. TOIVONEN (Eds): Practical applications of chlorophyll fluorescence in plant biology, 79-124. Dordrecht, The Netherlands, Kluwer Academic Publishers.

PANTEN, K.; BRAMLEY, R.; LARK, R.; BISHOP, T.; 2010: Enhancing the value of field experimentation through whole-of-block designs. Precision Agric. **11**, 198-213.

PANTEN, K.; BRAMLEY, R. G. V.; 2011: Viticultural experimentation using whole blocks: evaluation of three floor management options. Aust. J. Grape Wine Res. **17**, 136-146.

PAOLI, J. N.; TISSEYRE, B.; STRAUSS, O.; ROGER, J. M.; GUILLAUME, S.; 2005: Combination of heterogeneous data sets in Precision Viticulture. In: J. V. STAFFORD (Ed.): Precision Agriculture '05. Weimar, C.H.I.P.S.

SCHOEDL, K.; SCHUHMACHER, R.; FORNECK, A.; 2012: Correlating physiological parameters with biomarkers for UV-B stress indicators in *Vitis vinifera* (L.) cv. 'Pinot Noir' and 'Riesling' leaves, based on a two-year greenhouse study. J. Agric. Sci. (under revision).

SMITH, L.; WHIGHAM, P.; 1999: Spatial Aspects of Vineyard Management and Wine Grape Production. SIRC 99. University of Otago, New Zealand.

SOJA, G.; SOJA, A. M.; 2005: Recognizing the Sources of Stress in Wheat and Bean by Using Chlorophyll Fluorescence Induction Parameters as Inputs for Neural Network Models. Phyton **45**, 157-168.

STOLL, M.; JONES, H. G.; 2007: Thermal Imaging as a viable tool for monitoring plant stress. Int. J. Vine Wine Sci. **41**, 77-84.

STOLL, M.; SCHULTZ, H. R.; BERKELMANN-LOEHNERTZ, B.; 2008: Exploring the sensitivity of thermal imaging for *Plasmopara viticola* pathogen detection in grapevines under different water status. Funct. Plant Biol. **35**, 281-288.

STRASSER, R.; SRIVASTAVA, A.; TSIMILLI-MICHAEL, M.; 2000: The fluorescence transient as a tool to characterize and screen photosynthetic samples. Probing Photosynthesis: mechanisms, regulation and adaption. In: M. YUNUS; U. PATHRE; P. MOHANTY (Ed.): Probing photosynthesis: echanisms, regulation and adaption. London, Taylor and Francis.

TARDAGUILA, J.; BALUJA, J.; ARPON, L.; BALDA, P.; OLIVEIRA, M.; 2011: Variations of soil properties affect the vegetative growth and yield components of "Tempranillo" grapevines. Precision Agric. **12**, 762-773.

TROUGHT, M. C. T.; BRAMLEY, R. G. V.; 2011: Vineyard variability in Marlborough, New Zealand: characterising spatial and temporal changes in fruit composition and juice quality in the vineyard. Aust. J. Grape Wine Res. 17, 79-89.

VAN GAALEN, K. E.; FLANAGAN, L. B.; PEDDLE, D. R.; 2007: Photosynthesis, chlorophyll fluorescence and spectral reflectance in Sphagnum moss at varying water contents. Oecologia **153**, 19-28.

ZHANG, B.; KANG, S.; LI, F.; TONG, L.; DU, T.; 2010: Variation in vineyard evapotranspiration in an arid region of northwest China. Agric. Water Management **97**, 1898-1904.

ZRIBI, L.; FATMA, G.; FATMA, R.; SALWA, R.; HASSAN, N.; NÉJIB, R. M.; 2009: Application of chlorophyll fluorescence for the diagnosis of salt stress in tomato "*Solanum lycopersicum* (variety Rio Grande)". Sci. Hortic. **120**, 367-372.

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