Vitis **48** (2), 77–85 (2009)

# Statistical analysis of 'White Riesling' (*Vitis vinifera* ssp. *sativa* L.) clonal performance at 16 locations in the Rheinland-Pfalz region of Germany between 1971 and 2007

F. Laidig[1)], H. P. Piepho[2)] and W. Hofäcker[3)]

[1)] Bundesortenamt, Hannover, Germany
[2)] University of Hohenheim, Institute of Crop Production and Grassland Research, Stuttgart, Germany
[3)] Bad Kreuznach, Germany

## Summary

**Performance trials have been evaluated of 30 'White Riesling' clones grown at 16 locations in the Rheinland-Pfalz region between 1971 and 2007. A mixed linear model approach was used to handle the highly-unbalanced data structure. Environmental factors accounted for about 95 % of the variation for individual observations. Genotypic clone variation contributed only 0.65 % to the total variation for grape yield, 0.29 % for total soluble solids (TSS) and 0.22 % for acidity. F-tests for clonal differences showed significant F-values for each characteristic. Estimated clone means ranged from 107.4 to 130.8 kg·ar$^{-1}$ (1 ar = 100 m$^2$) for grape yield, from 72.0 to 75.2 °Oechsle for TSS and from 12.5 to 13.4 g·l$^{-1}$ for acidity. Significant mean differences were found only for clones located near the lower and upper extremes of the performance range. Long-term time trends of clonal performance are also present. On average over the 36 year period, grape yields increased by 2.00 kg·ar$^{-1}$ each year and TSS by 0.87 °Oechsle each year, whereas acidity decreased by 0.21 g·l$^{-1}$ each year.**

**No significant deviations of individual clones from the general long-term trends were verifiable for grape yield but some clones showed significant deviations for TSS and acidity.**

**A closer look at the linear trend for grape yield displayed a discontinuity around 1989. Before 1989 a linear gain of about 3.99 kg·ar$^{-1}$ was apparent whereas, after this time a very slight decrease of 0.28 kg·ar$^{-1}$ was observed. For mean daily temperature, the long-term trend was remarkably parallel to that of grape yield and TSS. For the Rheinland-Pfalz region, daily temperature increased significantly by 0.046 °C per year, whereas average daily sunshine showed a no significant change over time.**

K e y w o r d s : 'White Riesling' clones, performance trial, mixed model, mean comparison, long-term trend, variance component, stability.

## Introduction

Grapevine clones are of great importance to German viticulture. In viticulture a clone (Κλον: branch) consists of vegetatively propagated individuals from the same mother plant. All plants of a clone have the same genotype and belong to the same variety. During the process of clonal selection somatic mutations are used with the aim of getting healthier and higher-yielding vines which are better adapted to the growing environment especially in terms of soil and climate.

In Germany, more than 500 clones from 87 different grape varieties are known. There are 96 'White Riesling' clones registered in the National Variety List (Bundessortenamt 2000). 'White Riesling' used to be very favoured in German viticulture. After suffering some decreases in growing area it has increased again more recently, rising to 21,197 ha in 2006. This corresponds to about 33 % of the total area planted with white wine varieties in Germany.

Because of the prominent place of 'White Riesling', the performance of its various clones has been intensively evaluated in comparative trials during the last several decades. Sievers (1973) compared the performances of yield and must density of 35 clones (clone 239 Gm and 34 minor clones of 239 Gm) recorded from two locations in the Rheinhessen subregion between 1969 and 1971. For each clone and location observations from 8 vines were taken. Clonal means were compared using Duncan's multiple range test. Despite significant clonal differences, there was large variation in the yield data so that the author concluded that, from 3 years of results hardly any significant inferences could be drawn. To obtain reliable results on the performance of clones, Geisler and Stab (1958) suggested that at least 5 years and 4 replications were required and with observations from 25-30 vines. Moreover, they found that in the case of grape yield, the variation between vines measured by the coefficient of variation was about 5-times larger than for TSS, whereas the variation for TSS and acidity was at about the same. The recommendation of Geisler and Stab (1958) was based on the analysis of a uniformity trial for yield, must density and acidity that included 2,400 vines of 'White Riesling' at one location (Avelsbach, Mosel subregion) and over 4 years (1954-1957).

Weiling *et al.* (1977) compared a ten-year trial series (1961-1970) of 24 'Riesling' clones from two locations in the Nahe subregion with a three-year trial series (1968-1970) with an identical set of clones and locations applying univariate and multivariate analysis of variance and principal component analyses. Yield, TSS and acidity were

Correspondence to: Dr. F. Laidig, Bundessortenamt, Osterfelddamm 80, 30627 Hannover. Fax: +49-511-563362. E-mail: friedrich.laidig@bundessortenamt.de

Dedicated to Prof. Hanspeter Thöni on the occasion of his 75th birthday.

measured on 3 to 22 individual vines for each clone and location. In the three-year trial the analysis was based on 3 individual plants, whereas in the ten-year trial only the mean value from all plants was recorded. A comparison of the results from both trial series showed no satisfactory agreement. Differences occurred in the clone by year interaction, in the error variance and consequently in the ranking of clone means from both series. Data from only three years were not stable enough to provide reliable results for evaluating clones based on only three plants.

In a further study by WEILING *et al.* (1981) the performance of 14 'Riesling' clones was evaluated at six locations over 4 years (1973-1976). To control soil heterogeneity, the clones were arranged in Latin square designs with 14 replications. At 3 locations grape yield, TSS and acidity were assessed on 8 vines per plot. At each of the other locations 7, 6 or 3 vines per plot were grown. The total number of vines per clone at each location varied from 42 to 112. In contrast to the above-mentioned studies by WEILING (1971) and SIEVERS (1963) the trial layout allows efficient control of soil heterogeneity in two directions. Analysis of variance showed that trial errors were heterogeneous. Only 4 out of 6 locations showed homogenous error variances. In order to take into account heterogeneity in trial error variance, the clone means between locations were calculated by a weighting procedure using the formula:

$$\bar{\bar{y}} = \frac{\sum_i \frac{n_i}{\sigma_i^2 + r * \sigma_{po}^2} * \bar{y}_i}{\sum_i \frac{n_i}{\sigma_i^2 + r * \sigma_{po}^2}} \quad (1)$$

where y denotes the weighted clone mean, $\sigma_i^2$ denotes the error variance of the i-th location, $y_i$ the clone mean at the i-th location and $n_i$ the number of vines per clone at the i-th location. The number of replications is represented by r and the variance component for the interaction clone by location is denoted by $\sigma_{po}^2$ .

A comparison of the unweighted with the weighted clone means revealed different rankings for some clones. Clone means were compared at each location applying confidence intervals. Special attention was paid to the analysis of the clone by location interaction for grape yield in order to evaluate stability of clone performance across locations using a regression approach. The set of clones could be divided into two groups where one group contained medium to high yielding clones and the other low yielding ones.

The above clonal trial series were balanced with respect to clones, locations, years and numbers of replications per location. However, there were only 2 to 6 locations and only 3 years of data available. Especially for clones of the variety 'White Riesling', there is little variation available for the selection of new clones (BLESER *et al.* 2005). To draw reliable inferences about clonal performance in different environments, data for a larger number of locations and years is required. To analyse combined, unbalanced data originating from different trial series there are now available advanced statistical methods built into powerful statistical software applications (SMITH *et al*. 2005; PIEPHO and MÖHRING 2006). The aim of this paper is to apply mixed linear models to a set of rather unbalanced data from various multi-environmental clone trials carried out between 1971 and 2007. With this new statistical approach, inferences can be drawn from a broader range of environments. Clone breeders can benefit from these results by the provision of improved information to assist in the selection process. Also, the wine grower may profit from the results due to a more precise description of the performance properties of the various 'Riesling' clones (HOFÄCKER 1998) under a wide range of environments, so assisting with the choice of clones suitable for planting. Furthermore, indicators of the climatic conditions during the period, 1971-2007 will be compared with possible changes in clonal performance during the period of investigation.

## Material and Methods

Data: Grape yield (kg·ar$^{-1}$), TSS (°Oechsle) and acidity (g/l) were assessed as response characteristics. The yield was expressed in kg per ar (kg·ar$^{-1}$), 1 ar is 100 m$^2$. A yield of 1 kg·ar$^{-1}$ is equivalent to 100 kg·ar$^{-1}$. Total soluble solids (TSS) were measured in °Oechsle. 4 °Oechsle are approximately 1° Brix. Thirty clones (Tab. 1) of 'White Riesling' were included from 16 locations in the Rheinland-Pfalz region (Tab. 2). Data from individual trials came from different trial series with different sets of clones and trial layouts. Only the mean value for the replicates of each clone in each trial was available for analysis. The number of individual vines varied from 10 to 122 and replication from 1 to 14 (Tab. 2). Characteristics for most of the trials were measured on a pooled sample taken from the vines in each replication. When only one replicate was present in a trial, the individual vines were measured. The complete data set described in WEILING (1981) was included in this study but one location was excluded (Ungstein).

Meteorological data from 6 different weather stations representative of the 16 trial locations in Rheinland-Pfalz were provided by the German National Meteorological Service (Deutscher Wetterdienst). Average daily temperature (°C) and daily sunshine (hours) were included. Mean values from daily records were calculated for the period from 15 of July to 31 of October for all 6 weather stations and these were used for further analysis.

Statistical software: Data analysis was carried out using procedures from the statistical software package SAS Version 9.1.

Estimation of clone means: A linear mixed model approach was applied to calculate clone means for all characteristics. The model applied is:

$$y_{ijk} = \mu + G_i + L_j + Y_k + LY_{jk} + GL_{ij} + GY_{ik} + e_{ijk} \quad (2)$$

where: $y_{ijk}$ is the observed characteristic for clone i at location j in year k, $\mu$ is a general mean, G is the genotypic effect, L and Y are the main effects of location and year and LY is their interaction effect. All effects mentioned so far were regarded as fixed. GL and GY are the interaction effects of genotype by location and genotype by year, which are considered as random. The residual effect e is composed of the interaction term GLY plus the error of

Table 1

'Riesling' clones, number of years, locations and trial means and their mean values for yield, total soluble solids (TSS) and acidity

| Clone | Number of | | | Least square means | | |
|---|---|---|---|---|---|---|
| | Locations | Years | Trial means[a)] | Yield (kg·ar$^{-1}$) | TSS (°Oechsle) | Acidity (g·l$^{-1}$) |
| 110 Gm | 4 | 13 | 24 | 127.9 | 74.2 | 12.9 |
| 198 Gm | 5 | 19 | 24 | 123.4 | 74.7 | 12.7 |
| 239 Gm | 14 | 37 | 108 | 118.8 | 73.8 | 12.7 |
| 34 Trier | 3 | 12 | 22 | 115.6 | 73.3 | 12.8 |
| 37 Trier | 14 | 36 | 124 | 124.7 | 72.3 | 12.9 |
| A 2 | 4 | 17 | 31 | 113.1 | 74.8 | 12.9 |
| BW | 6 | 16 | 54 | 125.1 | 72.0 | 13.1 |
| Bernkastel 68 | 13 | 37 | 110 | 111.0 | 73.5 | 13.0 |
| DH 20 | 3 | 9 | 10 | 122.6 | 73.5 | 12.6 |
| DN 378 | 14 | 36 | 107 | 121.3 | 73.4 | 12.6 |
| DN 391 | 3 | 14 | 25 | 119.3 | 74.2 | 12.8 |
| DN 500 | 8 | 21 | 46 | 124.1 | 74.7 | 13.0 |
| FR 52 | 4 | 14 | 28 | 119.7 | 74.5 | 12.6 |
| Heinz 65 | 14 | 37 | 115 | 125.3 | 73.7 | 12.7 |
| Krötz 22 | 7 | 21 | 53 | 121.6 | 73.7 | 13.0 |
| M 122 | 6 | 16 | 54 | 114.7 | 73.7 | 12.9 |
| N 90 | 16 | 37 | 127 | 114.9 | 73.4 | 12.9 |
| Population | 7 | 20 | 63 | 113.6 | 73.1 | 13.1 |
| R 1 | 7 | 16 | 65 | 116.6 | 73.3 | 12.8 |
| Schlöder 40 | 7 | 18 | 44 | 127.4 | 73.5 | 12.8 |
| Schäffer 3 | 3 | 14 | 15 | 116.8 | 73.7 | 13.0 |
| St. 7 | 6 | 16 | 54 | 123.3 | 72.9 | 12.7 |
| Trautwein 356 | 13 | 35 | 115 | 121.7 | 73.2 | 12.8 |
| Veit 11 | 3 | 14 | 25 | 107.4 | 74.2 | 12.8 |
| WZ 2090 | 8 | 26 | 63 | 120.3 | 73.9 | 12.6 |
| We 158 | 5 | 21 | 35 | 113.7 | 73.1 | 12.8 |
| Weis 1 | 4 | 14 | 33 | 119.9 | 73.4 | 13.0 |
| Weis 17 | 4 | 15 | 23 | 130.8 | 73.0 | 13.4 |
| Weis 21 | 12 | 37 | 105 | 128.5 | 72.9 | 13.3 |
| Weis 29 | 2 | 18 | 20 | 114.8 | 75.2 | 12.5 |
| mean | - | - | - | 119.9 | 73.6 | 12.9 |

[a)] Total number of trial means 1722.

the trial mean of clone i at location j in year k. It cannot be assumed that residual variances are homogeneous because different numbers of vines and replications were present at each location. Therefore, heterogeneous residual variances Var ($e_{ijk}$) = $\sigma^2_j$, are allowed for each location j. For multiple comparisons of estimated means confidence intervals were calculated at the significance level of $\alpha = 0.01$ using the procedure as described by Piepho (2000), which can be applied also to unbalanced data.

Estimation of variance components: To evaluate the influence of environments and clones on the variability of yield, TSS and acidity, variance components of the effects in model (2) were estimated using the REML- option in SAS PROC VARCOMP. Due to limitations of SAS PROC VARCOMP and of computing capacity, homogeneity of the residual component was assumed.

Stability of clone performance across locations: The regression of clone by location means over years on the location means was calculated using PROC MIXED as a measure of the stability of individual clones with respect to location. The underlying model was:

Table 2

Trial locations, subregions, estimated location means and error variances, number of vines and replications

| Location | Subregion | Location means | | | Residual variance | | | Trial parameters[a)] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Number of | | |
| | | Yield $(kg \cdot ar^{-1})^2$ | TSS $(°Oechsle)^2$ | Acidity $(g \cdot l^{-1})^2$ | Yield $(kg \cdot ar^{-1})^2$ | TSS $(°Oechsle)^2$ | Acidity $(g \cdot l^{-1})^2$ | vines per clone | reps | vines per rep |
| Avelsbach | Mosel | 102.2 | 79.4 | 11.8 | 127.40 | 3.62 | 0.09 | 120/40 | 4/2 | 30/20 |
| Filzen | Mosel | 110.7 | 65.6 | 14.5 | 40.21 | 1.14 | 0.10 | 80 | 4 | 20 |
| Leiwen | Mosel | 100.1 | 65.9 | 14.9 | 34.85 | 0.32 | 0.04 | 122 | 14 | 8 |
| Oberemmel | Mosel | 110.9 | 60.4 | 16.5 | 29.08 | 1.25 | 0.11 | 100 | 5 | 20 |
| Ockfen | Mosel | 85.7 | 67.1 | 15.7 | 24.99 | 1.71 | 0.11 | 122 | 14 | 8 |
| Serrig | Mosel | 80.9 | 77.3 | 12.5 | 31.76 | 1.28 | 0.04 | 84 | 14 | 7 |
| Trier | Mosel | 104.3 | 66.4 | 12.5 | 11.97 | 0.42 | 0.04 | 98 | 14 | 7 |
| Altenbamberg | Nahe | 122.1 | 80.9 | 10.5 | 444.03 | 7.85 | 0.22 | 10 | 1 | 10 |
| Bad Kreuznach | Nahe | 87.8 | 87.7 | 10.0 | 458.59 | 15.78 | 0.51 | 10 | 1 | 10 |
| Niederhausen | Nahe | 83.4 | 64.0 | 15.1 | 18.17 | 1.19 | 0.05 | 112 | 14 | 8 |
| Bechtheim | Rheinhessen | 161.1 | 78.2 | 9.5 | 312.74 | 3.23 | 0.21 | 10 | 1 | 10 |
| Gau Odernheim | Rheinhessen | 230.4 | 73.4 | 12.7 | 316.21 | 3.95 | 0.06 | 10 | 1 | 10 |
| Herrnsheim | Rheinhessen | 148.2 | 77.1 | 11.0 | 191.41 | 6.44 | 0.21 | 40/10 | 4/1 | 10/10 |
| Nierstein | Rheinhessen | 133.8 | 90.6 | 8.2 | 130.04 | 2.75 | 0.15 | 80 | 4 | 20 |
| Kallstadt | Pfalz | 197.9 | 81.5 | 11.0 | 317.72 | 3.74 | 0.13 | 40/10 | 4/1 | 10/10 |
| Kirchheim | Pfalz | 171.2 | 78.7 | 12.8 | 227.67 | 3.66 | 0.12 | 10 | 1 | 10 |

[a)] '/' indicates that different numbers of replications and vines per replication occurred.

$$y_{ij} = a_i + b_i * x_j + e_{ij} \quad (3)$$

where $y_{ij}$ is the clone by location over years least square mean and $x_j$ is the least square mean for location over years and clones. The regression coefficient for clone i is denoted by $b_i$ and is the stability parameter. The constant for clone i is $a_i$. If clone i has a regression coefficient of $b_i < 1$ then it is considered to show a more-than-averagely stable response to increasing fertility levels of locations. If $b_i > 1$ then clone i is considered to react more sensitively - in other words, it is less stable (HOFÄCKER, 1998). The hypothesis was tested whether slopes are identical for all clones vs. whether slopes are not all identical.

Test of linear time trend of clone performance: The question whether clone performance changed during the period 1971-2007 was investigated by the model:

$$y_{ik} = a_i + (\mathbf{g} + \mathbf{d_i}) * \mathbf{t_k} + e'_{ik} \quad (4)$$

where $y_{ik}$ is the clone by year over locations least square mean for clone i in year k, $a_i$ is the constant for clone i, $\mathbf{t_k}$ is the covariate time at year k, **g** the common regression coefficient and $\mathbf{d_i}$ is the deviation of the regression coefficient of clone i from the common regression line **g**. The error term is partitioned as $e'_{ik} = u_k + e_{ik}$ , where $u_k$ is the random deviation from the regression line of the mean value at year k and $e_{ik}$ denotes the random deviation of clone i from the year mean. First, the hypothesis was tested that slopes $\mathbf{d_i}$ are homogeneous and then that the common slope **g** is not equal to zero using PROC MIXED.

Test for discontinuous linear time trend: The common time trend **g** in model (4) was assumed to be linear. However, to allow for a broken time trend composed of two connected straight lines with different slopes, model (4) was adjusted to:

$$y_{ik} = a_i + (\mathbf{g_1} + \mathbf{x} * \mathbf{g_2}) * \mathbf{t_k} + e'_{ik} \quad (5)$$

A dummy variable **x** was introduced with $\mathbf{x} = 1$ if $\mathbf{t_k} > 1988$ and $\mathbf{x} = 0$ if $\mathbf{t_k} \leq 1988$, which defines the assumed break point of the regression line on the time axis. The intercept for clone i is given by the constant $a_i$. Then the parameter for the slope is $\mathbf{g_1}$, if $\mathbf{t_k} \leq 1988$ and $\mathbf{g_1} + \mathbf{g_2}$, if $\mathbf{t_k} > 1988$. This means that $\mathbf{g_2}$ represents the change of slope of the regression line at the break point. As in model (4), the error term is $e'_{ik} = u_k + e_{ik}$, however for $e_{ik}$ heterogeneous error variances were allowed for both sections of the regression line as indicated by x.

## Results

Environmental and genotypic by environmental variation: Clonal data show considerable variability across locations and years. The extent of variation depends on many factors such as soil, management practices, climatic conditions, genetic potential of

Table 3

Variance component estimates from 'Riesling' clone performance trials, 1971 to 2007

| Component[a)] | Yield | | | | TSS | | | | Acidity | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Estimate | Confidence limits[b)] | | Estimate | Estimate | Confidence limits[b)] | | Estimate | Estimate | Confidence limits[b)] | | Estimate |
| | $(kg \cdot ar^{-1})^2$ | lower | upper | (% of total) | $(°Oechsle)^2$ | lower | upper | (% of total) | $(g \cdot l^{-1})^2$ | lower | upper | (% of total) |
| L | 1673.1 | 835.6 | 4877.1 | 46.1 | 26.6 | 10.9 | 136.5 | 14.7 | 1.23 | 0.52 | 5.80 | 6.7 |
| Y | 983.0 | 555.2 | 2196.1 | 27.1 | 112.9 | 68.5 | 220.0 | 62.2 | 13.90 | 8.87 | 24.90 | 76.1 |
| LxY | 782.6 | 582.7 | 1107.0 | 21.6 | 38.0 | 28.7 | 52.2 | 20.9 | 2.92 | 2.21 | 4.05 | 16.0 |
| G | 25.8 | 14.0 | 62.8 | 0.7 | 0.5 | 0.3 | 1.1 | 0.3 | 0.03 | 0.02 | 0.07 | 0.2 |
| GxL | 32.7 | 23.4 | 49.0 | 0.9 | 0.4 | 0.2 | 0.7 | 0.2 | 0.03 | 0.03 | 0.05 | 0.2 |
| GxY | 10.1 | 4.5 | 40.1 | 0.3 | 0.5 | 0.3 | 0.8 | 0.3 | 0.03 | 0.02 | 0.04 | 0.2 |
| e | 120.6 | 108.9 | 134.4 | 3.3 | 2.7 | 2.5 | 3.0 | 1.5 | 0.11 | 0.10 | 0.13 | 0.6 |

[a)] Location (L), year (Y), location by year (LxY), genotype (G), genotype by location (GxL), genotype by year (GxY), residual (e).
[b)] $1 - \alpha = 0.95$.

clones and the age of the vines. In Tab. 3, estimates of variance components and their confidence limits ($1 - \alpha = 0.95$) for grape yield, TSS and acidity are presented. Environmental factors, location, year and their interactions explain about 95 % of the total variation. However, locations and years have different influences on the variations of yield and of TSS. Whereas for yield almost half of the variation is caused by differences from location to location, for TSS, variation from year to year explained nearly two thirds of the total variability. However, differences between genotypic values of clones are rather small. They account only for 0.65 % for yield, 0.29 % for TSS and 0.22 % for acidity. The residual component composed of the interaction of clone by location by year plus the error of the trial mean is the dominant part of the clone by environment interaction. Fortunately, only the magnitude of the clone by environment interaction terms is of importance for the precision of mean comparisons, the environmental components do not influence the precision of the clone mean comparisons.

Comparison of clonal performance: F-tests for clonal differences were significant for each characteristic at $p < 0.0001$. Relatively low values of genotypic variance components indicate that only moderate differences between clone means can be expected. In Fig. 1 a-c the least square means and their confidence intervals related to differences are plotted. Yield means vary from 107.4 to 130.8 $kg \cdot ar^{-1}$, TSS from 72.0 to 75.2 °Oechlse and acidity from 12.5 to 13.4 $g \cdot l^{-1}$. Means with overlapping confidence intervals are not significantly different at $\alpha = 0.01$. In Fig. 2 a, for example, the mean of 'Veit 11' is not significantly different from 'R1' and 'Schäffer 3' but it differs significantly from '239 Gm'. Means of clones grown at a large number of different locations and over several years have small confidence intervals as, for instance, is the case with 'N 90', '239 Gm' or 'DN 378'. Locations with high residual variances contribute more to enlarged confidence intervals than locations with smaller ones. Estimates of residual variances for yield vary between 11.17 for 'Trier' and 458.59 for 'Bad Kreuznach' and for TSS from 0.42 for

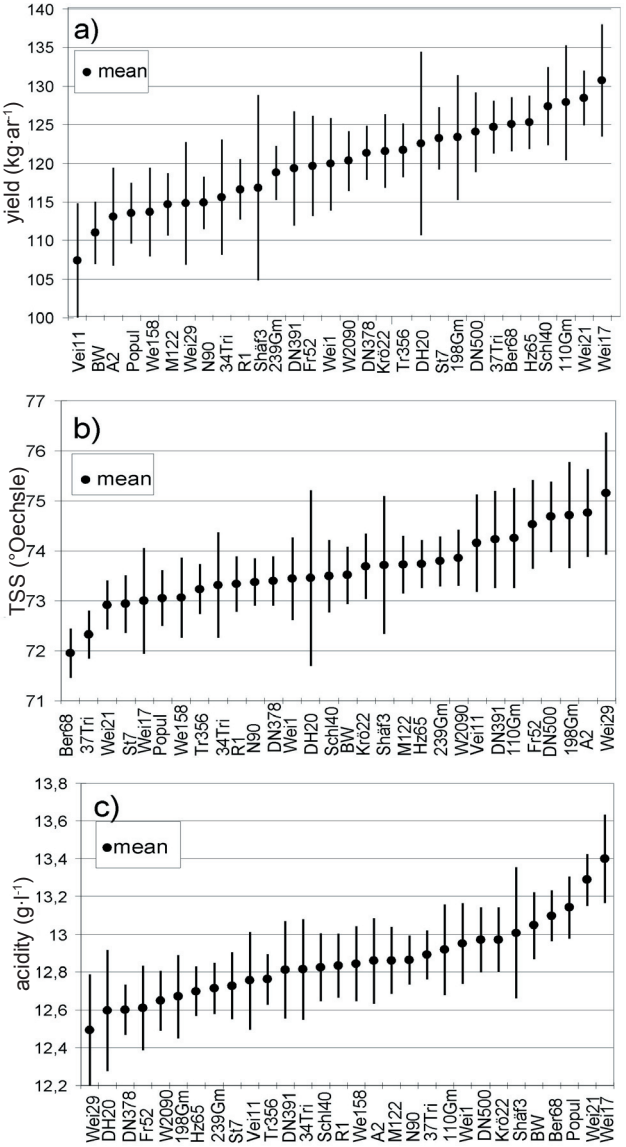


Fig. 1: Comparison of mean values for **a**) yield, **b**) total soluble solids (TSS) and **c**) acidity with confidence limits ($1 - \alpha = 0.99$).

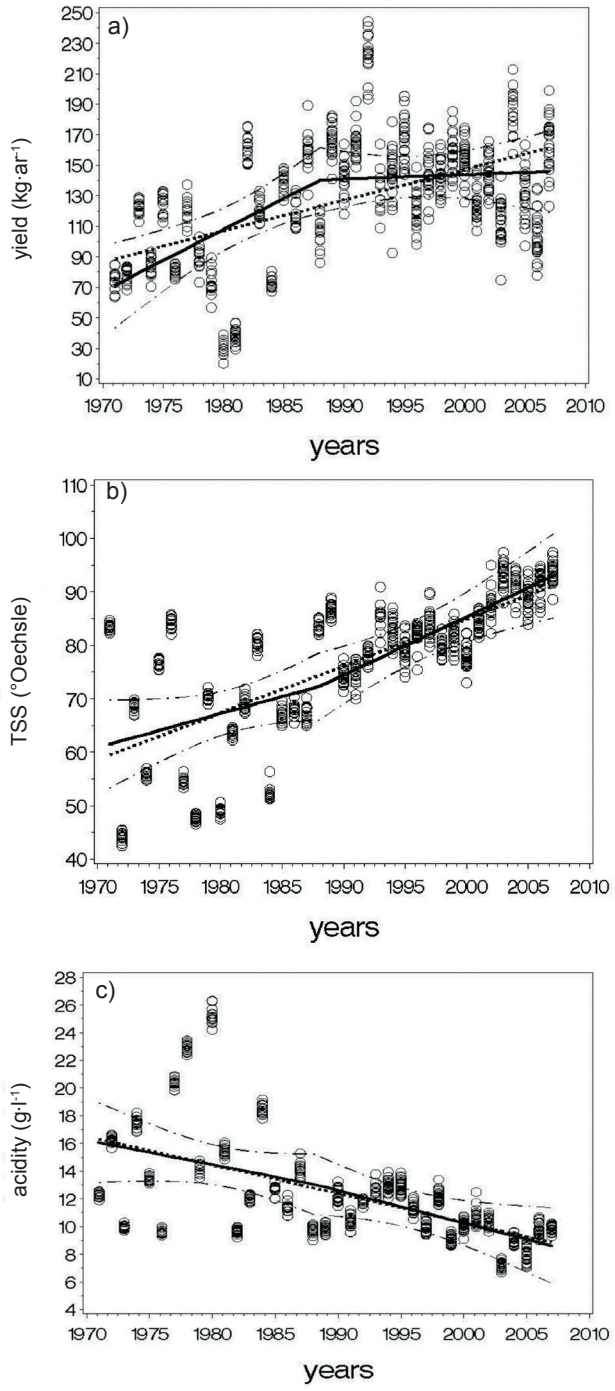


Fig. 2: Linear time trend (dashed line) and discontinuous linear time trend (solid line) with confidence bands (1- α = 0.99) for **a**) grape yield, **b**) total soluble solids (TSS) and **c**) acidity of 'Riesling' clones during the period 1971 to 2007.

'Trier' and 15.78 for 'Bad Kreuznach', whereas for acidity they range between 0.05 for 'Niederhausen' and 0.51 for 'Bad Kreuznach' (Tab. 2). Comparison of clone means shows that most of the clonal differences are not significant except for clones that are present in many years and locations, and clones with means located towards the upper or lower extremes of the scale. The majority of clonal means located in a broad, middle range do not differ significantly (Fig. 1 a-c).

Stability of clonal performance: Differences in clonal performance are of primary importance. However, besides a high level of yield and TSS, the stability of clonal performance across environments is of special interest to vine growers. Those clones are to be preferred that have high average yields, and yet are less variable across environments. Tests for difference in slopes for stability parameter $b_i$ in equation (3) was not significant for yield ($p = 0.02$), TSS ($p = 0.02$) or acidity ($p = 0.21$). From the data available it cannot be concluded that clones react significantly differently ($p < 0.01$) to varying growing conditions from location to location.

Time trend in clonal performance: For some of the clones listed in Tab. 1, data are available for more than 30 years. The question whether a long range time trend (**g** of equation (4) not equal to zero) is present, has been evaluated. F-tests for **g** were significant for yield ($p < 0.001$), TSS ($p < 0.001$) and acidity ($p = 0.001$). Average grape yield for clones increased by **g** = 2.00 kg·ar$^{-1}$, TSS by **g** = 0.87 °Oechsle, whereas acidity decreased by **g** = -0.21 g·l$^{-1}$ per year (Fig. 2 a-c). Deviation of time trends for individual clones $\mathbf{d}_i$ from their common trend **g** were not significantly different for yield ($p = 0.0274$), however for TSS ($p < 0.0001$) and acidity ($p < 0.0001$). A t-test for individual clones indicated that for TSS the deviations $\mathbf{d}_i$ were significantly different from zero ($p < 0.01$) for clones '239 Gm' (-0.11 g·l$^{-1}$), 'Schlöder 40' (+ 0.17 g·l$^{-1}$) and 'Weis 17' (+0.20 g/l) and for acidity the deviations of clones 'Trautwein 356' (-0.02 °Oechsle) and 'Veit 11' (+ 0.08 °Oechsle) were significant.

Test of discontinuous time trend: Inspection of the plot of clone by year means for yield against years as represented in Fig. 2 a suggests that there is a break in the trend around 1989. A test of the hypothesis of a linear *vs.* a discontinuous linear time trend for yield, TSS and acidity was not significant at $p < 0.01$ (Tab. 4). Only for yield is some evidence of a break apparent.

For yield the average yearly increase before 1989 was 3.99 kg·ar$^{-1}$ and, after this there was a slight, but not-significant ($p = 0.069$), decrease of about 0.28 kg·ar$^{-1}$. When looking at Fig. 2 a, clone yields are apparently less variable from year to year after 1988 than during the previous period. For TSS no significant change of trend was observed. The yearly increase was 0.62 °Oechsle before 1989 and 1.08 °Oechsle afterwards. As for TSS, variation of the means around the regression line is smaller after 1989 than in the earlier years. For acidity there is a negative trend with time. Acidity decreased by about 0.17 g·l$^{-1}$ before 1989 and afterwards by about 0.18 g·l$^{-1}$ per year.

Temperatures and daily sunshine: As can be seen from Fig. 3a there was a clear increase in daily mean temperature during 1971-2007. The average temperature rose by 0.046 °C per year which corresponds to a total increase of 1.7 °C between 1971 and 2007. The regression coefficient was highly significant ($p < 0.001$). Daily sunshine shows no clear change over time (Fig. 3 b).The regression coefficient of -0.01 h per year was not significantly different from zero ($p = 0.25$).

## Discussion

The mixed linear model approach allows evaluation of clonal performance trials from unbalanced datasets and

Table 4

Slopes ($g_1$ before 1989, $g_1+g_2$ after 1988) for linear time trend and significance levels of F-tests for yield, total soluble solids and acidity (TSS) for 'Riesling' clones

| Regression slope | Yield (kg·ar$^{-1}$) | | TSS (°Oechsle) | | Acidity (g·l$^{-1}$) | |
|---|---|---|---|---|---|---|
| | Estimate | Prob>F | Estimate | Prob>F | Estimate | Prob>F |
| $g_1$ [a)] | +3.99 | <.0004 | +0.62 | <.0001 | -0.18 | <.0001 |
| $g_2$ [b)] | -3.71 | <.0693 | +0.46 | 0.4420 | -0.06 | 0.7880 |
| ($g_1 + g_2$ [b)] | +0.28 | 0.7862 | +1.08 | 0.0006 | -0.24 | 0.0335 |

[a)] 1971-1988, [b)] 1989-2007.

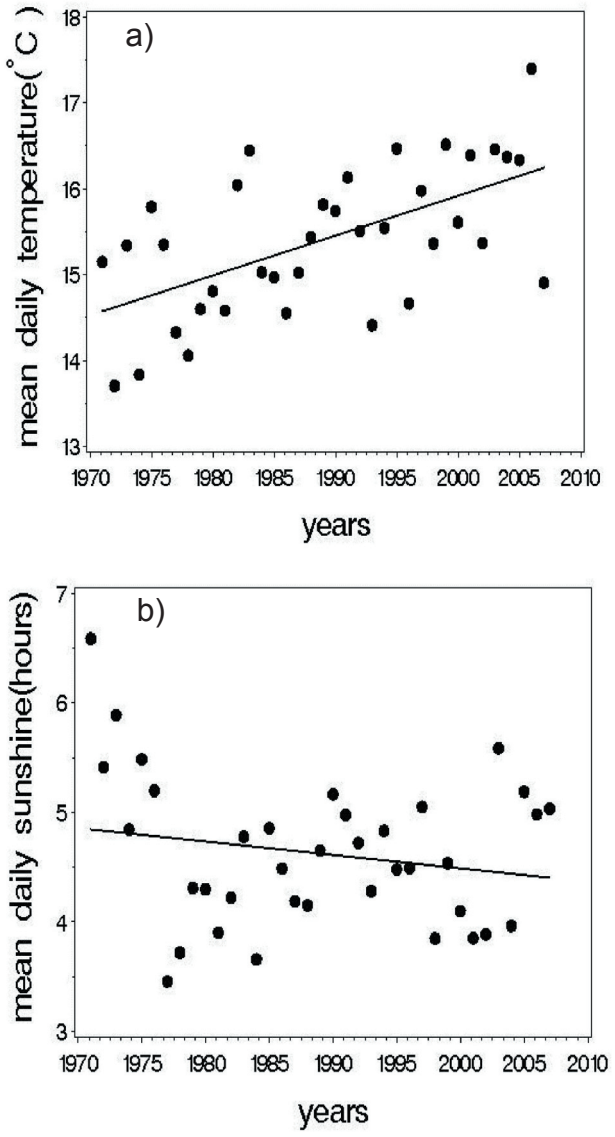


Fig. 3: Trends of **a**) mean daily temperature and **b**) daily sun shine 1971 to 2007.

also a full consideration of their underlying error structures. It provides a very flexible framework that is able to account for multiple sources of random variation (Piepho *et al*. 2003). Inferences drawn from mixed model analyses are generally more efficient than from ANOVA approaches based on fixed effects models (Smith *et al.* 2005, Piepho and Möhring 2006). Clone data used in Geisler and Stab (1958), Sievers (1973), Weiling *et al*. (1977, 1981) and Hofäcker (1998) are balanced with respect to the factors: clones, locations and years. In this study 1722 observations were available; this means that only 10 % of the cells from a fully-balanced data set were occupied. When datasets from several sources are combined, the range of environments is broadened allowing for more reliable information to evaluate clonal performance. Loss of balance renders the statistical analysis somewhat more involved but this is more than compensated for by a broader inferential basis.

In this study, significance tests of clone effects and confidence limits for estimated clone means do not consider only the heterogeneous error variances for locations $\sigma_j^2$ (Tab. 1), but also the covariance structure generated by the random effects of genotype by location $GL_{ij}$ and genotype by year $GY_{ik}$ interactions. Geisler and Stab (1958), Sievers (1973) and Weiling *et al.* (1977 and 1981) applied fixed effects models of the ANOVA approach, where clone effects were tested against error variances based on within-trial variation neglecting variation arising from random effects of genotype by environmental interaction. In the literature cited here, different error terms based on within-trial variation were chosen for testing clone effects. Sievers (1973) and Weiling *et al.* (1977) in a ten-year study used error mean squares based on variation between individual vines, whereas Geisler and Stab (1958) and Weiling *et al.* (1977) in a three-year study and Weiling *et al.* (1981) used plot means from pooled vines arising from one or more replicates to calculate error mean squares. Inferences drawn from data analysis depend upon the model type chosen. For the fixed effects model, standard errors for clone mean differences tend to be smaller because they are based merely on within-trial variation. Hence, significant results are more likely than from the mixed model approach. Conclusions drawn from a fixed effects model are valid only for the specific environments included in the study. For a mixed effects model, however, conclusions can be drawn for a population of target environments, if locations included can be regarded as a random sample from that population. Conclusions drawn from the results in this study are valid for the vine growing areas of the Rheinland-Pfalz region. By evaluating the magnitude of variance component estimates and their relative size among individual components of 'Riesling' clones, it must be recognised that clone genotypes represent only that part of the variation that is available within a particular variety. Registered clones must be non-distinct with respect to their morphological characteristics. Expression of morphological characteristics of clones should correspond to the genotype of the variety 'White Riesling'. Hence, large variations of genotypic

variance and interactions with the environment are not to be expected. The size of estimated variance components from a long-term study on the variability of yield for cultivars from 32 agricultural crops in German official variety trials (Laidig *et.al.* 2008) as compared with the corresponding figures in Tab. 3 for clones, demonstrates the different variability structure. Yield variability for agricultural varieties from all 32 species (*vs*. grape yield for 'Riesling' clones) for the genotypic variance is of size 6.3 % (0.7 %), for interaction of genotype by location 1.9 % (0.9 %), genotype by year 1.2 % (0.3 %) and for residual 6.0 % (3.3 %) of total variation. Environmental variation for varieties of agricultural crops accounts for about 75 % of total variability compared to almost 95 % in 'Riesling' clones. Genotypic variation of agricultural varieties is about 9-times larger than that for the 'Riesling' clones investigated in this study.

Estimates of variance components are subject to large errors and hence have wide confidence intervals except for the residual components (Tab. 3), so care is needed when interpreting the estimates of Tab. 3.

This study has shown that significant long-term trends exist. These are for increasing grape yield and TSS but for decreasing acidity (Fig. 2a, b, c). For grape yield, the slopes for the individual clones do not differ significantly from one another; *i.e*. all 'Riesling' clones follow about the same time trend in this characteristic. However, for TSS and for acidity the time trends differ significantly. The result of significantly different slopes of individual clones for TSS and acidity should be treated with caution because many of the clones were not tested in each year. The number of testing-years for individual clones ranged from 9 to 37 (Tab. 1). The question arises as to just which factors caused the trend patterns observed. Genetic improvement of clones by continued breeding, progress in management practices and changing climatic conditions can all be considered possible explanations. It is less likely that breeding was of much importance here because the potential for generating large variations between 'White Riesling' clones is small compared with the clones of other varieties (Bleser *et.al.* 2005). To obtain an indication as to whether the trends observed were influenced by genetic improvement, the relationship between the estimated mean values and the years in which the various clones were registered was considered. However, there was no obvious evidence of any dependence. Hence, consideration of an additional, specific term for a genetic trend in model (4) is not justified. The trend for increasing daily mean temperature (Fig. 3 a) provides favourable conditions for higher TSS. The well known negative correlation between TSS and acidity could be an explanation for the decreasing acidity. It is even more likely that acidity decreases more rapidly during the ripening period due to climatic changes leading to increased temperatures. The dominant factor underlying the strong increase in grape yield of 2.00 $kg \cdot ar^{-1}$ per year may also be attributed to gradually improving fertilizer applications and to better pesticides and application technologies as well as to improving viticultural techniques. The positive linear time trend in grape yield was subject to a remarkable change after 1988 (Fig. 2 a) without significantly influencing the continuous linear trend for acidity. For TSS the positive time trend seems to be re-enforced after 1988. Its estimated slope increased from 0.62 to 1.08 °Oechsle per year. The main reason is likely a change in the management practice around 1988 in favour of higher wine quality and lower grape yield. Lower numbers of buds, shoots and grapes per shoot as well as a higher leaf/fruit ratio may have caused the change observed.

An extension of the analyses presented in this paper should be considered. For example, instead of a two-stage analysis, where clone by year means are computed in the first stage, and these are then subjected to mixed model analysis in the second stage, the year by location by clone classification could be analysed in a single step by REML. Also, locations can be grouped into four growing subregions as represented in Tab. 1, and the model could be extended accordingly (Piepho and Möhring 2005). The regression on the environmental mean could be replaced by a factor-analysis model (Piepho 1997), thus accounting for the unbalancedness of the dataset. This approach was attempted as an alternative to the applied regression model but the iteration process for calculating the likelihood functions did not converge. These, more sophisticated analyses turned out to be difficult or impossible for our dataset because of the sparseness of the three-way classification and, also the large number of clones.

## Acknowledgements

The authors thank the “Zentralstelle für Klonenselektion”, Trier, the former “Landesanstalt für Rebenzüchtung Alzey”, the “Dienstleistungszentrum Ländlicher Raum Rheinhessen-Nahe-Hunsrück” for kindly providing the clone data. We also thank K. Frühauf from the German Meteorological Service, Geisenheim, for preparing the weather data.

## References

Bleser, E.; Konrad, H.; Rühl, E. H.; 2005: Perspektiven der Klonzüchtung. Deutsches Wein-Jahrbuch 2005, **56**, 116 -121.

Bundessortenamt; 2000: Beschreibende Sortenliste Reben.

Geisler, G.; Stab, J.; 1958: Versuchsanstellung im Weinbau. Vitis **1**, 257-281.

Hofäcker, W.; 1998: Charakterisierung und Beurteilung von Selektionsmerkmalen in der Rebenzüchtung. Mitt. Klosterneuburg **48**, 107-117.

Laidig, F.; Drobek, T.; Meyer, U.; 2008: Genotypic and environmental variability of yield for cultivars from crops in German official variety trials. Plant Breed. **127**, 541-547.

Piepho, H. P.; 1997: Analyzing genotype-environment data by mixed models with multiplicative effects. Biometrics **53**, 761-766.

Piepho, H. P.; 2000: Vertrauensintervalle für multiple Vergleiche bei ungleichen Standardfehlern der Differenzen. Z. Agrarinformatik **8**, 54-58.

Piepho, H. P.; Büchse, A.; Emrich, K.; 2003: A hitchhiker’s guide to the mixed model analysis of randomized experiments. J. Agron.Crop Sci. **189**, 310-322.

Piepho, H. P., Möhring, J.; 2005: Best linear unbiased prediction for subdivided target regions. Crop Sci. **45**, 1151-1159.

Piepho, H. P., Möhring, J.; 2006: Selection in cultivar trials – is it ignorable? Crop Sci. **46**, 193-202.

SAS Institute Inc., SAS/STAT Users Guide, Vers. 9.1, Cary, NC, USA.

Sievers, E.; 1973: Erste Resultate der Prüfung neuer Riesling-Klone. I. Die „Kleinklone“ aus dem Klon 239 Geisenheim. Weinberg und Keller **20**, 233-247.

Smith, A. B.; Cullis, B. R.; Thompson, R.; 2005: The analysis of crop cultivar breeding and evaluation trials: an overview of current mixed model approaches. J. Agricult. Sci. **143**, 449-462.

Weiling, F.; Unger, C.; Goedecke, H.; Schöffling, H.; 1977: Vergleich der Aussagekraft eines zehnjährigen und eines dreijährigen Leistungsversuches mit 24 Riesling-Klonen an zwei Standorten der Nahe. Mitt. Klosterneuburg **27**, 47-69.

Weiling, H.; Schöffling, H.; Unger, C.; 1981: Ein Leistungsversuch mit 14 Riesling-Klonen an sechs Standorten verschiedener Weinbaugebiete über vier Jahre mit jeweils 14 Wiederholungen. Mitt. Klosterneuburg **31**, 1-19.

*Received July 9, 2008*