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The effect of vineyard long-term monoculture soil on production of volatile compounds and photosynthetic apparatus in grapevine leaves

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Summary

The effect of soil collected from long-term (1007 years of duration of monoculture) and short-term (55 years) grapevine monoculture on production of volatile organic compounds (VOC's) and on functional parameters of photosystem II in grapevine leaves was analyzed. Grapevine plantlets grown in tested soils showed differences in VOC's production after five months cultivation. Chlorophyll *a* fluorescence measurements by JIP-test revealed that the photosystem II was less efficient but the fluorescence intensity increased in plant growing in soil from the long-term monoculture compared to plants growing in the short term monoculture soil. *Pseudomonas* spp. carrying the biocontrol genes *phlD* and *hcnAB* were isolated from long-term monoculture soil. A consortium of ten of these isolates was added to the short term monoculture soil. The plants grown in this inoculated soil showed similar changes in fluorescence intensity and photosystem efficacy as the plants growing in long term monoculture. In this study, simple tools for measurement of a "soil effect" by measuring only a leaf have been tested successfully. They have allowed exhibiting the influence of long-term monoculture on plant physiology.

K e y w o r d s : Monoculture, *Vitis, Pseudomonas,* Chlorophyll *a* fluorescence, volatile compounds.

Introduction

Soil is one of the most important factors, which influences directly the plant growth. In the vineyard, a soil, together with geographic location, climate, grapevine cultivar, rootstock, enological and viticultural techniques, is a valuable part of an interactive ecosystem called "terroir" (Seguin 1988, Falcetti 1994).

Positive effect of long-term grapevine monocultures on the amount of bacteria genus *Pseudomonas* spp. carrying the natural antimicrobial biosynthetic genes *phlD* and *hcnAB* responsible for production of 2,4-diacetylphloroglucinol (Phl) and hydrogen cyanide (HCN), has been shown in our previous study (Svercel *et al.* 2009, 2010). These root-associated bacteria are very important biocontrol agents (Haas and Défago 2005). An altering effect of pseudomonads on grapevine plants has been shown, e.g. biological control of crown gall (Khmel *et al.* 1998) and biological control of gray mold (Barka *et al*. 2000). Furthermore, *Pseudomonas* spp., certain *Erwinia* strains and vesicular arbuscular mycorrhizal (VAM) fungus *Glomus mosseae* appeared promising for reducing chlorosis occurrence of grapevine grafted on medium lime-tolerant rootstocks (Bavaresco and Fogher 1996 a and b). Moreover, *Pseudomonas* inoculation enlarged the chlorophyll concentration in grapevine leaves compared with untreated plants (Bavaresco and Fogher 1996 a). The photosynthetic efficiency could be measured due to a fluorescence of chlorophyll *a* (Chl *a*) and this measure provides information on the relationship between the structure and the function of the photosystem II (PS II) reaction centre (RC) and core complexes (Rosenqvist and Van Kooten 2003). Fluorescence measurements have been used largely, e.g. in agronomy, forestry, marine environment, ecotoxicology, plant physiology and plant breeding (Deell and Toivonen 2003). The polyphasic rise of the Chl *a* fluorescence transient (OJIP labelled phases) could be analyzed with a tool called JIP-test. This tool has been developed to investigate *in vivo* the "vitality" of plants and the adaptive behaviour of the photosynthetic apparatus (Strasser and Strasser, 1995, Tsimilli-Michael *et al.* 1995, 1996, Srivastava and Strasser 1996, Christen *et al.* 2007). However, the effect of long-term monoculture soil (or soil bacteria) on PS II is quite unknown.

The formation of volatile organic compounds VOC's has been shown to be essential (to the taste and aroma of wines) not only in the grapevine berries (Lund and Bohlmann 2006), but subsequently in grapevine leaves (van den Boom *et al*. 2004). Many VOC's produced by leaves were determined as important mediators of tritrophic interactions such as predator-herbivore-plant interactions or parasitoid-herbivore-plant interactions (van den Boom *et al.* 2004). Similarly, as by PSII, the effect of soil on production of VOC's especially in leaves is rather unknown.

In this study, an influence of long-term monoculture soil on a plant as one of the "terroir" parameters, by measuring only a leaf was analyzed by an application of simple tools. Two tools have been used to achieve this aim: i) the fingerprints of VOC's from grapevine leaves to elucidate differences between the plants maintained in long-term vs. short-term monoculture soils with similar properties, and ii) the measurements of Chl *a* fluorescence transient of grapevine planted in above mentioned soils and observe functional behaviour differences of the PS II of the plants in used soils. To answer a question, whether pseudomonads have an influence on PS II of the plants, soil from short-term monoculture was inoculated with *phlD*$^+$ *hcnAB*$^+$ pseudomonads isolated from long-term vineyard soil.

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Material and Methods

Soil sampling and plant: Soils were collected in June 2004 from short-term (55 years of continual grapevine monoculture) and adjacent long-term (1007 years) vineyard in the region of Bevaix/Neuchâtel (NE) in Switzerland, and chemical and physical soil properties were analyzed (Tab. 1). Soil samples were taken randomly from five points in the vineyards avoiding the border areas, from 10-30 cm depth using sterilized shovels. Each soil was kept in large plastic bags at 15 °C before use (approx. one year). Root residues and stones were removed and soil samples from the same site were pooled. A soil for pot plants without turf - Kübelpflanzenerde ohne Torf (Ricoter, Aarberg, Switzerland) was used as control in VOC's test.

Table 1

Characteristics[a] of used soils

Soils[b]	Neuchâtel	
Grapevine monoculture in years[c]	55	1007
Particle size distribution[d]		
Clay (%)	20.5	22.8
Silt (%)	47.1	52.9
Sand (%)	32.4	24.3
Exchange capacity		
[$BaCl_2$ triethanolamine]		
Saturation CEC[e] (cmol kg^{-1})	13.1	14.7
Saturation CEC (%)	99.6	97.2
K (%)	4.2	4.1
Ca (%)	82.7	82.4
Mg (%)	11.8	10.0
Na (%)	0.9	0.7
H (%)	0.3	2.8
Soluble elements		
B [hot water] (mg/kg)	1.2	1.3
Reserve elements		
[NH_4-Ac.+EDTA 1:10] (mg kg^{-1})		
P	104.5	78.6
K	301.6	318.3
Ca	75652.0	66773.0
Mg	629.3	613.5
Oligo elements		
[NH_4-Ac.+EDTA 1:10] (mg kg^{-1})		
Cu	161.3	162.6
Fe	308.0	290.0
Zn	19.1	20.1
Mn	325.0	327.0
Organic matter [titration] (%)	1.4	1.5
pH [water]	7.9	7.9
$CaCO_3$ [total] (%)	27.0	18.0
N total [Kjeldahl] (%)	0.1	0.1

[a] Soils were analyzed by the Swiss soil testing service, Nyon (Switzerland).

[b] Additionally, as control soil was used soil for pot plants without turf - Kübelpflanzenerde ohne Torf (Ricoter, Aarberg, Switzerland) with following properties: pH 7.3, apparent weight 620g l^{-1} (CEN), and consisting of land soil, cattle compost, broken expanded clay, wood fibers TORESA and basic mineral fertilization.

[c] The years of grapevine monoculture were identified from the following sources: Neuchâtel short (document no. 4608 of founding of the vineyard); Neuchâtel long (document "Abbaye de Bevaix - Prieuré" in the "Musée d'Art et d'Histoire" in Neuchâtel).

[d] Vineyard soils were classified as silt loam.

[e] CEC, cation exchange capacity.

Grapevine plants (*Vitis riparia* x *Vitis rupestris* 3309 accession RAC 1.1) were *in vitro* propagated on ENTAV medium (Galzy 1990). Plantlets were adapted in a period of 12 days to build the cuticle (6 days in a plastic covered tube followed by 6 d in an uncovered tube). Grapevine plantlets were transferred to soil-filled 420 cm^3 plastic pots with drainage holes at the bottom. Plants were grown at 22 °C, 70 % relative humidity, using a 16h/8h light/dark photoperiod and were watered with distilled H_2O to maintain soil water content of 20 %.

VOC's fingerprints of grapevine leaves: A mass spectrometry-based electronic nose (SMart Nose®, Marin-Epagnier, Switzerland) was used to generate fingerprints of VOC's (Pillonel *et al.* 2003), from grapevine leaves collected from the plants maintained three months and five months in long-term and short-term vineyard soils and in control soil. A 4-5 cm long leaf freshly cut from a living plant was placed in a 10 ml brown-glass headspace vial. The vial was closed by a hermetic cap with a silicone septum. The sample was heated at 90 °C during 5 min. The headspace of the sample was then injected in the Smart Nose® injector to record a "VOC's fingerprint" of each sample. For each trial 10 plants from each soil were tested by VOC's fingerprinting.

Isolation and inoculation of the soils with pseudomonad isolates: Single *Pseudomonas* spp. were isolated according Ramette *et al.* (2003) from soil collected from the long-term vineyard. Ten pseudomonad isolates positively tested on *phlD* (McSpadden Gardener *et al.* 2000) and *hcnAB* (Svercel *et al.* 2007) biocontrol genes by specific PCR were selected. Equal amount of each isolate suspension were taken and mixed together. Grapevine plants were inoculated with 10 ml of bacterial suspension with a concentration of about 10^8 cells per ml immediately after planting into soil coming from short-term grapevine monoculture and maintained until the fluorescence measurements were made.

Fluorescence measurements: Chl *a* fluorescence transients of dark-adapted attached grapevine leaves were measured using a Handy-PEA® chlorophyll fluorometer (Handy-*Plant Efficiency Analyser*, Hansatech Instruments, King's Lynn, Norfolk, UK). The transients were induced by 1-sec illumination with an array of six light-emitting diodes providing a maximum light intensity of 3,000 µmol m^{-2} s^{-1} and a homogeneous irradiation over a 4 mm diameter leaf area. The fast fluorescence kinetics (F0 to FM) was recorded from 10 µs to 1 s. The fluorescence intensity at 50 µs was considered as F0 (Strasser and Strasser 1995, Christen *et al.* 2007). Five plants per soil were chosen and 4 fluorescence measurements were performed on four different apparently healthy leaves per plant. The measurements were performed on plants maintained in soils three or five months, respectively.

Analysis of the fluorescence transients using the JIP-test: Raw fluorescence OJIP transients were transferred to a HandyPEA program spreadsheet (supplied with the instrument) and the data were treated according to the equations of the JIP-test parameters (Tab. 2; Strasser and Tsimilli-Michael 2001; for review see Strasser *et al.* 2004). These parameters provided several structural and functional information (e.g. specific and phenomenological fluxes, quantum yields or vitality indexes) and permitted to quantify the PS II behaviour of different treatments on plants.

Table 2

List of abbreviations used in fluorescence measurement[a]

ABS/CS	Absorption per CS (measured by absorption techniques or approximated by F_0 or F_M)
DIo/CS	Heat dissipation at time zero, per cross section of the leaf tissue
F_0	Fluorescence intensity at 50 μs ($F_0 = F_1$)
F_2	Fluorescence intensity at 100 μs
F_M	Maximal fluorescence intensity
N	Turnover number of Q_A reduction and re-oxidation
S_m	Pool size of electron carriers (relative area between F_M – maximal fluorescence intensity and F_t)
TR_0/CS	Calculated trapping at time zero, per CS
V_I	Relative variable fluorescence at 30 ms

[a] after Strasser and Tsimilli-Michael (2001), for review see Strasser *et al*. (2004).

Statistics: Ion mass intensity data were analyzed by FDA (Factorial Discriminant Analysis) and this approach was used to classify the soil type according to the Atomic Mass Units (AMU) detected with the mass spectrometer-based electronic nose. AMUs lower than 40 were eliminated because they surely correspond to fragment ions. AMUs exhibited a very low signal were also eliminated in order to avoid the analysis of noise. The FDA was applied to the rest of the signal data (AMU) resulting from the mass spectrometer-based electronic nose. FDA was carried out on the 16 highest discriminant power scores of the raw signal data. In FDA, the qualitative groups to be discriminated were the soil type (control, long-term and short-term). A criterion of the FDA efficiency is the proportion of correctly classified observations in validation sets. The correctly classified observations were counted and expressed in percentages. All the statistical procedures were carried out using the ExcelStat 9.0 environment.

Data collected from fluorescence measurement were analyzed with analysis of variance (ANOVA) using Systat version 10.0 (Systat Inc., Evanston, IL). Means were separated using Fisher's protected (P = 0.05) least significant difference (LSD) test.

Results

Volatile organic compound fingerprints of grapevine leaves: Grapevine leaves from plants maintained in variant soils were assessed with the mass spectrometer-based electronic nose SMart Nose®, generating fingerprints of VOCs. FDA applied on selected AMU data allowed to classify correctly a mean of 80 % of the soil according to the age of the vineyard. The confusion matrix was processed in order to validate the FDA model. Control soil was classified with 90 % accuracy, long-term soil with 85 % accuracy and short-term soil with an accuracy of 65 % (Tab. 3). Factorial maps

Table 3

Confusion matrices of FDA aiming at discriminating the soil type (control soil, long-term and short-term vineyard soil)

Soil type	Control	Long-term	Short-term	% Correct classification
Control	18	1	1	90
Long-term	1	17	2	85
Short-term	5	2	13	65
Total	24	20	16	80

according to the first two factorial scores presented the discrimination pattern between the soil types (Fig. 1). The first factorial score (F1) was responsible for 75.63 % of the discrimination and the second factorial score (F2) for 24.37 % of the discrimination. F1 allowed segregating the control soil from the long-term soil. The short-term soil was discriminated from the other soils with both F1 and F2. The AMUs important for the discrimination according to the first factorial score were AMU41 and AMU72, with correlation coefficient of 0.535 and 0.569, respectively. AMU58 and AMU71 (correlation coefficient of 0.439 and 0.501, respectively) were important for the discrimination according to the second factorial score (Tab. 4). In conclusion, with FDA, it was possible to discriminate between VOC's from leaves from short and long-term monoculture and control soil mostly after five months of cultivation (Fig. 1). However, these differences were not clear after three months of cultivation (data not shown).

Fluorescence measurements: Chl *a* were performed on dark-adapted attached leaves of grapevine plantlets growing in a soil collected from short-term, long-term vineyard and short-term inoculated with pseudomonad isolates from long-term soil, respectively. The relative differences between the plants maintained in short-term and long-term monoculture soils are presented in Fig. 2. Only few JIP-parameters were significantly different in the measurements done after five months of plant cultivation. The technical parameters i.e. the fluorescent intensity at 100 μs F_2, the relative variable fluorescence at 30 ms V_I [$V_I = (F_{30ms}-F_0)/(F_M-F_0)$], as well as the energy dissipation at time zero DIo/CS (heat dissipation per cross section = CS) [DIo/CS = (ABS/CS)-(TRo/CS)], were significantly higher for the plants in long-term monoculture than in short-term. Opposite results were obtained for the other two technical parameters *i.e.* the turnover number of Q_A reduction and re-oxidation N [$N = (S_m)(M_0/V_J)$], and the pool size of electron carriers S_m [$S_m = {}_{F0}\int^{FM} (F_M-F_t)/(F_M-F_0)$] Looking closely on

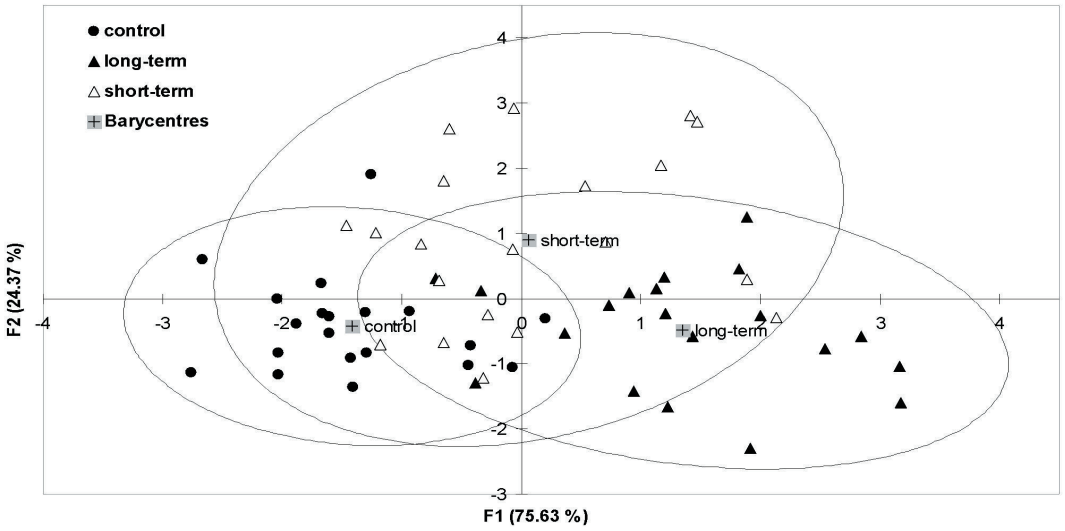

Fig. 1: FDA performed on signal data of Atomic Mass Units detected with the mass spectrometer-based electronic nose of leaf tissues collected from plants after five months of cultivation into tested soils. FDA map according to the first two factorial scores (F1 and F2) where the discriminated groups are the soil type (control ●, long-term ▲, short-term Δ and barycentres +).

T a b l e 4

Correlation between the first two factorial scores of the FDA and the measured atomic mass units and uni-dimensional test (class mean equality)

Atomic Mass Unit	Factorial Score of FDA		Uni-dimensional test (Class mean equality)	
	F1	F2	F-value	p-value
AMU41	0.535	0.335	7.030	0.002
AMU42	0.378	0.203	2.966	0.060
AMU43	0.220	0.107	0.919	0.405
AMU44	0.208	0.097	0.909	0.450
AMU46	0.213	0.094	0.839	0.437
AMU47	0.243	0.095	1.080	0.347
AMU57	0.337	0.253	2.626	0.081
AMU58	0.398	0.439	5.002	0.010
AMU64	-0.214	0.069	0.812	0.449
AMU70	0.287	0.287	2.209	0.119
AMU71	0.308	0.501	4.276	0.019
AMU72	0.569	0.167	6.876	0.002
AMU75	-0.133	0.005	0.291	0.749
AMU76	-0.305	0.022	1.615	0.208
AMU79	-0.161	0.072	0.475	0.624
AMU83	-0.048	0.311	0.898	0.413

the last mentioned parameter, the measured values of S_m exhibited differences between the plants in short-term, short-term inoculated and long-term monoculture soil (Fig. 2 II). The data measured after three months cultivation (trial A) revealed similar, but less contrasted tendencies (only parameters V_I and S_m were significantly different; data not shown).

The photosynthetic response of plants growing in the different soils was compared and the whole transients were further analyzed. The double normalization of the fluorescent rise in the range measurement (between F_0 and F_M) and between F_0 and F_J allowed to compare the relative variable fluorescence [$V = (F_t-F_0)/(F_M-F_0)$] of the different samples. The kinetic curve of long-term vineyard plant (or short-term inoculated respectively) was subtracted from the curve of short-term vineyard plant, provided qualitative information on specific characteristics of the PS II. The samples from long-term vineyard soil showed higher fluorescence intensity in the multiple turnover range (above 2 ms) than the samples from short-term vineyard (Fig. 3). This indicates biochemical inhibitions beyond the redox couple Q_A/Q_A^-. The inoculated plants exhibited higher fluorescence intensity than plants non-inoculated (short term), but lower than plants from long-term monoculture soil. The differences of the relative variable fluorescence between F_0 and F_J [$W_K = V/V_J$] showed evidence for the existence of the K band (300 to 400 μs) (Fig. 4). Plants maintained in the soils from long-term and inoculated short-term monoculture showed an increase in the K band indicating a partial blocking of the water splitting system (oxygen evolving complex, OEC). This enhanced K band was more marked in the samples kept in long-term monoculture soil.

Discussion

This is a first report of the effect of soil from long-term (1007 years) compared to soil from adjacent short-term (55 years) grapevine monoculture on the production of VOCs and responses of photosynthetic apparatus in the plant leaves.

The VOCs fingerprint was different for the three soil types. A mean of 80 % of the soil samples was correctly classified. Among the 16 most discriminative atomic mass units, four molecule fragments AMU41, MU58, AMU71 and AMU72 were well-correlated with the factorial scores (p-value < 0.05) and allowed a good discrimination us-

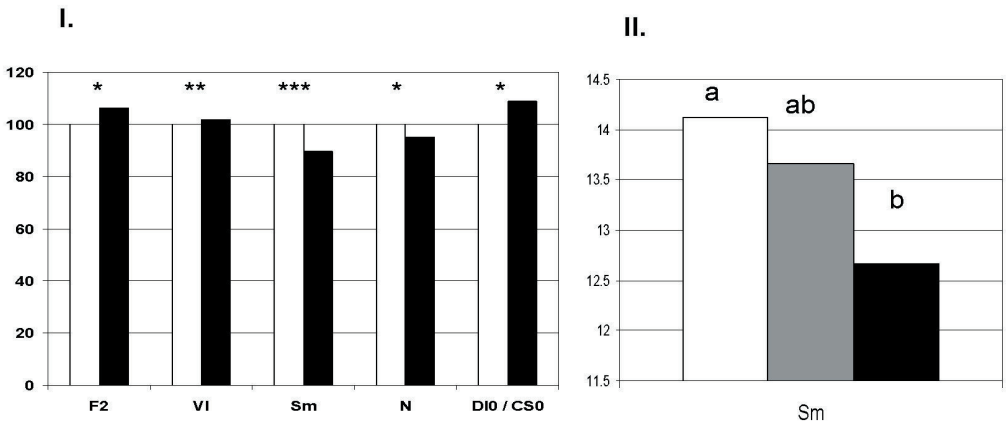

Fig. 2: **I**. Relative values of selected significant different JIP-test parameters of samples from short-term and long-term vineyard soils (black bars). For parameter expressions, see list of abbreviations. *, **, *** indicate that parameters were significantly different at *P* values ≤ 0.05, 0.01 and 0.001, respectively. The values are calculated from averages of 20 measurements taken from plants cultivated in soils after five months. **II**. Values of JIP-test parameter Sm (pool size of electron carriers) of samples from short-term (white bar), short-term inoculated with pseudomonads isolated from long-term (gray bar), and long-term (black bar) vineyard soils. Different letters indicate significant differences. The values are calculated from averages of 20 measurements taken from plants cultivated in soils after five months.

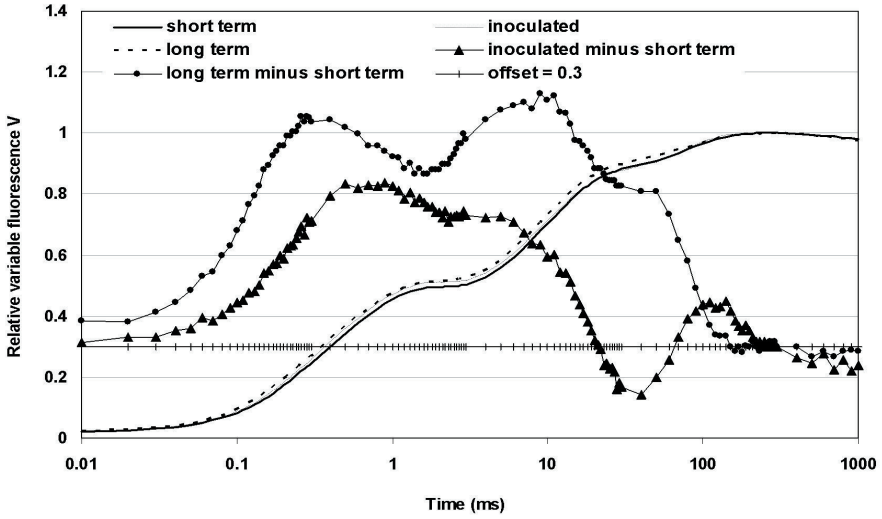

Fig. 3: Relative variable Chl *a* fluorescence after double normalization between $F_0 = 0$ and $F_M = 1$ of the fluorescence rise. The difference $\Delta V_{long\text{-}short}$ (●) is represented by the subtraction of the curve of samples from long-term monoculture soil (bold dashed line) minus the curve of samples from short-term monoculture soil (bold continuous line). The difference $\Delta V_{inocul\text{-}short}$ (▲) is represented by the subtraction of the curve of samples from short-term monoculture soil inoculated with pseudomonads isolated from long-term soil (regular continuous line) minus the curve of samples from short-term monoculture soil (bold continuous line) . ΔV offset = 0.3. The values for normalized curves were calculated from averages of 20 measurements taken from 5 plants cultivated in soils after five months.

ing FDA. These molecules were identified as relevant in classifying the soils according to the age of the vineyard. An identification of these compounds (e.g. using GC-MS) would be interesting in order to define the physiological changes in the plantlets influenced by the type of soil. Furthermore, this non-destructive approach allows the determination of the influence of a long-term monoculture in vineyards without analyzing the soil bacterial population.

It could be demonstrated that the production of VOC's is different for the plant cultivated in the soil collected from long-term monoculture and short-term monoculture, and this could be detected by the SMart Nose®'s procedure. Further, the plants tested should have grown more than (or at least) five months in a tested soil to express clearly the soil's specificity.

Most of the publications have been focusing on the VOCs production in berries, because they are crucial for the taste and aroma of wines (Lund and Bohlmann 2006). High significant effect of climate, soil and grape cultivar with regard to wine behaviour and berry composition was shown by van Leeuwen *et al.* (2004). The impacts of climate and soil were greater than that of cultivar. Contrary to his study, very similar soils and same climate were used for the comparison. Some other authors have shown (e.g. van den Boom *et al.* 2004) that the VOC's production in mechanically damaged grapevine leaves could play an important role as pheromone attractants for insect predators and parasitoids and is a promising biocontrol agent. The mechanical damaged grapevine leaves (caused by spider mite) increased the produced amounts of some VOC's (e.g.

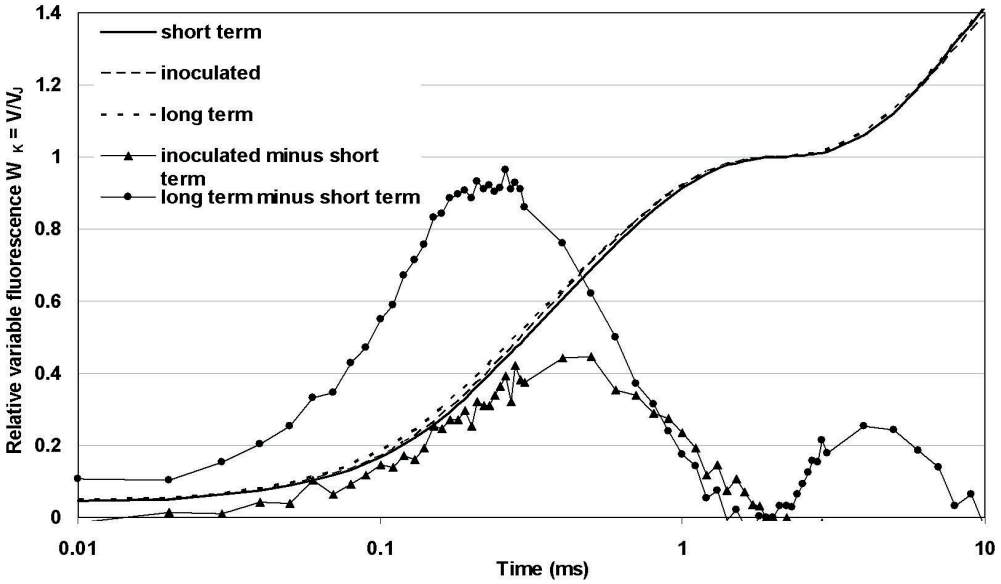

Fig. 4: Relative variable fluorescence W_K exhibiting the K band at about 300 – 400 µs. Fluorescence rises were initially double normalized between $F_0 = 0$ and Fj = 4. The difference $\Delta W_{K\ long\text{-}short}$ (●) is represented by the subtraction of the curve of samples from long-term monoculture soil (bold dashed line) minus the curve of samples from short-term monoculture soil (bold continuous line). The difference $\Delta W_{K\ inocul\text{-}short}$ (▲) is represented by the subtraction of the curve of samples from short-term monoculture soil inoculated with pseudomonads isolated from long-term soil (regular continuous line) minus the curve of samples from short-term monoculture soil (bold continuous line) . The values for normalized curves were calculated from averages of 20 measurements taken from 5 plants cultivated in soils five months.

4,8-dimethyl-1,3,7-nonatriene) in a hundred fold. From this point of view it will be interesting to see, which VOC's were produced differently by plant leaves in our used soil. This part still remains to examine. Unfortunately, just little published information about the factors influencing the VOC's production in grapevine leaves could be found. AGELOPOULOS *et al.* (2000) studied factors such as developmental stage of plant, photophase duration, and foliage weight in intact potato plants and discovered that all of those factors affect the production of VOC's qualitatively and quantitatively.

Fluorescence measurements of Chl *a* are broadly used in plant science (DEELL and TOIVONEN 2003). Therefore, our aim in this study was to investigate this method as a potential tool for the test of longstanding vineyard soil effect. This method was easy-to-use and non-destructive. Differences between grapevines planted in long term, short-term and short-term inoculated soils in some few JIP-test parameters have been shown and suggested that a higher effect on PS II could be expected after longer period of cultivation of plants in the soils. The data revealed a less efficient photosynthetic activity for the plants kept in long-term monoculture than for plants in short-term. Interestingly, an inoculation of short-term soil with *phlD*$^+$ (allele K) pseudomonads isolated from long-term soil increased the fluorescence intensity of measured grapevines planted relatively to the short-term soil. This result has to be interpreted with the hypothesis of the JIP-test, that the absorption is proportional to the Chl *a* concentration. That means that for the same Chl *a* concentration, PS II of plants in long-term (or inoculated plants) was less efficient than plants in short-term monoculture soil. According to the study of BAVARESCO and Fogher (1996 a), an enhanced Chl *a* concentration was observed after inoculation with *Pseudomonas fluorescens* strains. Therefore, the efficiency of the PS II of the inoculated plants could be even lower as measured, if the Chl *a* concentration is higher. However, BAVARESCO and FOGHER (1996 a) have not checked used *Pseudomonas* strain for Phl production. Phl is known to increase the efflux (i.e., exudation) and to decrease the influx of root amino acids (PHILLIPS *et al.* 2004). The influence of these changes on PS II is still unknown. Due to the novelty of our results the comparison with other studies is very difficult. Most of the studies investigated the relationship between nutrition, water, pollution and photosynthetic apparatus of grapevine leaves.

As already mentioned, there are many factors, which are hidden behind the term of terroir and the study of the effect of all parameters in a single experiment is difficult (VAN LEEUWEN *et al.* 2004). Many authors have assessed mostly the impact of a single parameter of terroir on grape quality: climate (GLADSTONES 1992), soil (SEGUIN 1975, VAN LEEUWEN and SEGUIN 1994), cultivar (RIOU 1994, HUGLIN and SCHNEIDER 1998), or rootstock (MAY 1997). Some of them investigated combined effects of two terroir parameters: soil and climate (DUTEU *et al.* 1981) and soil and cultivar (VAN LEEUWEN 1995) or three terroir parameters: soil, climate and cultivar (RANKINE *et al.* 1971, VAN LEEUWEN *et al*. 2004). In our study, one of the so-called terroir parameter soil was described to exhibit the importance of grapevine monoculture longevity. Neighbouring vineyards (same climate) with same viticultural and enological practices and with similar soil properties were chosen (Tab. 1). Only the duration of monoculture was different. Differences in VOC's production and different behaviour of some JIP-test parameters from recorded fluorescence transients

between short and long-term monocultures were detected. Further experiments are required to elucidate the cause of these differences. In the next step, it would be necessary to identify those VOC's and provide more fluorescence measurements using short-term and long-term vineyard soils from different locations.

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References

Agelopoulos, N. G.; Chamberlain, K.; Pickett, J. A.; 2000: Factors affecting volatile emissions of intact potato plants, *Solanum Tuberosum*: variability of quantities and stability of ratios. J. Chem. Ecol. **26**, 497-511.

Barka, E. A.; Belarbi, A.; Hachet, C.; Nowak, J.; Audran, J. C.; 2000: Enhancement of in vitro growth and resistance to gray mould of *Vitis vinifera* co-cultured with plant growth-promoting rhizobacteria. FEMS Microbiol. Lett. **186**, 91-95.

Bavaresco, L.; Fogher, C.; 1996a: Lime-chlorosis occurrence and leaf mineral composition of grapevine treated by root microorganisms. J. Plant. Nutr. **19**, 87-98.

Bavaresco, L.; Fogher, C.; 1996b: Effect of root infection with *Pseudomonas fluorescens* and *Glomus mosseae* on severity of lime-induced chlorosis in *Vitis vinifera* L. cv. 'Pinot Blanc'. J. Plant. Nutr. **19**, 1319-1329.

Christen, D.; Schönmann, S.; Jermini, M.; Strasser, R. J.; Défago, G.; 2007: Characterization and early detection of grapevine (*Vitis vinifera*) stress responses to esca disease by *in situ* chlorophyll fluorescence and comparison with drought stress. Env. Exp. Bot. **60**, 504-514.

Deell, J. R.; Toivonen, P. M. A.; 2003: Practical Applications of Chlorophyll Fluorescence in Plant Biology. Kluwer Acad. Publ., Dordrecht.

Duteau, J.; Guilloux, M.; Seguin, G.; 1981: Influence des facteurs naturels sur la maturation du raisin, en 1979, à Pomerol et Saint-Emilion. Conn. Vigne Vin. **15**, 1-27.

Falcetti, M.; 1994: Le terroir. Qu'est-ce qu'un terroir? Pourquoi l'étudier? Pourquoi l'enseigner? Bull. O I V (Off. Int. Vigne Vin) **67**, 246-275.

Galzy, R.; 1990: Remarques sur la nutrition carbonée de la vigne cultivée *in vitro*. Bull. O I V (Off. Int. Vigne Vin) **707**, 6-20.

Gladstones, J.; 1992: Viticulture and Environment. Winetitles, Adelaide.

Haas, D.; Défago, G.; 2005: Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat. Rev. Microbiol. **3**, 307-319.

Huglin, P.; Schneider, C.; 1998: Biologie et Écologie de la Vigne. Lavoisier Tec & Doc. Paris.

Khmel, I. A.; Sorokina, T. A.; Lemanova, N. B.; Lipasova, V. A.; Metlinsky, O. Z.; Burdeinaya, T. V.; Chernin, L. S.; 1998: Biological control of crown gall in grapevine and raspberry by two *Pseudomonas* spp. with a wide spectrum of antagonistic activity. Biocontr. Sci. Technol. **8**, 45-57.

Lund, S. T.; Bohlmann, J.; 2006: The molecular basis for wine grape quality - a volatile subject. Science **311**, 804-805.

May, P.; 1997: Using Grapevine Rootstocks. The Australian Perspective. Winetitles, Adelaide.

McSpadden Gardener, B. B.; Schroeder, K. L.; Kalloger, S. E.; Raaijmakers, J. M.; Thomashow, L. S.; Weller, D. M.; 2000: Genotypic and phenotypic diversity of *phlD*-containing *Pseudomonas* strains isolated from the rhizosphere of wheat. Appl. Environ. Microb. **66**, 1939-1946.

Phillips, D. A.; Fox, T. C.; King, M. D.; Bhuvaneswari, T. V.; Teuber, L. R.; 2004: Microbial products trigger amino acid exudation from plant roots. Plant Physiol. **136**, 2887-2894.

Pillonel, L.; Ampuero, S.; Tabacchi, R.; Bosset, J. O.; 2003: Analytical methods for the determination of the geographic origin of Emmental cheese: volatile compounds by GC/MS-FID and electronic nose. Eur. Food Res. Technol. **216**, 174-178.

Ramette, A.; Moënne-Loccoz, Y.; Défago, G.; 2003: Prevalence of fluorescent pseudomonads producing antifungal phloroglucinols and/or hydrogen cyanide in soils naturally suppressive or conducive to tobacco black root rot. FEMS Microbiol. Ecol. **44**, 35-43.

Rankine, B.; Fornachon, J.; Boehm, E.; Cellier, K.; 1971: Influence of grape variety, climate and soil on grape composition and on the composition and quality of table wines. Vitis **10**, 33-50.

Riou, C.; 1994: Le Déterminisme Climatique de la Maturation du Raisin: Application au Zonage de la Teneur en Sucres dans la Communauté Européenne. Office des Publications Officielles des Communautés Européennes, Luxembourg.

Rosenqvist, E.; van Kooten, O.; 2003: Chlorophyll fluorescence: A general description and nomenclature. In: J. R. DeEll; P. M. A. Toivonen (Eds.): Practical applications of chlorophyll fluorescence in plant biology, 31-77. Kluwer Academic Publishers, Dordrecht.

Seguin, G.; 1975: Alimentation en eau de la vigne et composition chimique des moûts dans les Grands Crus du Médoc. Phénomènes de régulation. Conn. Vigne Vin. **9**, 23-24.

Seguin, G.; 1988: Ecosystems of the great red wines produced in the maritime climate of Bordeaux. In: L. Fuller-Perrine (Ed.): Proc. Symp. Maritime Climate Winegrowing, 36-53. Dep. Hortic. Sci., Cornell Univ., Geneva, NY.

Srivastava, A.; Strasser, R. J.; 1996: Stress and stress management of land plants during a regular day. J. Plant Physiol. **148**, 445-455.

Strasser, B. J.; Strasser, R. J.; 1995: Measuring fast fluorescence transient to address environmental questions: the JIP-test. In: P. Mathis (Ed.): Photosynthesis Congress, Montpellier, France, 977-980. Kluwer Acad. Publ., Dordrecht.

Strasser, R. J.; Srivastava, A.; Tsimilli-Michael, M.; 2004: Analysis of the chlorophyll *a* fluorescence transient, In: G. C. Papageorgiou and Govindjee (Ed.): Advances in Photosynthesis and Respiration, vol. 19; Chlorophyll Fluorescence a Signature of Photosynthesi., 321-362. Kluwer Acad. Publ., Dordrecht.

Strasser, R. J.; Tsimilli-Michael, M.; 2001: Stress in plants, from daily rhythm to global changes, detected and quantified by the JIP-test. Chim Nouvelle (SRC) **75**, 3321-3326.

Svercel, M.; Christen, D.; Möenne-loccoz , Y.; Duffy, B.; Défago, G.; 2009: Effect of long-term vineyard monoculture on rhizosphere populations of pseudomonads carrying the antimicrobial biosynthetic genes *phlD* and/or *hcnAB*. FEMS Microbiol. Ecol. **68**, 25-36.

Svercel, M.; Duffy, B.; Défago, G.; 2007: PCR amplification of hydrogen cyanide biosynthetic locus *hcnAB* in *Pseudomonas* spp. J. Microbiol. Meth. **70**, 209-213.

Svercel, M.; Hamelin, J.; Duffy, B.; Möenne-loccoz , Y.; Défago, G.; 2010: Distribution of *Pseudomonas* populations harboring *phlD* or *hcnAB* biocontrol genes is related to depth in vineyard soils. Soil Biol. Biochem. **42**, 466-472.

Tsimilli-Michael, M.; Krüger, G. H. J.; Strasser, R. J.; 1995: Suboptimality as driving force for adaptation: A study about the correlation of excitation light intensity and the dynamic fluorescence emission in plants. In: P. Mathis (Ed.): Photosynthesis: from Light to Biosphere. Proceedings of the Xth International Photosynthesis Congress, 981-984. Montpellier, France, Kluwer Acad. Publ., Dordrecht.

Tsimilli-Michael, M.; Krüger, G. H. J.; Strasser, R. J.; 1996: About the perpetual state changes implants approaching harmony with the environment. Archs. Sci., Genève **49**, 173-203.

van den Boom, C.; van Beek, T. A.; Posthumus, M. A.; de Groot, A.; Dicke, M.; 2004: Qualitative and quantitative variation among volatile profiles induced by *Tetranynchus urticae* feeding on plants from various families. J. Chem. Ecol. **30**, 69-89.

VAN LEEUWEN, C.; SEGUIN, G.; 1994: Incidences de l'alimentation en eau de la vigne, appréciée par l'état hydrique de feuillage, sur le développement de l'appareil végétatif et la maturation du raisin (*Vitis vinifera* variété Cabernet franc, Saint-Emilion, 1990). J. Int. Sci. Vigne Vin. **28**, 81-110.

VAN LEEUWEN, C.; 1995: Comportement de deux cépages (*Vitis vinifera* variété Merlot et variété Cabernet franc) dans trois sols de la région viticole de Saint-Emilion. In: A. LONVAUD-FUNEL (Ed.): Oenologie 1995: 5e Symposium International d'Oenologie, 3-8. Lavoisier Tec. & Doc. Paris.

VAN LEEUWEN, C.; FRIANT, P.; CHONÉ, X.; TREGOAT, O.; KOUNDOURAS, S.; DUBOURDIEU, D.; 2004: Influence of climate, soil and cultivar on terroir. Am. J. Enol. Vitic. **55**, 207-217.

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