K-124165

Environmental plasticity of grapevine leaf phenolics: Microlocalization and functional analysis

CLOSING REPORT

RESULTS

The aim of the work was to explore how the phenolic plasticity of grapevine leaves contributed to long- and short-term acclimation to their changing environment, primarily but not restricted to light and temperature conditions. The work included registering and analysing responses in three different time frames,

- Long term acclimation during leaf development from bud-break to veraison.
- Impact of diurnal light and temperature variations on leaves.
- Short-term acclimation to sudden changes in light conditions as a result of training and thinning of the canopy.

In addition to performing the planned outdoor experiments in the vineyard, we also worked with model plants in order to explore various hypotheses related to acclimation to UV stress. Published works are summarized briefly in this report, while results included in yet unpublished manuscripts in progress are described in a more detailed way.

1. Long term acclimation during leaf development from bud-break to veraison

Vitis vinifera L. cv. Furmint plants were analysed at three different sites. The project planned to include other clone varieties, but only clone P.120 was sampled, because the other clones were not available at all three sites in disease- and stress-free conditions by the start of the project.
The three sites, as planned, were at:
(i) Pécs, Research Institute for Viticulture and Oenology of Univ Pécs (location: SztMiklós-hegy, GPS 46.071325, 18.155858)
(ii) Tarcal as part of the Tokaj growth region (location: Szarvas dűlő, GPS 48.107903, 21.368008)
(iii) Mád (location: Szepsy Estate, GPS 48.199523, 21.273092)

Meteorological data were collected at each site in order to characterize condition experienced by grapevine leaves between two distinct phenophases, from budbreak (mid-April) to veraison (late July - early August). Figure 1 shows that conditions were diverse, with several extremes, such as in total amount of rain (Fig.1D). In order to take into account conditions not only for the entire duration of the experiment, but also during a period closer to leaf assessment, meteorological parameters were also calculated for the last 7 days before on-site measurements, which took place at veraison. These weekly parameters showed more extremities in conditions, especially in precipitation (Fig.1H). Photosynthesis and related gas exchange parameters were measured with infrared gas analyser (IRGA) and varied between sites and also between years as shown in Fig.2. Leaf pigments contents were measured with the Dualex leaf clip on site, using chlorophyll, flavonoid and anthocyan indexes from both adaxial and abaxial leaf sides (data not shown). All on site measurements took place between 10-12 a.m. in order to exclude variations due to circadian changes. Following these, leaves were cut and frozen in liquid nitrogen immediately; then transferred, and stored at low temperature until being processed for the analysis of phenolic profiles with HPLC.

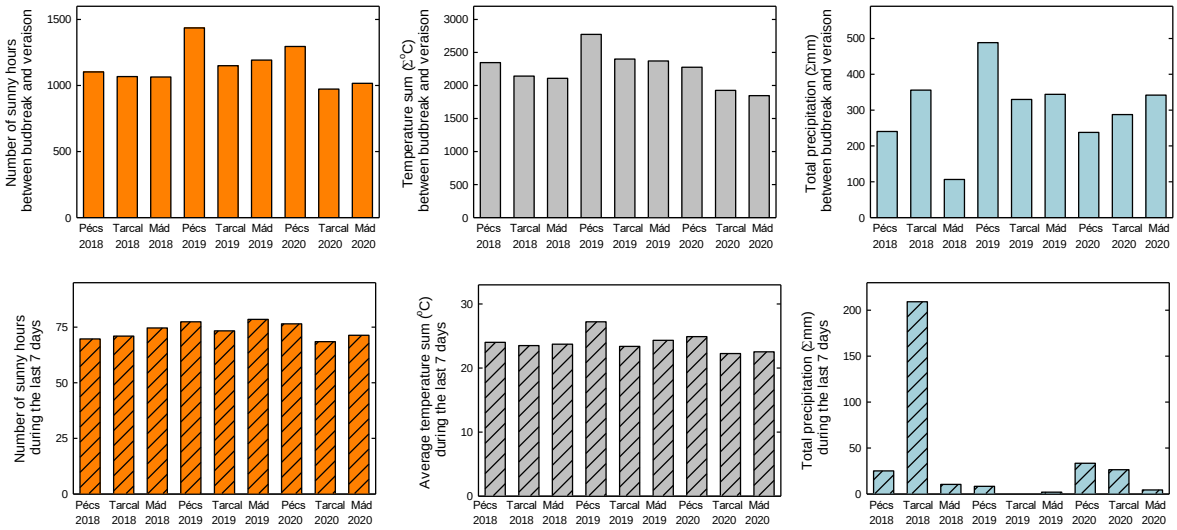

Figure 1. Meteorological conditions at the three locations of the experiment.

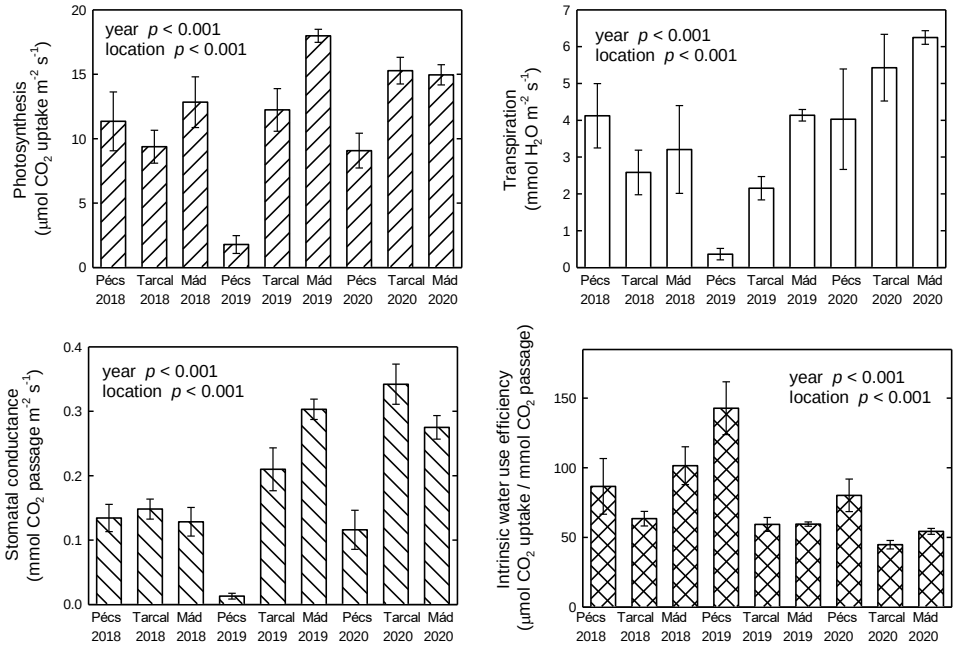

Figure 2. Photosynthesis and gas exchange related parameters of Furmint leaves.
Gas exchange parameters were measured with infrared gas analyser (IRGA) on site, in the vineyard. Columns and error bars correspond to means and standard deviations (n=20). Significant effects of location or year on means were analysed with two-factor ANOVA and verified with post-hoc tests, and results with *p* values are shown.

Photosynthesis and related gas exchange parameters varied with locations and also with years (Fig.2). However, factor interactions were also significant, indicating that location was not the sole driver of differences in any of the studied years. The lack of correlation between physiology parameters and soil properties, which are constant characteristics of locations confirmed this (data not shown).

The dominant phenolic acid in grapevine leaves was caftaric acid, and flavonoids were all flavon-3-ols, mainly quercetin- and to a smaller extent kaempferol-glycosides. Figure 3 illustrates typical component ratios, and changes in the amounts of major compounds are shown in Fig.4 with results of statistical analysis.

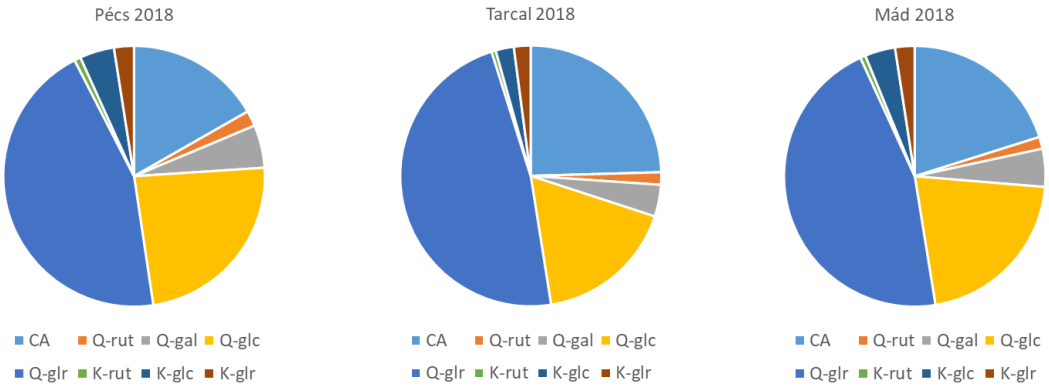

Figure 3. Phenolic composition of Furmint leaves (examples).

CA, caftaric acid; QUE-rut, quercetin-3-O-rutinoside; QUE-gal, quercetin-3-O-galactoside; QUE-glc, quercetin-3-O-glucoside; QUE-glr, quercetin-3-O-glucuronide; KAE-rut, kaempferol -3-O-rutinoside; KAE-glc, kaempferol -3-O-glucoside; KAE-glr, kaempferol-3-O-glucuronide.

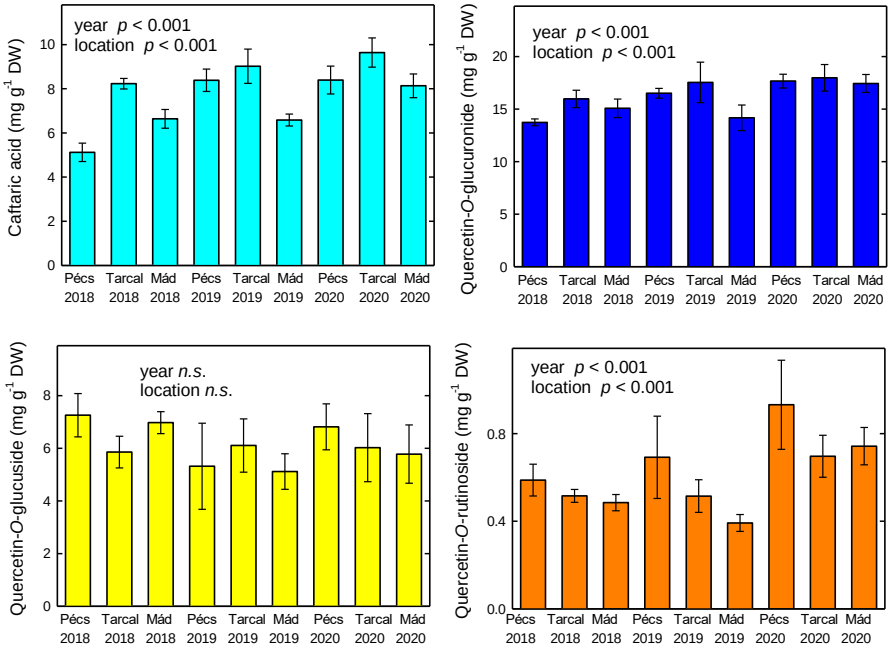

Figure 4. Comparisons of major phenolic components in Furmint leaves.

Columns and error bars correspond to means and standard deviations (n=5, pooled samples). Significant effects of location or year on means were analysed with two-factor ANOVA and verified with post-hoc tests, and results with *p* values are shown. *n.s.* = no significance

Although Furmint leaf phenolic compounds remained the same qualitatively, their amounts varied between years and locations (Fig.4). However, similarly to photosynthesis parameters, all significant variation were interactive and statistical analyses revealed neither location-, nor year-driven trends. Therefore, in order to link metabolite contents, meteorological and physiology parameters, we performed a correlation analysis (Fig.5). This identified two environmental factors as negative correlators affecting leaf physiology: the amount of sunlight throughout leaf development (between budbreak and veraison) and, as relatively shorter time-range effect, temperature during the last 7 days before leaf sampling. These two environmental parameters were both strong negative correlations of

photosynthesis, transpiration and stomata opening, but were positive correlators of intrinsic water use efficiency. This latter result indicates that the apparent negative effects on photosynthesis indicate downregulation as the avoidance of photoinhibition rather than actual damage.

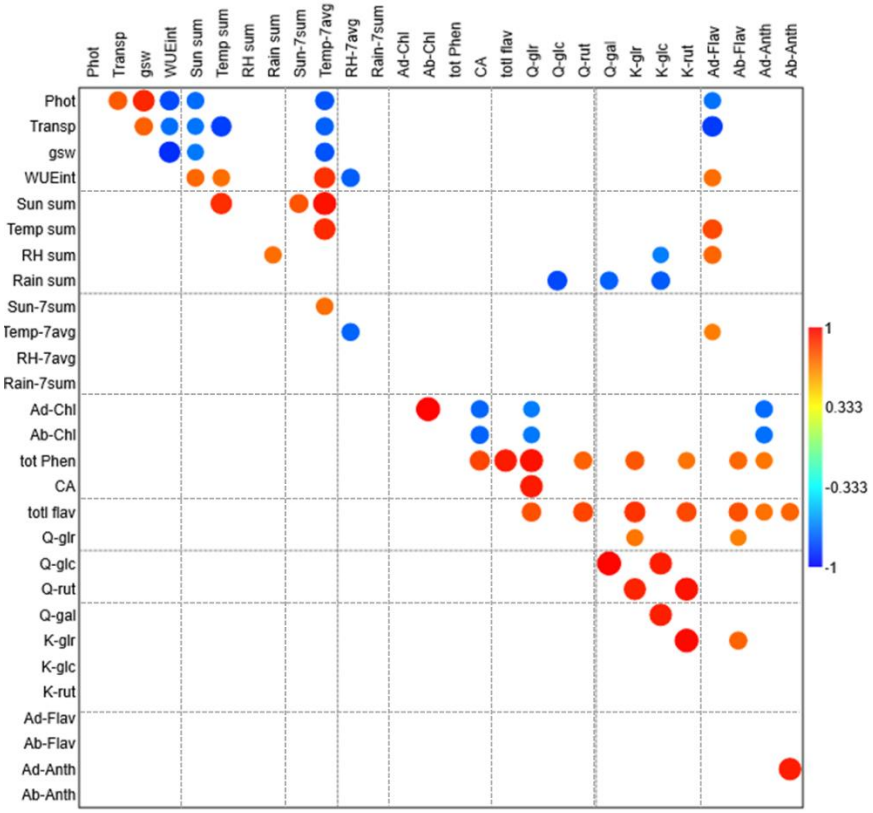

Figure 5. Correlations between photosynthesis, phenolic contents, and environmental conditions.

Pearson's correlation coefficients were calculated pair wise using means calculated for each year and location (n=9). Colours corresponding to positive (red) or negative (blue) correlations at $p < 0.05$ levels, as shown in the figure.
Phot, photosynthetic CO_2 uptake; Transp, water vapour transpiration; gsw, stomatal conductance to CO_2; WUEi, intrinsic water-use efficiency calculated as Phot/gsw;
Sun sum, sum of sunny hours between budbreak to veraison; Temp sum, temperature sum between budbreak to veraison; RH sum, relative humidity integrated between budbreak to veraison; Rain sum, total precipitation between budbreak to veraison; Sun-7sum, sum of sunny hours during the last 7 days; Temp-7avg, average temperature during the last 7 days; RH-7avg, average relative humidity during the last 7 days; Rain-7sum, precipitation during the last 7 days; Ad-Chl, adaxial chlorophyll index; Ab-Chl, abaxial chlorophyll index; tot phen, total phenolic content; CA, caftaric acid; tot flav, total flavonoid contents; QUE-glr, quercetin-3-O-glucuronide; QUE-glc, quercetin-3-O-glucoside; QUE-gal, quercetin-3-O-galactoside; QUE-rut, quercetin-3-O-rutinoside; KAE-glr, kaempferol-3-O-glucuronide; KAE-glc, kaempferol -3-O-glucoside; KAE-rut, kaempferol -3-O-rutinoside; Ad-Flav, adaxial flavonoid index; Ab-Flav, abaxial flavonoid index; Ad-Anth, adaxial anthocyanin index; Ab-Anth, abaxial anthocyanin index;

Surprisingly, amounts of phenolic compounds in leaves had no significant effect on either photosynthesis or other gas-exchange related parameters. This lack of close relationship between phenolic compounds and photosynthesis suggests that long-term acclimation is achieved via small adjustments in compound ratios rather than larger changes in total amounts. Adaxial flavonoid content, however, was positively correlated with temperature, both as long-term (temperature sum)

and shorter-term (temperature average during the last week). This response of flavonols to temperature was confirmed in shorter, diurnal scale, as detailed below. A publication is in progress and figures shown in this section are from the manuscript.

2. Impact of diurnal light and temperature variations on leaves.

Vitis vinifera L. cv. Pinot Noir vines, which were applied in this study, were grown in a west-east row direction in Pécs, Hungary (latitude: 46°07' N, longitude: 18°17' E, 200m elevation). The experiment was carried out on a cloudless sunny day, and diurnal changes in leaf phenolics were measured from samples taken every hour between 7 a.m. and 7 p.m. PAR, total UV irradiance, air temperature and humidity data were collected at the local meteorological station; leaf temperature and photosynthesis-related parameters were measured with infrared gas analyser; and UV-A and UV-B irradiance data were calculated using a model. As shown in Fig.6, environmental parameters changed gradually during the day, without any sudden changes. Photosynthesis and related parameters showed that south-facing sun-exposed grapevine leaves were well-acclimated to these conditions (Fig.7). Net CO_2 uptake, was high in the morning and declined later (Fig.7A) while transpiration followed a bell-shaped pattern and was maximal around solar noon (Fig.7B).

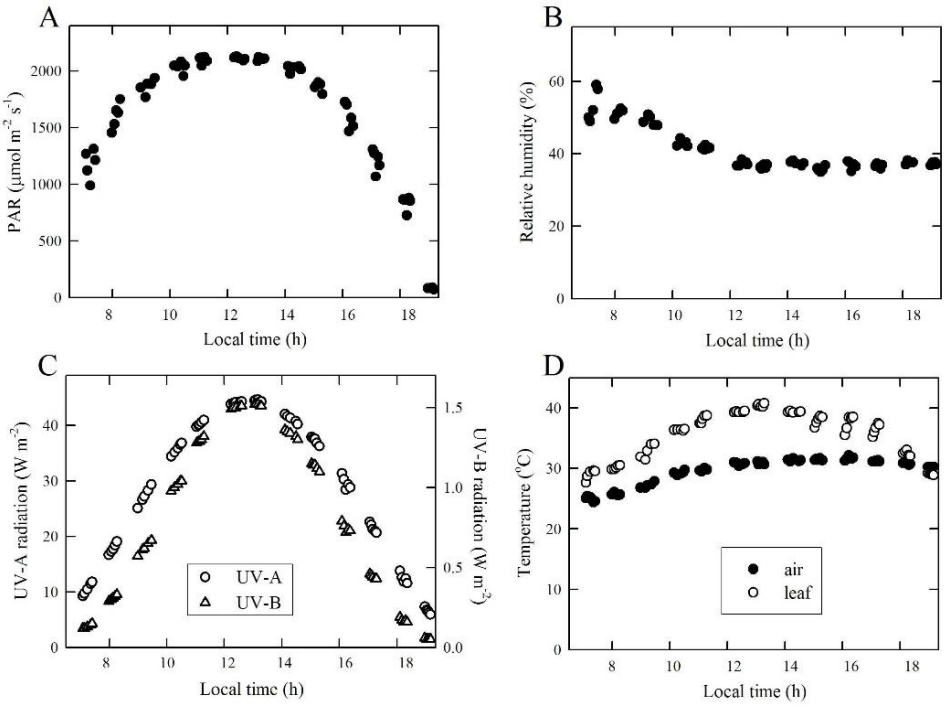

Figure 6. Changes in environmental parameters and leaf temperature during the experiment. (from Csepregi et al. 2019)

Leaf stomatal conductance for water vapour reached a peak level and then decreased gradually from late in the morning throughout the afternoon (Fig.7C), explaining the temporary increase in transpiration at mid-day and the decline of both photosynthesis and transpiration in the evening. Intrinsic water-use efficiency (WUEi), decreased during the first half of the day, reached a minimum around noon, and increased well above morning levels during the second half of the day (Fig.7D).

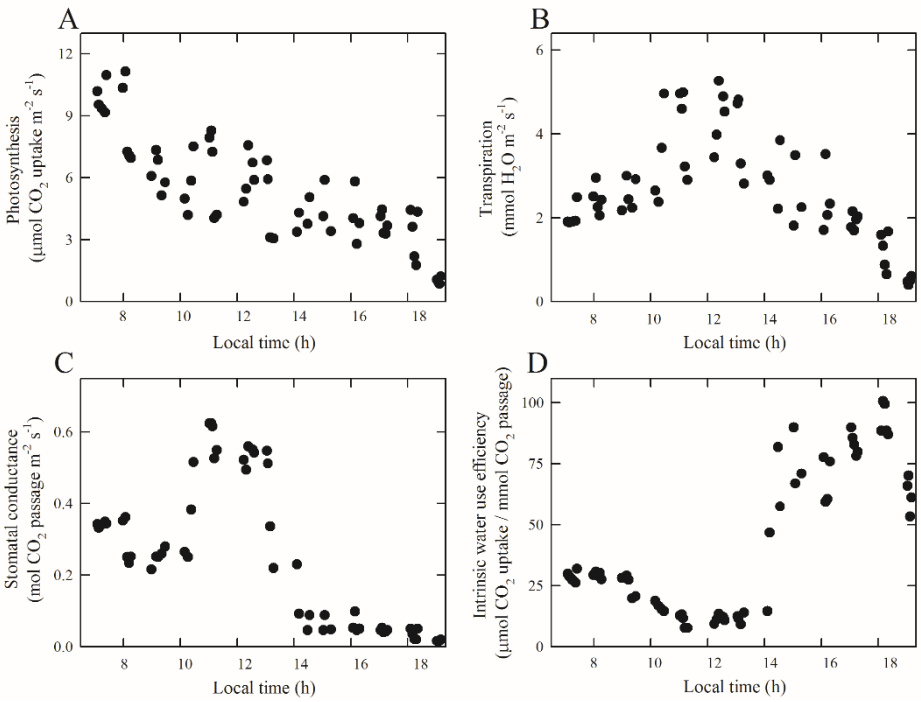

Figure 7. Changes in Pinot noir leaf photosynthesis and water use parameters during the experiment. (from Csepregi et al. 2019)

Phenolic profiles of Furmint (Fig.3) and Pinot noir (Fig.8) leaves were the same in major components, although ratios of these showed differences. For example, the relative amount of quercetin-3-*O*-glucuronide was larger in Pinot noir leaves. Kaempferol-rutinoside, a minor flavonol component was present in Furmint leaves but it was not detectable in Pinot noir. HPLC analysis of phenolic profiles showed that leaf phenolic acid content was the same during the day (caftaric acid in Fig.8). Flavonoid content showed a steady increase, due to an increase in the major flavonol component quercetin-3-*O*-glucuronide (QUE-glu in Fig.8).

Changes in phenolic contents were statistically compared to changes in environmental conditions, and correlation analysis (Fig.9) showed that epidermal UV absorbance, characterized by the Dualex flavonoid index, and total extractable phenolic contents were correlated to distinct environmental parameters. The former was positively correlated to irradiance and leaf temperature, while the latter was positively correlated to air temperature. There was a positive correlation between air temperature and amounts of the dominant flavonol component, quercetin-3-*O*-glucuronide. The only phenolic component statistically connected to the flavonoid index was quercetin-3-*O*-glucoside. This correlation was positive and both parameters decreased during the day, although changes in the amount of this flavonol component showed no correlation to environmental factors. Total antioxidant capacities of leaf extracts were positively correlated to solar UV, and leaf and air temperature, but not to photosynthetically active radiation. Positive correlations of quercetin-3-*O*-glucoside contents with the flavonoid index, with photosynthesis and with sub-stomatal CO_2 concentration suggest a special protective role of this flavonol. A short-term negative effect of solar UV-A and UV-B on photosynthetic CO_2 uptake was also identified, which was unrelated to changes in stomatal conductance.

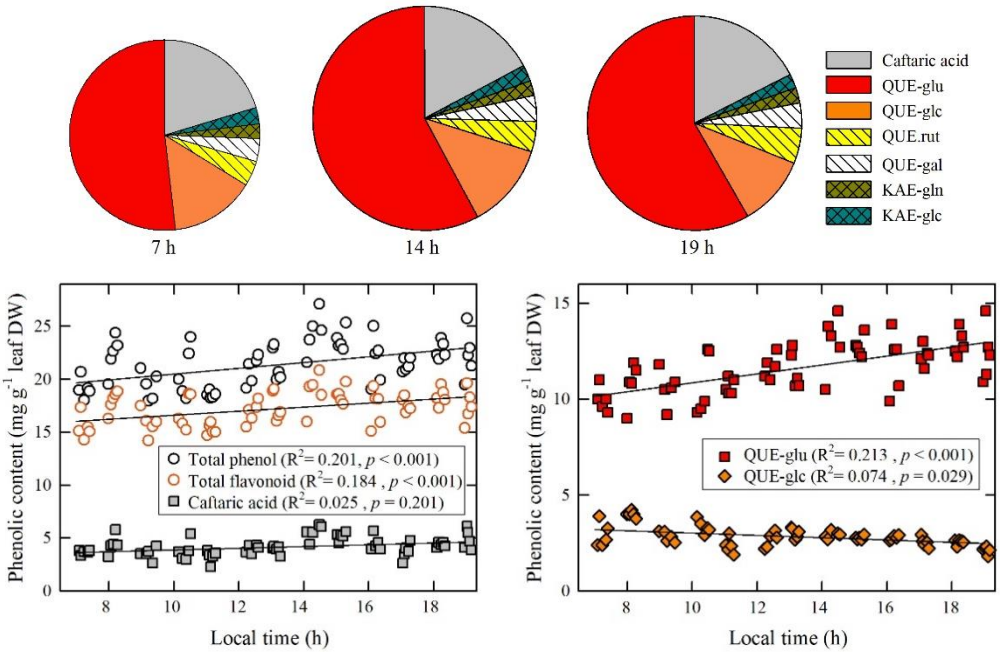

Figure 8. Changes in Pinot noir leaf phenolic profiles during the experiment. (redrawn from Csepregi et al. 2019).

Pie charts demonstrate typical phenolic compositions, and their areas are proportional to total amounts. Dots represent values measured with HPLC, and straight lines show results of linear fits for n=65 data. Numbers in parenthesis are the coefficient of determination R^2, and the *p* value characterizing the statistical significance of linear time dependence.

Our experiment demonstrated that high base levels of phenolic components, present in grapevine leaves as a result of long-term adaptation, are not constant during the day but are modulated by complex radiation and temperature signals originating in environmental factors (Csepregi et al. 2019). Contrary to the extensive changes in phenolic profiles observed in a variety of plants under modulated sunlight or low temperature (Barnes et al. 2016), we have shown that in grapevine leaves only a relatively small fraction of phenolic compounds was responsive to dynamic changes in the natural environment in our experiment ((Hideg et al. 2018, Csepregi et al. 2019).

In summary (Sections 1 and 2), we found that photosynthetic performance was affected by both long- and short-term changes in environmental conditions (Teszlák et al. 2018). Leaf phenolic profiles also respond to these changes, via adjustments of the amounts of flavonols, especially mono-glycosylated quercetins. Environmental factors driving changes in flavonol contents were precipitation (long-term) and temperature (short-term). However, flavonol content and photosynthesis were only correlated during short-term changes, and studies of diurnal variations suggest that a conversion between flavonol forms (from glucoside to glucuronide) may contribute to better photosynthetic performance. We found no indication for a connection between amounts of the major phenolic acid in grapevine leaves (caftaric acid) and either photosynthetic performance or environmental conditions.

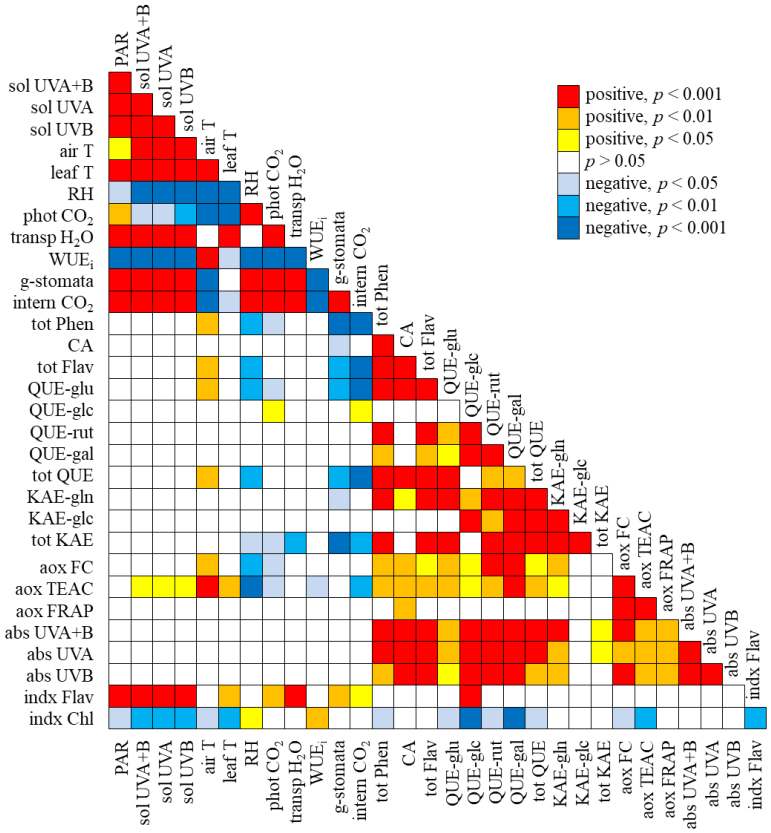

Figure 9. Correlations between environmental conditions, leaf physiology, metabolite content and antioxidant capacity parameters. (from Csepregi et al. 2019).
Pearson's correlation coefficients were calculated pair wise for two n=65 data sets. Colours corresponding to positive (red, orange, yellow) or negative (dark blue, cyan, pale blue) correlations at *p* levels are shown in the figure. White cells indicate the lack of significant correlation.
Environmental conditions: PAR, photosynthetically active radiation; sol UVA+B, solar UV radiation 280–400 nm; sol UVA, solar UV-A radiation 315–400 nm; sol UVB, solar UV-B radiation 280–315 nm; air T, air temperature; RH, relative air humidity.
Leaf physiology parameters: leaf T, leaf temperature; phot CO_2, photosynthetic CO_2 uptake; transp H_2O, water vapour transpiration; WUEi, intrinsic water-use efficiency calculated as photosynthetic CO_2 uptake divided by stomatal conductance; g-stomata, stomatal conductance to CO_2; intern CO_2, internal sub-stomatal CO_2 concentration.
Metabolite contents determined with HPLC: tot Phen, total phenolic content; CA, caftaric acid; tot Flav, total flavonoid contents; QUE-glu, quercetin-3-O-glucuronide; QUE-glc, quercetin-3-O-glucoside; QUE-rut, quercetin-3-O-rutinoside; QUE-gal, quercetin-3-O-galactoside; KAE-glu, kaempferol-3-O-glucuronide; KAE-glc, kaempferol -3-O-glucoside.
Antioxidant capacities of leaf extracts: aox FC, total antioxidant capacity measured with the Folin-Ciocalteu method; aox TEAC, total antioxidant capacity measured with the TEAC method; aox FRAP, total antioxidant capacity measured with the FRAP
Other, metabolite-related parameters: abs UVA+B, total 280–400 nm absorption of leaf extracts; abs UVA, 315–400 nm absorption of leaf extracts; abs UVB, 280–315 nm absorption of leaf extracts; indx Flav, adaxial leaf flavonoid index; indx Chl, adaxial leaf chlorophyll index.

The above experiments explored leaf responses to gradual (long- or short-term) changes in the environment. These were complemented with studies of responses to a sudden change.

3. Short-term acclimation to sudden changes in light conditions as a result of training and thinning of the canopy.

This study used *Vitis vinifera* L. cv. Pinot Noir vines from the same vineyard as diurnal change experiments (Section 2). However, here we used sun leaves on the south-facing side of the canopy, and shade leaves in the middle of the canopy, all growing on the 7th-9th nodes. When leaves were approximately 85 days old (calculated from budbreak), all shoots with sun leaves were removed from half of the chosen plants according to standard vineyard practice, leading to a full exposure of shade leaves. This was carried out between 9 and 10 a.m. local time (UTC +2h). Two days later, shade leaves which suddenly exposed to sun leaf conditions (called "shade→sun leaves" in this study) were measured on site and then frozen for metabolite analyses. Typical photon flux densities were 1650 and 90 µmol photons m^{-2} s^{-1} in full sun exposed and shaded canopy areas, respectively. Thus, shade→sun leaves suffered an approx. 18-fold change in PAR, and an even larger change in solar UV exposure. For reference, sun leaves and shade leaves were also measured and collected from plants, which had undisturbed canopies.

Leaf gas exchange measurements showed that shade→sun leaves acclimated to new light and temperature conditions by increasing stomatal conductance, photosynthesis and evaporation. Although none of these parameters reached the level of those in sun leaves, the intrinsic water use efficiency of shade→sun was as good as those of sun leaves (Fig.10).

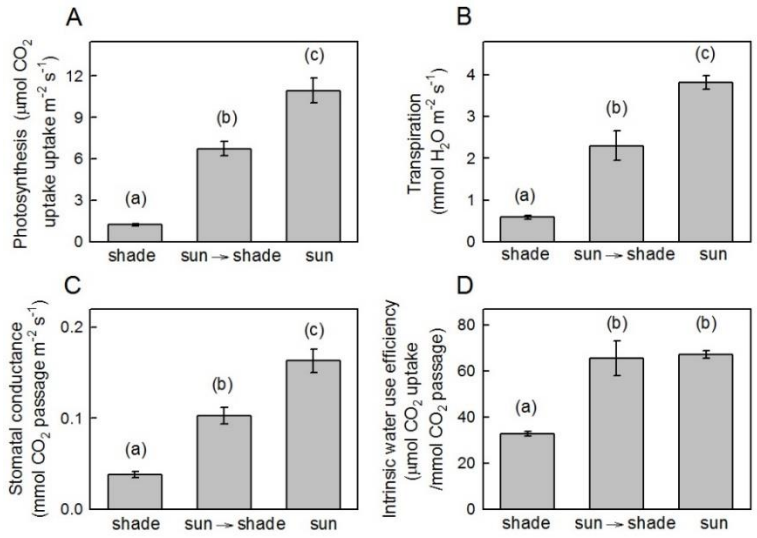

Figure 10. Changes in photosynthesis related parameters

Effects of sudden exposure of grapevine shade leaves to full sunlight (shade→sun) in comparison with leaves developed either in sunlight (sun leaves) or in canopy shading (shade leaves). Gas exchange parameters were measured with IRGA on site, in the vineyard. Columns and error bars correspond to means and standard deviations (n=20). Lower case letters in parenthesis indicate significantly ($p < 0.05$) different means as calculated with ANOVA and verified with post-hoc tests.

Survival of this sudden change without damage by photoinhibition was aided by efficient non-photochemical quenching in shade→sun leaves (Fig.11). There were no significant changes in either chlorophyll or epidermal pigment contents during the two days, and in this respect shade→sun leaves remained similar to shade leaves (Fig.12).

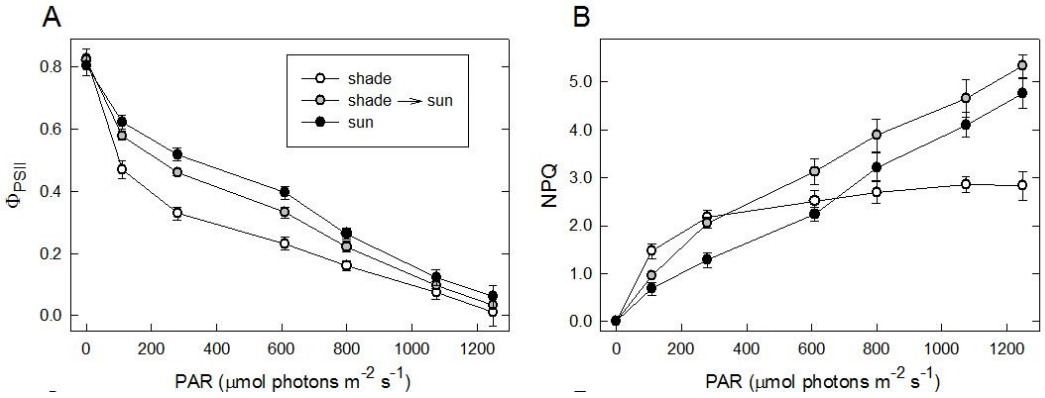

Figure 11. Changes in leaf photochemistry

Effects of sudden exposure of grapevine shade leaves to full sunlight (shade→sun) in comparison with leaves developed either in sunlight (sun leaves) or in canopy shading (shade leaves). Photochemical yields (ϕ_{PSII}) and non photochemical quenching (NPQ) were measured in the laboratory, using dark adapted leaves and the MAXI-version of the Imaging PAM (Heinz Walz GmbH, Effeltrich, Germany). Columns and error bars correspond to means and standard deviations (n=5).

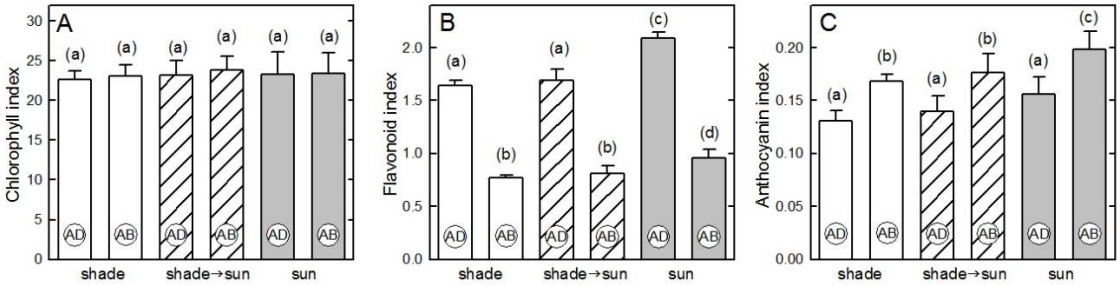

Figure 12. Changes in chlorophyll content and epidermal pigments

Effects of sudden exposure of grapevine shade leaves to full sunlight (shade→sun) in comparison with leaves developed either in sunlight (sun leaves) or in canopy shading (shade leaves). Adaxial (AD) and abaxial (AB) pigment contents were measured with the Dualex leaf clip sensor on site, in the vineyard. Columns and error bars correspond to means and standard deviations (n=20). Lower case letters in parenthesis indicate significantly ($p < 0.05$) different means as calculated with ANOVA and verified with post-hoc tests.

Results of HPLC profiling indicated that phenolic biosynthesis was not adjusted to the level of sun leaves in shade→sun leaves, and amounts of all studied components remained at the level of shade leaves. Moreover, the amount of caftaric acid slightly decreased (Fig.13).

If not phenolic compounds, which metabolites confer the acclimation to the sudden change in light conditions? Our experiments established the role of carotenoids and other non-enzymatic antioxidants in the process. HPLC analysis of carotenoids, which was performed for the project as part of a collaboration with the Centre for Agricultural Research (Martonvásár, Hungary), showed a marked increase in zeaxanthin (Fig.14A) content but no change in other constituents of the xanthophyll cycle (Figs. 14B, 14C) in shade→sun leaves as compared to shade leaves. This change resulted in a very low epoxidation index (Fig. 14F) and explains how shade→sun leaves adjusted NPQ to the level of sun leaves (Fig. 11B) while keeping photochemical yields higher than shade leaves (Fig. 11A). In addition to increasing the efficiency of energy dissipating capacities, a general upregulation of antioxidant capacities was expected, but only a few changes were observed. Alpa-tocopherol was marginally

higher in shade→sun leaves (Figs. 15A), but correlation analysis (Fig.16) confirmed this positive trend (Fig. 16). Also, shade→sun leaves showed high hydroxyl radical neutralizing antioxidant capacities, indicating a reinforced defence against potential ROS products of photoinhibition and UV-B-related oxidative stress (Figs. 15B).

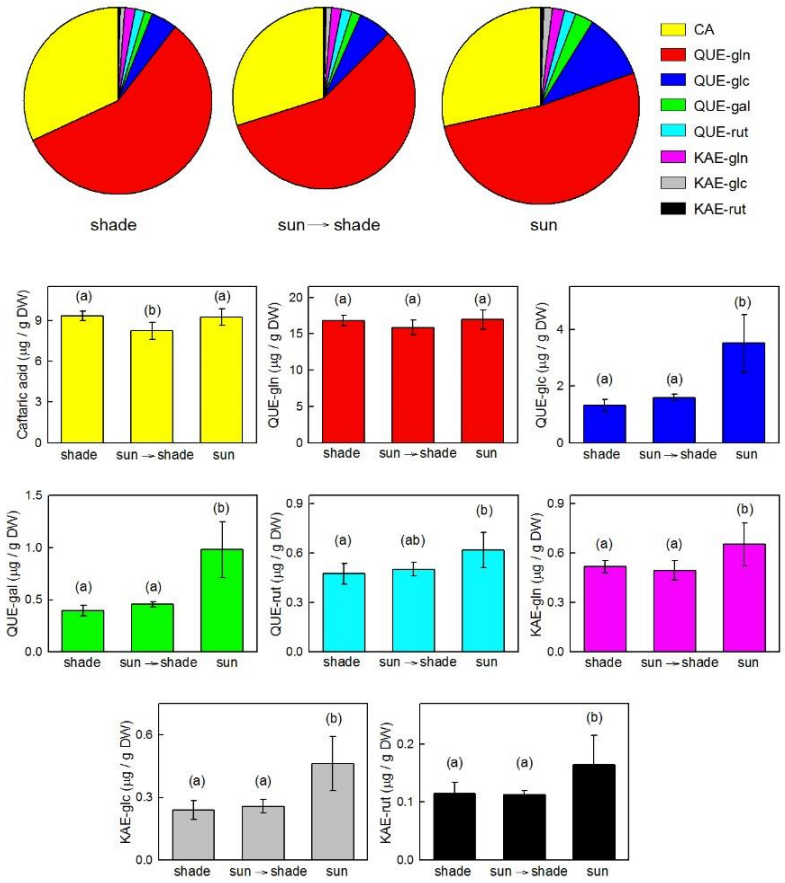

Figure 13. Changes in leaf phenolics

Effects of sudden exposure of grapevine shade leaves to full sunlight (shade→sun) in comparison with leaves developed either in sunlight (sun leaves) or in canopy shading (shade leaves). Pie charts demonstrate typical phenolic compositions, and their areas are proportional to total amounts. Bar charts show comparisons of amounts by compounds as determined with HPLC. Columns and error bars correspond to means and standard deviations (n=5, pooled leaf samples). Lower case letters in parenthesis indicate significantly ($p < 0.05$) different means as calculated with ANOVA and verified with post-hoc tests.

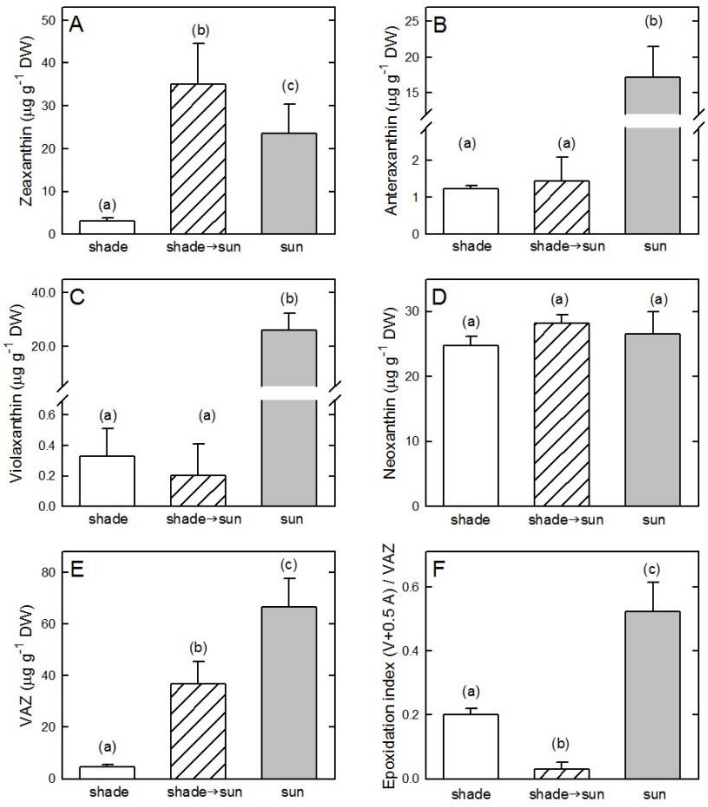

Figure 14. Changes in leaf carotenoids

Effects of sudden exposure of grapevine shade leaves to full sunlight (shade→sun) in comparison with leaves developed either in sunlight (sun leaves) or in canopy shading (shade leaves). Xanthophyll contents were determined with HPLC and related parameters were calculated from these data. Columns and error bars correspond to means and standard deviations (n=5, pooled leaf samples). Lower case letters in parenthesis indicate significantly ($p < 0.05$) different means as calculated with ANOVA and verified with post-hoc tests.

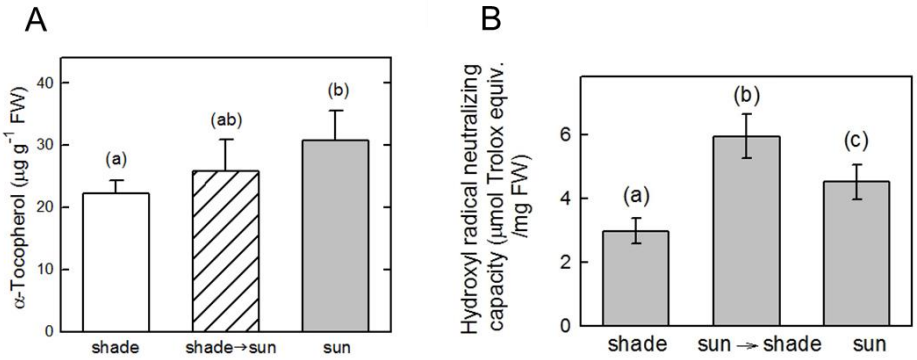

Figure 15. Changes in antioxidants

Effects of sudden exposure of grapevine shade leaves to full sunlight (shade→sun) in comparison with leaves developed either in sunlight (sun leaves) or in canopy shading (shade leaves). (A) Alpha-tocopherol contents were measured with HPLC. (B) Hydroxyl radical scavenging capacities were determined with a fluorometric assay. Columns and error bars correspond to means and standard deviations (n=5, pooled leaf samples). Lower case letters in parenthesis indicate significantly ($p < 0.05$) different means as calculated with ANOVA and verified with post-hoc tests.

Although light is an important factor in acclimation to abiotic stress condition (Janda et al. 2020), correlation analysis showed that although several metabolites responded to the sudden change in light conditions, only a few were directly irradiance driven (Fig.16). The amount of xanthophyll cycle pigments, especially zeaxanthin had strong positive correlation with PAR. A negative correlation between PAR (or photosynthesis) with α-carotene indicates a rearrangement of metabolic pathways. Leaves with higher photosynthetic activity had lower levels of oxidized chlorophyll, but lower amounts of and the β-carotene isomer 9-cis-β-carotene, suggesting the consumption of these compounds as antioxidants.

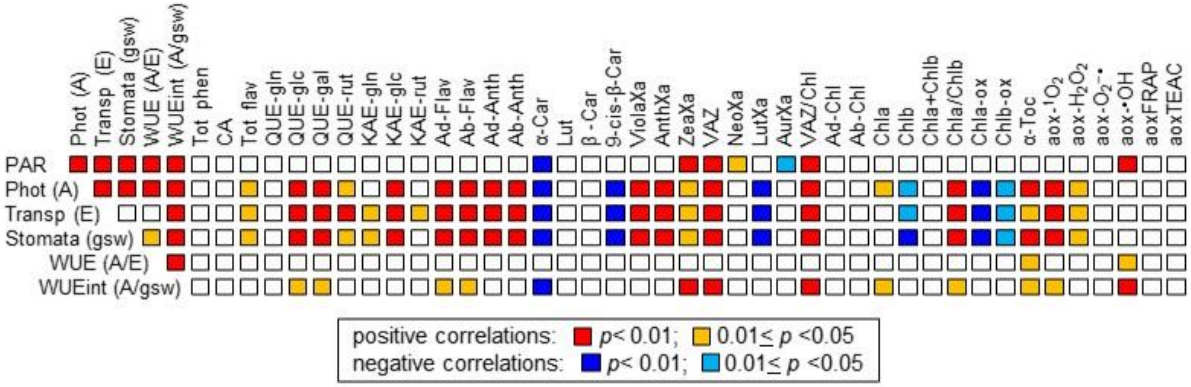

Figure 16. Correlations between metabolite contents, light conditions, and photosynthesis
Pearson's correlation coefficients were calculated pair wise for two n=65 data sets. Colours corresponding to positive (red, orange) or negative (dark blue, cyan) correlations at the indicated *p* levels are shown in the figure. White cells indicate the lack of significant correlation.
PAR, photosynthetically active radiation; Phot (A), photosynthetic CO_2 uptake; Transp (E), water vapour transpiration; stomata gsw, stomatal conductance to CO_2; WUE, water use efficiency; WUEi, intrinsic water-use efficiency; tot phen, total phenolic content; CA, caftaric acid; tot flav, total flavonoid contents; QUE-gln, quercetin-3-O-glucuronide; QUE-glc, quercetin-3-O-glucoside; QUE-gal, quercetin-3-O-galactoside; QUE-rut, quercetin-3-O-rutinoside; KAE-gln, kaempferol-3-O-glucuronide; KAE-glc, kaempferol -3-O-glucoside; KAE-rut, kaempferol -3-O-rutinoside; Ad-Flav, adaxial flavonoid index; Ab-Flav, abaxial flavonoid index; Ad-Anth, adaxial anthocyanin index; Ab-Anth, abaxial anthocyanin index; α-Car, α-carotene; Lut, lutein; β-Car, β- carotene; ViolaXa, violaxanthin; AnthXa, antheraxanthin; ZeaXa, zeaxanthin; VAZ, sum of violaxanthin, antheraxanthin and zeaxanthin content; NeoXa, neoxanthin; LutXa, luteoxanthin; AurXa, auroxanthin; Ad-Chl, adaxial chlorophyll index; Ab-Chl, abaxial chlorophyll index; Chla and Chlb, chlorofill-a and -b content determined by HPLC; Chla-ox and Chlb-ox, oxidized chlorofill-a and -b; α-Toc, α-tocopherol; aox-$^1O_2$, singlet oxygen reactive antioxidants; aox-H_2O_2, H_2O_2 reactive antioxidants; aox-$O_2^{-\bullet}$, superoxide radical reactive antioxidants; $^{\bullet}OH$, hydroxyl radical reactive antioxidants; aox FRAP, total antioxidant capacity measured with the FRAP method; aox TEAC, total antioxidant capacity measured with the TEAC method.

The above results demonstrated known differences between shade and sun leaves, but also added new dimensions, such as adding ROS specific antioxidant capacities to the analysis. The work demonstrated a specific metabolic plasticity of grapevine leaves to cope with a sudden, nearly 20-fold change in light intensity. An interesting aspect of metabolite analysis is the negative correlation between flavonoid and carotenoid contents (data not shown), indicating the necessity of a trade-off as part of acclimation. A publication is in progress and figures shown in this section are from the manuscript.

4. Microlocalization of phenolic compounds

Leaf extracts provide quality information of the overall capacity of metabolites as antioxidants or UV absorbers. Localization, however, is a critical factor in the realization of these features in leaves. The optical sensor Dualex was developed and marketed as a non-invasive tool for the assessment of epidermal flavonoids and anthocyanins. Due to technical limitations (the availability of stable UV-emitting LED), this method utilizes 375 nm UV-A radiation to assess epidermal flavonoids; and thus, it is less sensitive to the presence of phenolic acids. The application of this optical technique assumes that a detectable change in flavonoids would also imply a change in phenolic acids, because the two compound groups have common steps in their biosynthesis (Goulas 2004). Taking the example of major grapevine leaves phenolics (Figs. 3, 8, 13), it is plausible to assume that quercetin-glucuronide, which absorbs UV-A and UV-B equally (Csepregi & Hideg 2018) would contribute to UV-B screening to a smaller extent that caftaric acid, which features twice as high UV-B than UV-A absorption (Csepregi & Hideg 2018). On the other hand, a counterargument involves acknowledging the above differences but assumes that actual roles in planta rather depend on amounts than absorption capacities.

A collaboration with the Department of Biochemistry and Medical Chemistry (Medical School, University of Pécs) enabled us to verify the first hypothesis. The spatial heterogeneity of phenolic compounds in grapevine leaves was explored with MALDI TOF MS (matrix-assisted laser desorption ionization time-of-flight mass spectrometry) imaging, and showed that caftaric acid was localized in adaxial epidermal layers, while major flavonoids were found in deeper, mesophyll layers (Fig. 17).

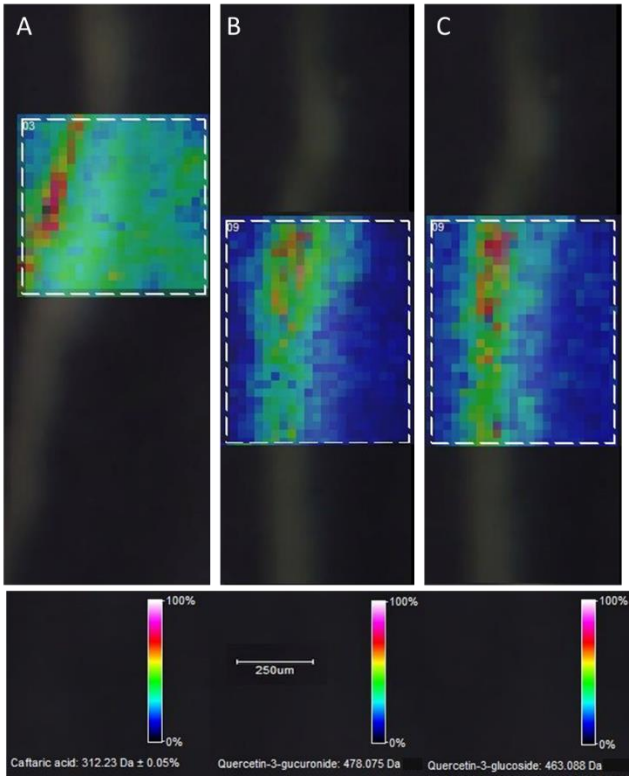

Figure 17. Microlocalizations of major phenolic compounds in grapevine leaf cross-sections. Faded grey images show the positions of leaf sections, and false colour coding of highlighted areas corresponds to relative amounts. Adaxial leaf surfaces are facing towards the left side of the images. Images are shown in raw formats. Compounds and molecular masses are indicated in the lower panels.

This finding explains why the amount of caftaric acid was unresponsive to environmental changes in long term acclimation (Fig.5), during gradual changes during the day (Fig.9), and did not participate in the acclimation to a sudden change in light conditions either (Fig.16). Caftaric acid appears as a stable defence line as UV absorber in the epidermis, as opposed to flavonoids, which contribute to acclimation as antioxidants or putative secondary absorbers. Our results also showed that acclimation involves the adjustment of component ratios among flavonols, for example a temperature (and not irradiance) driven increase in quercetin-glucuronide content was part of acclimation to gradual daily environmental variations (Figs.8 and 9), but this compound was not a significant correlator of long-term acclimation (Fig.5). In the latter experiment, quercetin-glucoside and other minor flavonol components were negatively correlated to precipitation (Fig.5). These characteristics of metabolic responses to gradual, long-term (months, Section 1) or repeated short-term (daily, in Section 2) changes are different from alarm reactions provoked by a sudden change in light conditions, as shown in Section 3. When conditions changed suddenly, photosynthesis was positively correlated to levels of several flavonols (Fig.16). Model experiments in controlled environments (acclimation to UV as single factor in a growth chamber) showed that quercetin-derivatives also serve as substrates to stress responsive phenolic peroxidases (Rácz et al. 2020, Rácz & Hideg 2021), and such indirect antioxidant functions may also be assumed in grapevine leaves.

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