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# UV-A screening in plants determined using a new portable fluorimeter

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## Abstract

UV screening by plant surfaces can be determined by exposing plant organs to UV radiation and measuring the chlorophyll (Chl) fluorescence elicited. From this fluorescence, the UV transmittance can be derived: the more intense the screening the lower the reporter Chl fluorescence and the lower the UV transmittance. The relationships between UV screening at 375 nm, as determined in the field by a portable UV-A-PAM fluorimeter, and UV screening at 314 and 360 nm, measured in the laboratory with the non-portable XE-PAM fluorimeter, were investigated in leaves of grapevine (*Vitis vinifera* L. cv. Bacchus) and barley (*Hordeum vulgare* cv. Ricarda), as well as in white grape berries. With leaves, linear trends were observed between XE-PAM measurements at 314 nm and UV-A-PAM measurements at 375 nm but the relationship between transmittance at 360 and 375 nm in barley was curved: a simple model calculation suggests that this curvi-linearity arises from particularly weak absorbance of barley flavonoids at 375 nm relative to absorbance at 360 nm. Transmittance values at 314 nm plotted against 375 nm yielded a much smaller slope in grapevine leaves than in barley leaves, which was attributed to screening in the short-wavelength UV by hydroxycinnamic acids in the former but not in the latter species. With grape berries, a poor correlation was detected between transmittances at 314 and 375 nm which might arise from high scattering of UV radiation at the berry surface. Such artefacts appear to be confined to the UV-B region, as berry transmittance at 360 nm correlated very well with that at 375 nm. Thus, assessment of UV screening in the field at short UV wavelengths using 375 nm readings from a UV-A-PAM fluorimeter is possible provided that information is available on the relationship between the transmittance at the UV wavelength of interest and at 375 nm for the sample tissue being investigated.

*Additional key words*: apigenin; berry; comparison of devices; flavonoids; *Hordeum*; leaf; luteolin; transmittance; *Vitis*.

## Introduction

Many higher plants accumulate UV-absorbing phenolics in the leaf epidermis; thus, hazardous effects of natural UV-radiation are minimized (Caldwell *et al.* 1983, Tevini *et al.* 1991, Bornman and Teramura 1993, Jordan 1996). Two major classes of UV screening phenolics are known: hydroxycinnamic acids absorbing predominantly in the UV-B (280–315 nm), and flavonoids exhibiting a broader absorbance range including the UV-A (315–400 nm; Cockell and Knowland 1999).

Information about the capacity of epidermal UV screening is a prerequisite not only for understanding plant performance in natural environments but also for predicting the productivity of crop plants under various climatic conditions. The efficacy of UV screening *in vivo* cannot be assessed simply from UV absorbance of extracted phenolics (Liakoura *et al.* 2003, Kolb and Pfündel 2005). Therefore, determining epidermal screening needs more direct methods such as absorbance spectrometry on isolated epidermal strips (Grammatikopoulos *et al.* 1999, Markstädter *et al.* 2001), radiometry inside the leaf using micro fibre optics (Bornman and Vogelmann 1988, Day *et al.* 1992, Cen und Bornman 1993), or UV fluorimetry with intact leaves to measure chlorophyll (Chl) fluorescence elicited by UV radiation from which the UV transmittance of the epidermal layer can be derived (Bilger *et al.* 1997, Barnes *et al.* 2000, Burchard *et al.* 2000, Mazza *et al.* 2000). Of these three methods, the first is limited to species displaying easily-removable epidermis, the second requires special apparatus confined to a few specialized laboratories, but the third, fluorimetry, is relatively simple to use and is not restricted to plant species with easily-removable epidermis. Despite their wide-ranging potential, most spectrofluorimeters are bulky and, hence, normally laboratory based.

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Two portable fluorimeters, permitting repeated measurements of samples under field conditions, have recently been developed: the UV-A-PAM fluorimeter which uses a radiation guide as an optical link between the instrument and the sample (Bilger *et al.* 2001, Kolb *et al.* 2003, Krause *et al.* 2003), and the *Dualex* fluorimeter in which the sample is sandwiched between excitation and detection units (Goulas *et al.* 2004). In this paper, the portable UV-A-PAM instrument was used. Generally, UV screening is assessed by a dual-wavelength excitation approach, as originally introduced by Bilger *et al.* (1997), which derives UV-transmittance values of the screening epidermis from the yields of Chl fluorescence excited by UV and visible radiation. In both instruments, detection of UV screening is limited to the long-wavelength UV-A due to the lack of commercially-available light emitting diodes (LED) with shorter wavelength radiation. UV screening at shorter wavelengths, where the potential of irreversible plant damage increases (Caldwell and Flint 1997), may possibly be estimated with portable fluorimeter if clear relationships exist between UV screening determined with LED excitation and that at other wavelengths. With this rationale in mind, the present work with grape and barley leaves, as well as white-grape berry skins, compares UV transmittance values at 375 nm, derived from fluorescence data obtained with the portable UV-A-PAM fluorimeter, with transmittance values at 316 and 360 nm similarly derived in the laboratory with the non-portable XE-PAM fluorimeter.

## Materials and methods

**Plants and data gathering**: Three-years-old grafted vines (*Vitis vinifera* cv. Bacchus) and barley plants (*Hordeum vulgare* cv. Ricarda) grown from seeds were cultivated in pots in a shaded glasshouse in which UV radiation was virtually absent, and irradiance was 15 % of the outdoor one as described by Kolb *et al.* (2001) and Kolb and Pfündel (2005). Additionally, leaves from Bacchus plants located in sloped vineyards close to Würzburg were used for the experiments of Fig. 3*A,B*. In both plant species, fully developed leaves were investigated and, in barley, only F-1 leaves (the leaves below flag leaves) of about eight-weeks-old plants were examined. Also, white Bacchus berries were studied in which the sugar contents (data not shown) indicated that the grapes had entered the final phase of ripening (cf. Coombe 1976). Plant material exhibiting various degrees of UV screening was obtained by exposing berries and leaves of greenhouse-grown plants to outdoor conditions for different time intervals as described by Kolb *et al.* (2001). In the case of vineyard-grown Bacchus leaves, shaded and exposed leaves were investigated.

Data were always recorded after sunset. At experimental sites, epidermal UV transmittance values were derived from measurements of Chl fluorescence with the portable UV-A-PAM fluorimeter which excites $F_0$ Chl fluorescence at 375 and 470 nm (*Gademann Messgeräte*, Würzburg, Germany; see separate description in section below). The XE-PAM fluorimeter provides the flexibility of selecting different wavelengths' ranges with the help of band-pass filters for excitation of Chl fluorescence with pulse-modulated radiation derived from a xenon discharge lamp but is too cumbersome to use in the field. Therefore, to measure UV screening at identical positions with the XE-PAM fluorimeter (*Walz*, Effeltrich, Germany), samples were transferred to the laboratory in a dark and moist container.

**The XE-PAM fluorimeter** applies 1 µs flashes at sufficiently low frequency to assess the basal fluorescence yield, $F_0$ (Schreiber *et al.* 1993). Excitation was in the UV-B (314 nm, bandwidth 24 nm), UV-A (360 nm, bandwidth 28 nm), or visible (490 nm, bandwidth 165 nm) range, and fluorescence was detected at wavelengths above 690 nm (Bilger *et al.* 1997, Kolb *et al.* 2001, Markstädter *et al.* 2001). In the fluorimeter, the sample surface is adjusted at an angle of 45° to both the excitation source and to the fluorescence detector. For XE-PAM measurements, disks of 0.7 $cm^2$ were punched out from leaves, and caps of 2 mm thickness were cut off the grape berries. Exposed berry sides and adaxial (upper) leaf sides were investigated but, in the case of grape leaves, also some abaxial (lower) leaf sides were probed.

**Derivation of transmittance values**: These values for UV radiation, denoted $T_{UV}$, were estimated using subsequent equation:

$$T_{UV} = (F_{UV}/F_{VIS})\,(F_{M,UV}/F_{M,VIS})^{-1} \quad (1)$$

where the $F_{UV}$ and the $F_{VIS}$ stands for fluorescence of UV screening samples excited by 314, 360 [UV], and 490 [VIS] nm wavelengths (XE-PAM fluorimeter) or 375 [UV] and 470 [VIS] nm wavelengths (UV-A-PAM fluorimeter). The $F_{M,UV}$ and $F_{M,VIS}$ values were derived using the same excitation conditions applied to non-screened mesophyll tissue, but in the case of the UV-A-PAM instrument, a different approach was taken involving the use of a fluorescence standard provided by *Walz*.

It proved impossible with the leaves used in the present study to prepare non-screened mesophyll tissue by stripping off the epidermis. Therefore, in XE-PAM measurements, the $F_{M,UV}/F_{M,VIS}$ ratios used to calculate UV transmittance in grape leaves were measured with epidermis-free mesophyll tissue of *Pisum sativum* L., mutant Argenteum, which does possess a loosely attached leaf epidermis (Hoch *et al.* 1980). Similarly, the same ratio for barley leaves was determined with intact leaves from a flavonoid-deficient barley mutant (mutant Ant 287; Reuber *et al.* 1996). For measurements of $F_{M,UV}/F_{M,VIS}$

with berries, the caps were flipped over so that the unshielded pulp was exposed to the excitation beam. For each of the three mesophyll references, a single mean value for $F_{M,UV}/F_{M,VIS}$ was employed to calculate transmittance values ($23<n<45$, standard error <5 % of the respective mean in all cases). For all plant samples, transmittance values derived from UV-A-PAM measurements were computed using $F_{M,UV}/F_{M,VIS}$ ratios established by the *Walz* fluorescence standard (Bilger *et al.* 2001). With excitation at 314 nm, the XE-PAM fluorimeter determined $F_{M,UV}/F_{M,VIS}$ ratios relative to that of the *Walz* fluorescence standard of 1.2, 0.9, and 0.3 for epidermis-free *P. sativum* mutant Argenteum leaves, barley mutant Ant 2287 leaves, and berry pulp, respectively. Using 360 nm as the wavelength of excitation radiation, the corresponding ratios were 1.9, 1.7, and 1.1.

**The UV-A-PAM fluorimeter** is a micro-processor controlled portable device featuring its own keypad, LC-display, and data logger, and can be used, therefore, in stand-alone mode in field experiments. Alternatively, it can also be operated under laboratory conditions linked to a PC using special software. A flexible fluid light tube with high UV-A transmittance links the instrument and sample for carrying excitation radiation to the sample and fluorescence back to the instrument. During measurements the free end of the light guide was gently pressed against the surface of the leaves or berries.

The UV-A-PAM fluorimeter applies low-intensity pulse-modulated measuring radiation from light emitting diodes (LED) at 375 (10 nm bandwidth) and 470 (25 nm bandwidth) nm for monitoring fluorescence yield at wavelengths above 650 nm, normally corresponding to the minimal fluorescence yield, $F_0$ (for nomenclature and an introduction to the basics of PAM fluorimetry see Schreiber 2004). Measured parameters are $F_{UV}$ (with 375 nm excitation) and $F_{VIS}$ (with 470 nm excitation). As the two excitation wavelengths are rapidly alternating, the two signals are always directly comparable, even in the case of fluorescence changes (*e.g.* induced by changes in ambient irradiance).

For assessment of UV screening, the instrument originally was calibrated to give identical values of $F_{UV}$ and $F_{VIS}$ with a leaf of *Vicia faba*, from which the epidermis was removed. For this purpose the instrument provides the baseline-adjustment function, with which the 470 nm excitation radiation can be appropriately trimmed using suitable mesophyll references so that $F_{M,UV}/F_{M,VIS} = 1$ and, consequently,

$T_{UV} = (F_{UV}/F_{VIS})\ (F_{M,UV}/F_{M,VIS})^{-1} = F_{UV}/F_{VIS}$ (2, see also preceding section).

This means that after appropriate calibration the ratio $F_{UV}/F_{VIS}$ may be taken as a direct measure of UV-A transmittance.

Together with the instrument, a blue-plastic fluorescence standard (*Walz*) is provided: the fluorescence properties of this standard resemble those of *V. faba* mesophyll, as after calibration with this standard, $F_{UV}/F_{VIS}$ was close to unity. While this standard may serve for the routine re-calibration of the instrument in the case of changes in the relative intensities of 375 and 470 nm LEDs, comparative measurements with the laboratory-based XE-PAM fluorimeter have shown that a calibration using mesophyll of the same or a closely related type of leaf is more reliable (see above and Discussion).

## Results

An important advantage of the portable UV-A-PAM fluorimeter is its optical design based on a flexible light tube, which allows repetitive and non-intrusive assessment of UV screening properties of different areas of the same sample in its natural environment over extended periods of time. The following experiments, with grape berries and barley leaves, illustrate these advantages (Fig. 1). The instrument readily determines spatial heterogeneities of UV screening within plant organs without affecting the plants' integrity. It is clear that only the exposed surface of grape berries possesses efficient UV screening, whereas the non-exposed sides are rather UV transparent (Fig. 1*A*). Consistent with the results of Wagner *et al.* (2003), a marked gradient of UV screening in barley leaves was observed, with lowest and highest UV screening occurring close to the base and within the tip region, respectively (Fig. 1*C*, *insert*).

Such heterogeneities potentially interfere with determination of temporal changes of UV screening. Therefore, to investigate how natural radiation induces UV screening, we confined ourselves to defined areas of the plant organs, namely the most exposed surface of grape berries and the central position of barley leaves (Fig. 1*B*,*C*). The UV-A-PAM fluorimeter permitted to repeatedly probe the same areas during acclimation of greenhouse-grown plants to outdoor conditions. Consistent with earlier results from grape leaves (Kolb *et al.* 2001), an increase of UV screening in grape berries started only after a lag phase of about one day (Fig. 1*B*). In clear contrast, substantial improvement of UV screening was observed in barley leaves during the first day of outdoor exposure (Fig. 1*C*). During the experiment with barley leaves, weather conditions changed from clear to overcast skies after day 4. The data obtained with the UV-A-PAM fluorimeter showed unambiguously that the overcast skies stopped the ongoing build-up of UV screening (Fig. 1*C*).

Technical constraints confine the UV excitation by the UV-A-PAM fluorimeter to the long-wavelength (375 nm) UV-A. The deleterious potential of UV radiation, however, is much higher at shorter wavelengths (Caldwell and Flint 1997). Fig. 2 shows the absorbance

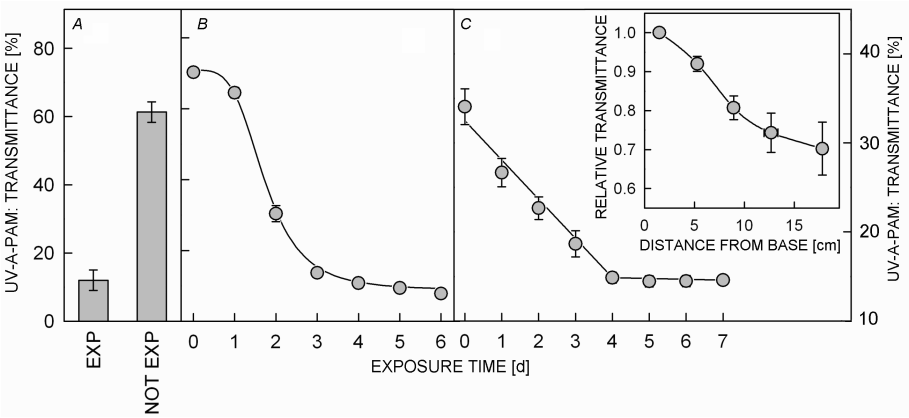


Fig. 1. UV screening, indicated by decreased UV transmittance values, measured in white grape berries (*A*, *B*) and barley leaves (*C*). All transmittance values were derived from UV-A-PAM measurements. Heterogeneous distribution of UV screening was investigated in plants acclimated to outdoor conditions: panel *A* compares berry skin transmittance of sun-exposed berry sides (EXP) with non-exposed sides (NOT EXP; data derived from Kolb *et al.* 2003). *The insert* in *C* depicts the lateral gradient of UV screening in barley leaves with transmittance values of individual leaves normalized to the measurement closest to the leaf base. Panels *B* and *C* depict induction of UV screening by natural radiation of exposed sides of berries and of the central area of barley leaves, respectively; in both cases, acclimation of greenhouse-grown plants was studied. Sunny conditions prevailed during the entire experiment in *B*. In *C*, the initial 4 d were sunny and days 5 to 7 were overcast. *Bars* indicate standard errors of means of $n = 10$, 22, 10, and $3<n<8$ individual measurements in *A*, *B*, *C*, and *insert to C*, respectively.

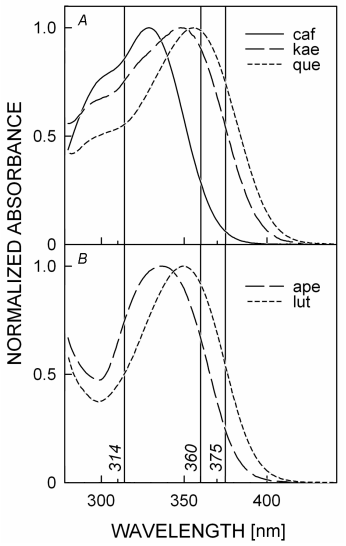


Fig. 2. Absorbance spectra of principal phenolics in grapevine (*A*) and barley (*B*). Positions of maximum UV excitation of the XE-PAM (314 and 360 nm) and the UV-A-PAM fluorimeter (375 nm) are indicated by vertical lines. ape, (monohydroxylated) apigenin derivative; caf, *trans*-caffeic acid (a hydroxycinnamic acid derivative); kae, (monohydroxylated) kaempferolderivative; lut, (*ortho*-dihydroxylated) luteolin derivative; que, (*ortho*-dihydroxylated) quercetin derivative (for information on grape and barley phenolics see Kolb *et al.* 2003 and Kolb and Pfündel 2005).

spectra of the principal phenolics involved in UV screening in grapevine and barley. At 375 nm, the excitation wavelength employed by the UV-A-PAM fluorimeter, absorbance by these compounds ranged between 5 and 75 % of the maximal absorbance observed when shorter UV wavelengths were employed. These large variations probably result in different relationships for different samples between UV-A-PAM data and UV screening at shorter wavelengths. To test this idea, measurements were obtained with the UV-A-PAM and XE-PAM fluorimeters to allow, in principle, determine screening in the entire UV-A and UV-B region. Fig. 3 shows the relationships derived from such measurements with grape and barley leaves, as well as with grape berries.

For leaves of both species, we observed reasonable linear trends between XE-PAM and UV-A-PAM data (Fig. 3*A–D*): the plot of transmittance data at 360 nm *vs.* 375 nm in barley leaves, however, shows a curved relationship (Fig. 3*D*). Much of the curved relationship can be explained by a simple model (Fig. 3*D*, *solid curved line*) which accounts not only for the different absorbance at 360 and 375 nm of the main flavonoids in barley (Fig. 2*B*) but also for the small and constant fraction of UV screening in barley that consists of absorption by apigenin derivatives plus a largely variable fraction of absorption arising from largely variable concentrations of luteolin derivatives (see legend of Fig. 3). When transmittance in the UV-B (at 314 nm) is compared with UV-A-PAM data, the slopes of regression lines were much smaller than 1 and the slope of the regression line obtained with grape leaves was about 50 % of that of barley leaves (Fig. 3*A,C*).

In berries, the relationships between XE-PAM and UV-A-PAM data were unique: while the correlation between transmittance at 314 and 375 nm was poor and the regression line extrapolated clearly above the origin, an almost perfect correlation was obtained between transmittances at 360 and 375 nm (Fig. 3*E,F*).

## Discussion

Portability and use of a flexible light guide are features of the UV-A-PAM fluorimeter that allows conveniently explore UV screening in attached leaves and berries in the field (Fig. 1). Because UV-A-PAM measurements do not affect the integrity of plant organs, temporal variations of UV screening could be reliably monitored by repeated examination of identical locations on given samples. In this way, the UV-A-PAM fluorimeter provides an excellent tool to study dynamic spatio-temporal variations of UV transmittance under field conditions.

The current version of the UV-A-PAM fluorimeter permits the derivation of estimates of UV screening only at 375 nm. The relationships between this data and UV screening at shorter wavelengths, determined by the XE-PAM fluorimeter, varies depending on the XE-PAM wavelength employed and the material being examined (Fig. 3). To understand these variations, at least three factors must be considered:

(*1*) Fluorescence excitation and signal detection occurs at different angles relative to the sample surface in the two fluorimeters. In principle, the different geometries could affect the measured ratios of fluorescence excited by UV and visible radiation.

(*2*) With the XE-PAM fluorimeter, UV transmittance was calculated with $F_{M,UV}/F_{M,VIS}$ ratios measured using various plant materials as the mesophyll reference. With the UV-A-PAM fluorimeter, transmittance was calculated with a fluorescence standard to represent $F_{M,UV}/F_{M,VIS}$. Certainly, the calculation of transmittance values will be affected by these differently calculated $F_{M,UV}/F_{M,VIS}$ values.

(*3*) Absorbance of UV screening phenolics at excitation wavelengths of the UV-A-PAM fluorimeter differs from that at XE-PAM wavelengths (Fig. 2). Therefore, species-dependent differences in spectral characteristics of phenolics could also affect the relationship between results obtained with the two fluorimeters. Theoretically, this effect could be minimised by using a XE-PAM excitation window centred at 375 nm, the wavelength of peak emission of the UV-A-PAM. However, we are not aware of filters that permit a corresponding modification of the XE-PAM.

Ounis *et al.* (2001) demonstrated that the ratio of fluorescence excited by UV and visible radiation in tobacco leaves is virtually unaffected by the angle of incidence of the excitation beam; therefore, in the case of leaves, it is unlikely that different optical geometries affect the relationships between the data obtained with the two fluorimeters. With the XE-PAM fluorimeter using UV-A excitation, we found that the $F_{M,UV}/F_{M,VIS}$ ratios of our mesophyll references for leaves were higher than that of the fluorescence standard (see Materials and methods). Hence, it appears possible that leaf transmittance obtained with the UV-A-PAM fluorimeter was overestimated. Therefore it is necessary to always measure appropriate mesophyll samples in addition to the routine calibration of the UV-A-PAM fluorimeter with a fluorescence standard (Krause *et al.* 2003).

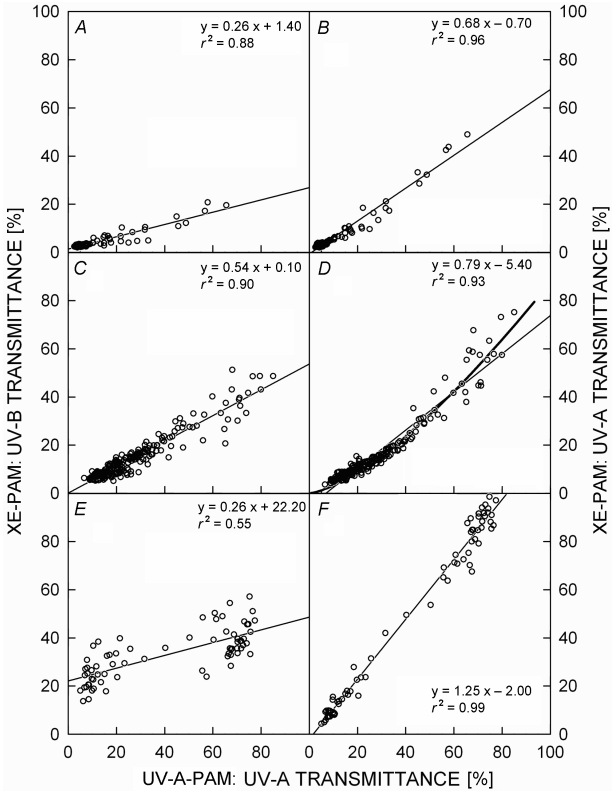


Fig. 3. Comparison of the results obtained with two types of UV fluorimeters. Transmittance values in the UV-B (314 nm) and UV-A (360 nm) determined with a XE-PAM fluorimeter are plotted against UV-A transmittance (375 nm) determined with a UV-A-PAM fluorimeter in *the left-hand* and *right-hand panels*, respectively. The experimental materials used were grape leaves (*A* and *B*), barley leaves (*C* and *D*), and white grape berries (*E* and *F*). *Straight lines* result from linear regression analyses for which equations and coefficients of determination are given. *The curved line* in panel *D* results from a model which is based on the observation that UV screening in barley leaves consists of a moderate and constant component due to apigenin derivatives plus a variable component arising from variable concentrations of luteolin derivatives (Kolb and Pfündel 2005). For the calculation, the absorbance at 360 nm of apigenin derivatives was set at 0.1 and that of luteolin derivatives was varied from 0 to 3. From absorbance at 360 nm, values for 375 nm were computed using a 375/360 nm absorbance ratios of 0.3 and 0.7 for apigenin and luteolin derivatives, respectively, which both agree reasonably well with absorbance spectra of the main apigenin and luteolin derivatives in barley (Fig. 2). Finally, all absorbance data were transformed into transmittance values.

The different $F_{M,UV}/F_{M,VIS}$ ratios, however, do not explain why the slope between UV-B transmittance and transmittance derived by the UV-A-PAM fluorimeter was

much smaller in grape leaves than in barley leaves (Fig. 3*A*,*C*). Almost certainly, this difference results from significant UV screening by hydroxycinnamic acids, present in grapevine but not in barley (Kolb and Pfündel 2005), that absorb poorly at 375 nm which is the UV excitation wavelength of the UV-A-PAM fluorimeter (Fig. 2).

In the UV-A region, the relationship between XE-PAM and UV-A-PAM data exhibited a curvilinear trend in the case of barley leaves (Fig. 3*D*). That the curvilinear relationship probably originates in different absorbance characteristics of flavonoids at the UV-A wavelengths of the XE-PAM (360 nm) and UV-A-PAM (375 nm) fluorimeters is suggested by a model curve which fits reasonably well to the data (see curved line in Fig. 3*D*). This model is based simply on the different absorbance at 360 and 375 nm of the principal apigenin and luteolin derivatives in barley and, additionally, considers the absorption provided by both the variable and the constant contents of luteolin and apigenin derivatives, respectively (see Kolb and Pfündel 2005). Curvature of our model plots decreases with increasing ratios of flavonoid absorbance at 375 nm relative to that at 360 nm (not shown). Generally, the absorbance spectra of grapevine flavonoids are red-shifted relative to those of barley flavonoids (Fig. 2) resulting in higher 375/360 nm absorbance ratios for grapevine than for barley flavonoids (Fig. 2). Therefore, we suggest that curvilinearity was not evident in grape leaves (Fig. 3*B*) because of the relatively high 375/360 nm absorbance ratios of grapevine flavonoids.

The results obtained with grape berries were unique because, among all data sets, both the best and the worst correlations were obtained when XE-PAM data in the UV-B (314 nm) and UV-A (360 nm), respectively, were compared with transmittance derived from UV-A-PAM data (see Fig. 3*E*,*F*). Surface optics of grape berries differ from leaves in several aspects: they exhibit a curved rather that flat shape, UV-screening phenolics are accumulated in a multi-layered skin region below the epidermis rather than within the epidermis (Kolb *et al.* 2003) and they are covered by a wax layer which, according to data from other species (Honkavaara *et al.* 2002), is expected to reflect UV radiation better than leaves. Therefore, we cannot exclude the possibility that the different geometries of the two fluorimeters affect fluorescence measurements in grape berries in different ways. Furthermore, high intensities of reflected/scattered excitation radiation may reach the detector filters where they might, for example, excite filter fluorescence. This can significantly contribute to the total signal, especially when UV excited fluorescence is low in efficiently screened samples. The existence of such an artefact in the case of the XE-PAM fluorimeter, but not in the UV-A-PAM fluorimeter, could explain the plot between UV-B transmittance and UV-A-PAM data extrapolating clearly above the origin (Fig. 3*E*). The excellent relationship between the two instruments in the UV-A region (Fig. 3*F*) strongly suggests that such artefacts are mostly confined to UV-B excitation. The latter plot was also characterized by a particularly steep slope (compare Fig. 3*F* with 3*B* and *D*). The $F_{M,UV}/F_{M,VIS}$ ratio for UV-A excitation obtained with berry pulp was much lower than the corresponding $F_{M,UV}/F_{M,VIS}$ ratios from leaves (see Materials and methods). Possibly, the true $F_{M,UV}/F_{M,VIS}$ of the unshielded berry skin is higher than that obtained with berry pulp and, consequently, over-estimation of XE-PAM-derived transmittance by use of a $F_{M,UV}/F_{M,VIS}$ ratio which is too low might explain the steep slope observed in Fig. 3*F*.

In conclusion, for various biological samples different relationships exist between screening at 314 or 360 nm and 375 nm. Therefore, simple extrapolation of UV screening determined with the UV-A-PAM fluorimeter to shorter wavelengths in most cases is not feasible. However, once a reliable relationship between transmittance data at different wavelengths has been established, for example with the help of the XE-PAM fluorimeter, assessment of UV screening at shorter wavelengths by UV-A-PAM measurements is possible. This could be a considerable advantage for field measurements, where use of the XE-PAM fluorimeter would be too cumbersome.

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