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SEASONAL CHANGES IN BERRY FLUORESCENCE INDUCED BY DIFFERENT LEVELS OF UV-RADIATION

VARIATIONS SAISONNIERES DE LA FLUORESCENCE DES BAIES INDUIT PAR DIFFERENTS NIVEAUX DE RAYONNEMENT UV

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Abstract:

Plant stress is usually diagnosed via physiological measurements on leaves such as water potential, photosynthetic rate or chlorophyll fluorescence. Since stress in the case of grapevines is of concern with respect to the quality of the fruit, we attempted to use one of the above mentioned techniques, chlorophyll fluorescence, directly on the fruit itself in order to investigate stress responses *in vivo*. Berry fluorescence was measured in an experiment with different levels of UV-B and UV-A radiation in the field in 2000 in Geisenheim with the variety Riesling. UV radiation has been shown to affect chlorophyll and was thus likely to show differences in fluorescence signals. Berry fluorescence was measured periodically between veraison and harvest on dark-adapted berries (20min). For each berry, a light response curve was recorded followed by a recovery phase in the dark. The maximum quantum yield of PSII (qY) was determined after a saturation pulse at each light intensity. The qY decreased exponentially with increasing light intensity. The response level of the quantum yield of PSII decreased during ripening indicating a loss in chlorophyll and/or PSII capacity for all treatments. Effects of the different levels of UV-radiation on the light curve were only detected at the end of the ripening period, with higher quantum yield values recorded for the treatments protected against UV-A and UV-A+UV-B. Similar results were obtained after recovery in the dark. Changes in fluorescence signals were accompanied by a visual change in berry colour indicating changes in skin pigmentation, which may also have some effect on fruit quality.

Keywords: UV, berry fluorescence, maximum quantum yield of PSII, Riesling

Résumé :

Le stress chez les plantes est généralement diagnostiqué à partir de mesures physiologiques sur les feuilles comme par exemple le potentiel hydrique, le taux de photosynthèse ou la fluorescence de la chlorophylle. Dans le cas de la vigne, la qualité du raisin élanat en relation avec l'intensité de la contrainte, nous avons essayé d'utiliser une des mesures mentionnés, la fluorescence de la chlorophylle, directement sur la baie de façon à rechercher les réponses au stress *in vivo*. La fluorescence des baies a été mesurée dans une expérimentation avec différents niveaux de rayonnement UV-B et UV-A installée au champs pendant l'année 2000 à Geisenheim avec le cépage Riesling. Il a été démontré que le rayonnement UV influence la chlorophylle et induit des modifications dans les signaux de fluorescence. La fluorescence des baies a été mesurée périodiquement entre la véraison et la vendange sur des baies adaptées à l'obscurité (20min). Pour chaque baie on a enregistré une courbe de réponse à la lumière qui a été suivie par un phase de récupération à l'obscurité. Le rendement quantique maximal du PSII (qY) a été déterminé après un flash lumineux saturant pour chaque intensité de lumière. Le qY décroît exponentiellement avec l'augmentation de l'intensité de la lumière. Le niveau de réponse du qY changeait pendant la période de maturation en indiquant une perte de chlorophylle et/ou de la capacité du PSII pour tous les traitements. On a aussi constaté des effets des différents niveaux de rayonnement UV sur la courbe de réponse à la lumière seulement à la fin de la maturation où on a observé des qY plus élevés sur les traitements protégés des rayonnements UV-A et UV-A+UV-B. Des résultats similaires ont été obtenus après la récupération dans l'obscurité. Les modifications des signaux de la fluorescence ont été accompagnés par des altérations visuelles sur la couleur des baies ce qui indique des altérations de la pigmentation de la pellicule et qui peut aussi affecter la qualité du raisin.

Mots clés: UV, fluorescence de la baie, rendement quantique maximal du PSII, Riesling

INTRODUCTION

Increased UV-B radiation could damage terrestrial organisms. The UV-B waveband ranges from 280nm to 320nm, although only wavelengths greater than 290nm can reach the earth's surface. In sunlight, the rate of UV-B to photosynthetically active radiation (PAR, 400-700nm) fluctuates, primarily caused by changes in solar angle and thickness of the ozone layer. The depletion of the latter decreases UV-B screening. Estimates of changes in UV-B radiation over the past decades suggest an increase of about 8% per decade with possible monthly deviations from the mean value by up to +40% under clear sky conditions for latitudes between 30° and 45° North (McPeters et al. 1996), encompassing the latitudes where almost all grapes are grown in Europe. Research in the past few years has indicated an increase in UV-absorbing compounds as an almost ubiquitous response to increased UV-B radiation. Since most of these reactive components such as flavonoids, amino acids and carotenoids are important constituents of grapes with a marked effect on flavour development, some effects of UV-B radiation on grape composition can be expected (Schultz et al. 1998). Since UV-B can inactivate photosystem II and photosynthetic enzymes and reduce chlorophyll and carotenoid concentrations in plant tissues (Jansen et al. 1998), we thought of assessing PSII functioning in grape berries through fluorescence analyses to devise a non-destructive method for diagnosing UV-B stress-induced changes in berry composition.

MATERIALS AND METHODS

Growth conditions: The experiment was conducted in a 21-year old vineyard of Riesling grapevines clone 198 on 5 C rootstock at the State Research Institute at Geisenheim, Germany (50° N, 8° E) in 2000. The vines were pruned to 8 buds m⁻², shoots were positioned vertically, row orientation was East-West and row distance 2m.

UV - Treatments: Three weeks after bloom, UV-B, UV-B+UV-A absorbing- and a neutral non-absorbing (neutral) polyester film (1.1 m with, 0.1 mm thickness, Schleussner KG, Dreieich, Germany) was installed in blocks of 4.8m length on the south facing side of the canopy at an angle of 35° with respect to the foliage to cover the fruit zone (lower 75 cm of the canopy) in which approximately 20% of the leaves had been removed. The rest of the canopy remained unshielded (natural). The spectral characteristics of the film (bandwidth 300-1100 nm) were measured with a portable spectro-photometer (Li-Cor 1800, Lincoln, Nebraska, USA); there were only small fractions of UV-B or UV-A light transmitted. Light attenuation in the photosynthetic active region of the spectrum (400-700 nm) was 3-4%. Maximum and minimum air temperatures under the cover differed by less than 1° C from ambient. Those treatments were compared with two controls, one covered by a neutral film and the other under natural conditions.

Berry fluorescence: Berry fluorescence was measured periodically between veraison and harvest on 6 berries per treatment. Whole clusters were dark adapted for 20 minutes using sheets of black cloth. To avoid heating of the berries, the cloths were loosely fitted around the cluster and the base kept open to allow air circulation. The leaf-clip holder of a portable chlorophyll fluorometer (Mini-Pam, Walz, Effeltrich, Germany), adapted for measurements on berries, was installed underneath the cloth and supported by an elastic suspender attached to one of the canopy catch wires in order to conduct the measurements *in vivo* on attached berries. The berries were carefully inserted into the leaf-clip holder and a light response curve, consisting of nine consecutive measurements at increasing light intensities (from 0 to 1400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR), was then recorded. After termination of the last illumination period, a recovery phase in the absence of actinic light was assessed by 6 consecutive saturation pulses applied at 10, 30, 70, 150, 310 and 630 s after light off. The maximum quantum yield of PSII

(qY) was determined after a saturation pulse at each light intensity according to the expression of Genty *et al* (1989):

$$qY = (Fm' - F) / Fm'$$

where F and Fm' are the minimum and maximum fluorescence, respectively, of a sample adapted to a given light intensity.

RESULTS

Light and recovery response curves

Berry fluorescence was usually measured on clear days between veraison and harvest. The qY was highest for dark adapted berries and decreased rapidly with increasing light intensity irrespective of the state of berry development, attaining very low values at the higher PAR levels (Fig. 1). When the light response curves between measurement dates were compared, a gradual shift to lower qY values with time was observed, reaching very low values at harvest (fig. 1).

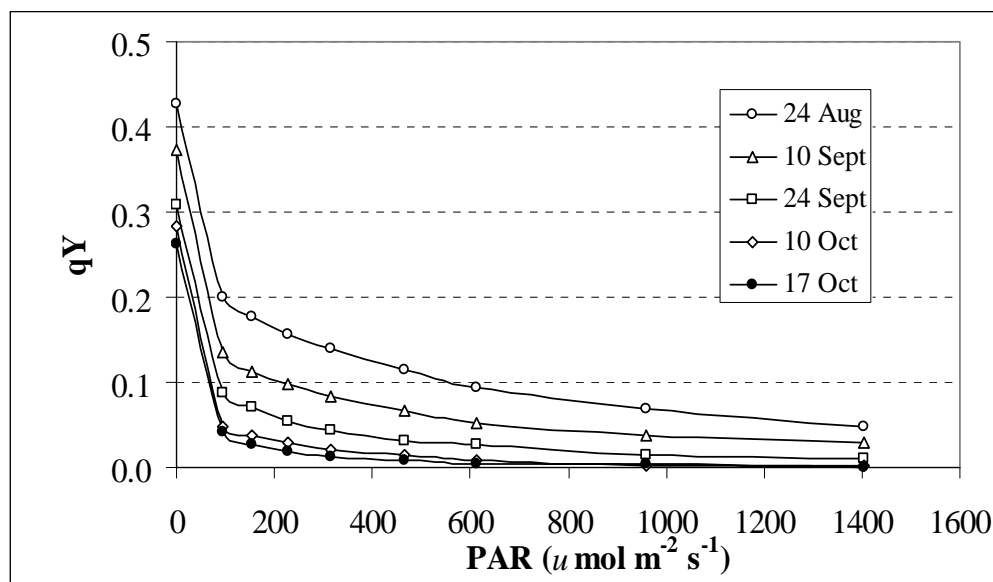


Figure 1 - Light response curve of the quantum yield of PSII (qY) recorded on previously dark-adapted berries of Riesling grapevines in Geisenheim. Data are averages of 4 treatments with 6 berries per treatment.

Figure 1 - Courbe de réponse à la lumière du rendement quantique du PSII (qY) enregistré sur les baies de Riesling préalablement adaptées à l'obscurité à Geisenheim. Les données sont des moyenne de 4 traitements avec 6 baies par traitement.

The recovery of qY in the absence of actinic light showed an opposite pattern to the light curve (Fig. 2). The qY increased with time reaching the highest values after the maximum length of the recovery period (630 s). These values were similar to those found at the beginning of the light curve for dark adapted samples. Similar to the results from the light curves, recovery values of qY also decreased during berry development.

The first two curves (veraison and 3 weeks later) were well separated at all recovery times, but in the second half of the ripening period, the curves presented smaller differences and were distinguishable only after 150 seconds.

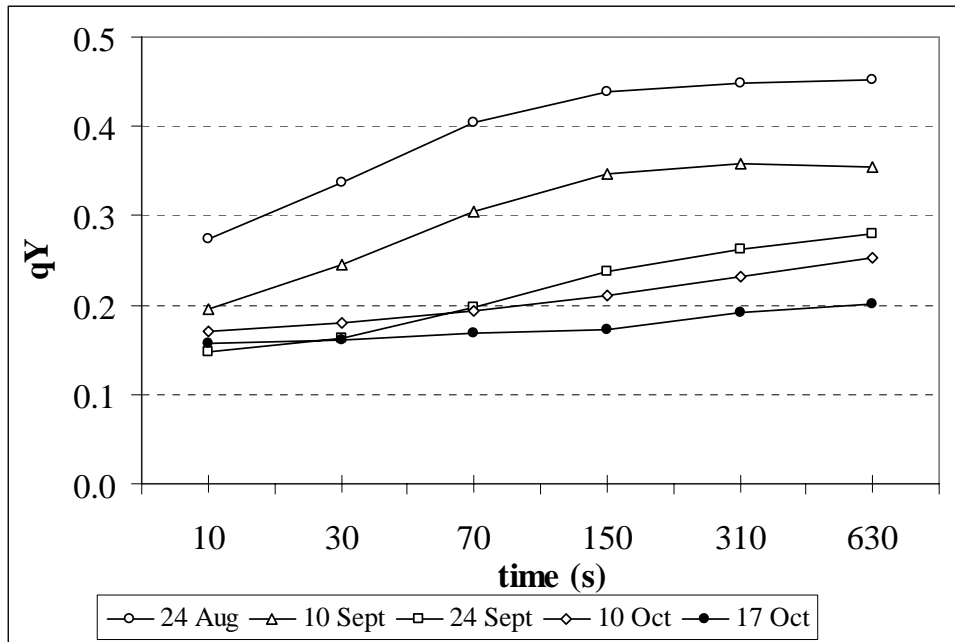


Figure 2 - Effect of time during the season on the recovery phase of the qY of Riesling berries in the dark recorded after a light response curve. Data are averages of 4 treatments with 6 berries per treatment.

Figure 2 - Effet du temps au cours de la saison sur la phase de récupération du qY des baies de Riesling à l'obscurité enregistré après une courbe de réponse à la lumière. Les données sont des moyennes de 4 traitements avec 6 baies par traitement.

UV Effects

The effects of different levels of UV-radiation on the light response curve of PSII activity were only detected at the end of the ripening period with the main differences at low light intensities (Fig. 3). The highest qY of dark adapted samples was recorded on berries from the UV-A+UV-B shielded treatment but differences were significant only with respect to the two control treatments (Fig. 4). Similar relative differences were obtained after recovery in the dark but the differences between treatments remained consistent throughout the recovery periods (Fig.5).

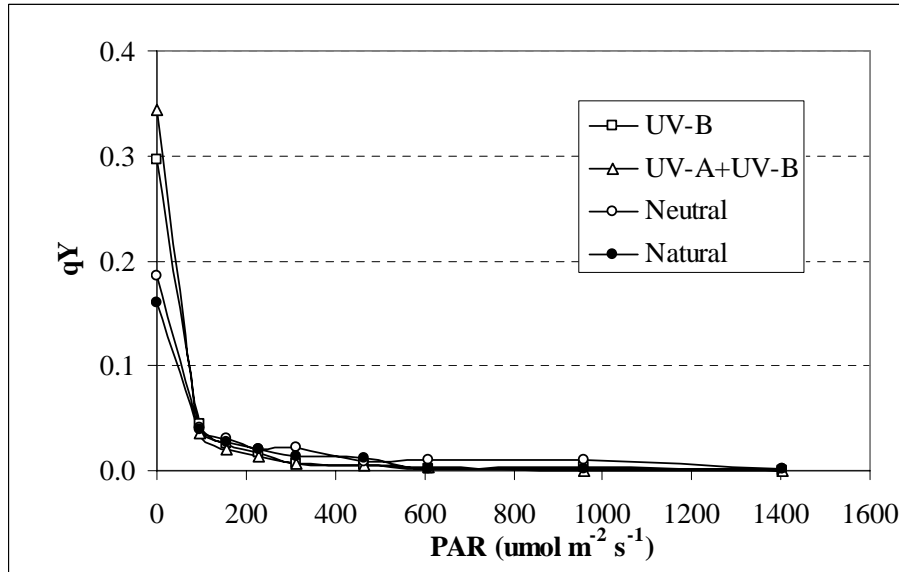


Figure 3 - Effect of UV radiation on the light response curve of PSII qY recorded at the end of the ripening period on dark-adapted berries of Riesling grapevines in Geisenheim. Data are averages of 6 berries per treatment.

Figure 3 - Effet du rayonnement UV sur la courbe de réponse du PSII qY enregistré à la fin de la période de maturation sur des baies de riesling adaptées à l'obscurité à Geisenheim. Les données sont des moyennes de 6 baies par traitement.

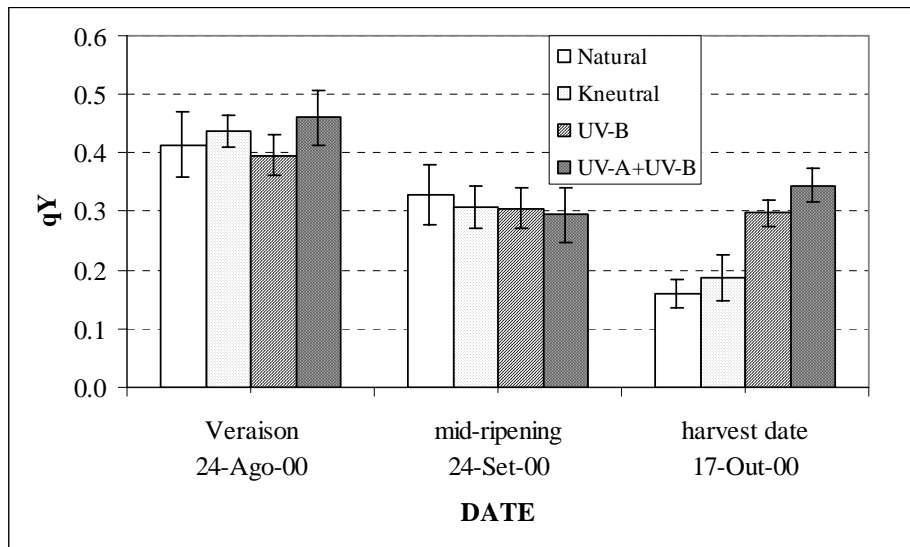


Figure 4 - Effects of UV radiation on the potential maximal quantum yield of PSII of dark-adapted Riesling berries. UV-B and UV-B+UV-A: treatments protected against UV-B and UV-B+UV-A radiation by an absorbing polyester film; Neutral = control covered with a non-absorbing polyester film; Natural = control under natural conditions (no film).

Figure 4 - Effets du rayonnement UVB sur le rendement quantique maximal potentiel du PSII de baies de Riesling adaptées à l'obscurité. UV-B et UV-B+UV-A: Traitements protégés contre le rayonnement UV-B et UV-B+UV-A par un film absorbant polyester; Neutral = témoin couvert d'un film polyester non absorbant; Natural = témoin sous conditions de rayonnement naturel (pas de film).

DISCUSSION

The present results show that fluorescence measurements can be conducted on the fruit itself after some adaptation of the leaf-clip holder. Maximum qY values between 0.4-0.5 were observed at the beginning of the measurements around veraison. At the same stage during the season leaves have qY values of between 0.75 and 0.83 (Schultz 1996). The differences can probably be attributed to a substantial loss in chlorophyll concentration in berries between flowering and veraison (Frieden et al. 1987). After veraison chlorophyll loss continues but at a much reduced rate (Frieden et al. 1987). Although qY also continued to decrease during berry maturation, the effect was much less pronounced for the treatments protected against UV radiation, indicating that some changes in the photosynthetic apparatus were induced by UV-light.

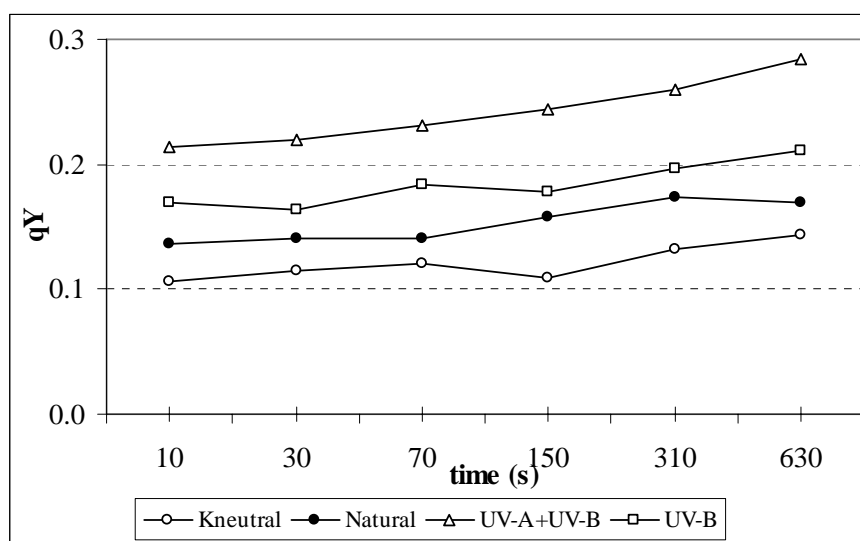


Figure 5 - Effect of UV radiation on the recovery phase of qY in the dark of the maximum quantum yield of PSII (qY) recorded at the end of the ripening period in dark adapted berries of Riesling grapevines. Data are averages of 6 berries per treatment.

Figure 5 - Effet du rayonnement UV sur la phase de récupération du qY à l'obscurité du rendement quantique maximal du PSII enregistré à la fin de la période de maturation sur des baies de riesling adaptées à l'obscurité. Les données sont des moyennes de 6 baies par traitement.

The rate of recovery of qY after exposure to high light slowed markedly during the ripening period. In mid-October, recovery was very slow suggesting long-lasting photo damage. However, the rate of recovery was essentially similar for all treatments yet with different initial qY values, indicating that the differences may be related mainly to changes in the pigment content or pigment composition.

Plants have effective ways of dealing with UV-radiation. Special enzymes (photolyases) can effectively repair many of the lesions caused by UV-B light if sufficient UV-A or visible light is present and temperatures are favourable (Britt 1996). With this in mind it is surprising that the treatment shielding against UV-B and UV-A radiation had the highest qY at the end of the season. This implies that UV-A may, under certain conditions, contribute to PSII damage and/or accelerate senescence. The changes in PSII activity are probably related to oxidative damage, which can lead to lipid peroxidation, chlorophyll and DNA damage due to the

production of free radicals (Caldwell et al. 1999). However, UV-radiation also stimulates antioxidant enzymes such as glutathione reductase, superoxide dismutase and ascorbate peroxidase and can induce gene expression of genes regulating different parts of the phenylpropanoid pathway causing changes in the synthesis of phenolics (Jansen et al. 1998). It is the combination of these responses which probably influenced fluorescence signals of the berries. The visual changes in berry colour accompanying the changes in fluorescence indirectly confirm this hypothesis. The alterations in skin pigmentation may also have some effect on fruit quality, since several pigments serve as precursors to aromatic components in wine (i.e. Razungles et al. 1993, Schultz et al. 1998).

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