Environmental factors correlated with the metabolite profile of *Vitis vinifera* cv. Pinot Noir berry skins along a European latitudinal gradient

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ABSTRACT

Mature berries of Pinot Noir grapevines were sampled across a latitudinal gradient in Europe, from southern Spain to central Germany. Our aim was to study the influence of latitude-dependent environmental factors on the metabolite composition (mainly phenolic compounds) of berry skins. Solar radiation variables were positively correlated with flavonols and flavanonols and, to a lesser extent, with stilbenes and cinnamic acids. The daily means of global and erythematic UV solar radiation over long periods (bud break-veraison, bud break-harvest and veraison-harvest), and the doses and daily means in shorter development periods (5-10 days before veraison and harvest) were the variables best correlated with the phenolic profile. The ratio between trihydroxylated and monohydroxylated flavonols, which was positively correlated with antioxidant capacity, was the berry skin variable best correlated with those radiation variables. Total flavanols and total anthocyanins did not show any correlation with radiation variables. Air temperature, degree days, rainfall and aridity indices showed fewer correlations with metabolite contents than radiation. Moreover, the latter correlations were restricted to the period veraison-harvest, where radiation, temperature and water availability variables were correlated, making it difficult to separate the possible individual effects of each type of variable. The data show that managing environmental factors, in particular global and UV radiation, through cultural practices during specific development periods, can be useful to promote the synthesis of valuable nutraceuticals and metabolites that influence wine quality.

Keywords: *Vitis vinifera cv. Pinot Noir, latitudinal gradient, phenolic composition, berry skins, solar radiation, ultraviolet radiation, hydroxylation ratios, Europe*

INTRODUCTION

Environmental factors, such as air temperature, ambient solar radiation (including UV) and photoperiod, vary with latitude. In turn, variations in these environmental factors may cause changes in physiological and/or biochemical characteristics of plants. Yet, this is not always the case as plant responses to latitudinal climatic conditions may be masked by, for example, local climatic factors, cultivational measures, or pest and diseases. Thus, there is a need for latitudinal studies that help to identify the environmental factors that impact most on plants, as well as the traits most affected. Such studies are important in terms of understanding ecological processes (especially in the context of climate change), but also have a direct relevance for the agricultural industry. A number of plant traits have been studied in relation to latitude, including plant height, seed production, growth, biomass production, photosynthesis rates, chlorophyll fluorescence, photosynthetic pigment composition, mineral nutrient contents and ratios, water relations and secondary metabolite contents.[1-8] Most of these traits have been measured in leaves, whereas only a few studies have used fruits. Latitude-related environmental variables that have been hypothesized to explain changes in plant traits include air temperature, degree days, rainfall, aridity indices, soil moisture, total solar radiation doses, and UV radiation doses. Most latitudinal studies have been carried out using wild species, while only a few studies have dealt with commercially interesting species, such as juniper,[3] ryegrass[7] and currant.[8] To our knowledge, no study has dissected the effects of latitudinal gradients, and the associated environmental parameters, on grapevine, although latitude is a recognized factor used, for example, to predict the suitability of territories for grapevine culture.[9]

Remarkably, the effects of latitude and associated environmental parameters on the phenolic composition of grapevine berries have not been studied, in spite of the fact that similar studies have been conducted on other species with less commercial impact.[3-5,7,8] This omission is even more remarkable, given that the phenolic compounds synthesized in grapevine berries decisively determine wine characteristics and quality, including the presence of important nutraceuticals and nutritionally-desirable antioxidants.[10,11] Berry skin is the main source of many of these phenolic compounds, including anthocyanins, flavonols and stilbenes.[12-14]

The present study was conducted on Pinot Noir grapevines. This variety is the tenth most cultivated grapevine worldwide, and the seventh fastest-expanding winegrape variety in the period 2000-2010.[15] Pinot Noir grapevines occupy more than 86,000 ha in the world (1.88% of the total grapevine acreage), especially in Europe, where it occupies 3% of the total acreage. Pinot Noir is especially adapted to cold climates, thus ascending to higher latitudes than other varieties. In fact, the European distribution of this cultivar ranges from southern Spain to central Germany. Given this wide ranging distribution, our aim was to identify the influence of latitude and associated environmental parameters (air temperature, global and UV radiation, rainfall and aridity) on the metabolite composition of berry skins of *Vitis vinifera* cv. Pinot Noir in Europe. This study will inform management of those environmental parameters that affect berry skin composition. In turn, a better understanding of the influence of these parameters can help improve wine quality.

MATERIALS AND METHODS

Collection sites and environmental variables

Berries of Pinot Noir grapevines (*Vitis vinifera* L.) were collected in 2013 from 11 localities in Spain, France, Italy, Hungary, Austria, Slovenia, the Czech Republic and Germany (Figure 1, Table 1). This represented a latitudinal gradient of almost 14º (36.7-50.0 ºN) and a linear distance of around 1,500 km, covering most of the commercial Pinot Noir growing latitudes in the Northern Hemisphere (35-55º).[16] Vineyard age varied between 6 and 30 years, and vineyard soils were mostly calcareous and neutral-alkaline (pH between 7.0 and 8.5). No fertilization or irrigation had been applied to the vineyards.

In each locality, berry samples were collected from three separate plants (replicates) at commercial maturity, always around noon-time, and on a sunny day. Collection dates varied from 31 July to 22 October, depending on the location. Three clusters were collected for each replicate. As row orientation varied between vineyards, clusters were always picked from a SE-orientated shoot. In situ, every berry was separated from its cluster by cutting the pedicel. Subsequently, berry density was determined as floatability in a NaCl solution series, which allowed for harvesting berries of a similar ripeness using a non-destructive method.[17,18] To reduce the variability that is normally found within a cluster, berries with a density between 140-160 g NaCl l^{-1} were selected, rinsed in distilled H_2O and immediately transported to the laboratory in a portable icebox. In the laboratory, berries were frozen in liquid nitrogen and kept at -80ºC until further analyses.

Relevant environmental data were obtained for each locality. Daily values of mean temperature, rainfall and ground-station global radiation (GGR) were obtained for the period bud break-harvest from the nearest meteorological observatory to each vineyard. For most vineyards, meteorological stations were located less than 200 m from the actual vineyards. Remaining stations were located less than 20 km away, except in the case of Lednice (Czech Republic) where the station for GGR measurement was located 50 km from the vineyard. In the latter cases, it was ascertained that meteorological stations were located at a similar latitude and altitude as the respective vineyards, which makes the assumption that data were homogeneous. Based on these data, two aridity indices were calculated: the ratio Rainfall/ETP, where ETP is the potential evapotranspiration computed according to Hargraves formula (based on solar global radiation and mean air temperature), and the Gaussen Index (the ratio between rainfall and twice the mean daily temperature). In addition, daily values of DSSF (Downward Surface Shortwave Flux) global radiation and TEMIS-derived erythematic UV radiation (T UVery) were obtained for the period bud break-harvest. Daily DSSF was calculated by integrating the 30 minutes of data downloaded from the LandSaf web page (http://landsaf.meteo.pt). The data in this archive take into account the differences in the day-length of the various locations. T UVery was downloaded from the ESA-TEMIS web page (http://www.temis.nl) and estimated on the basis of Meteosat data (to assess cloud cover), SCIAMACHY data (to assess O_3 column) and a radiative transfer model.[19] The degree days (using 10ºC as base temperature) and the daily doses of GGR, DSSF and T UVery were integrated over three different periods: bud break-veraison, bud break-harvest, and veraison-harvest. Additionally, DSSF and T UVery doses were integrated for 5 and 10 days before veraison, and for 5 and 10 days before harvest, because the periods around veraison and prior to harvest are important for the synthesis of phenolic compounds in grapevine berries and, thus, for their commercial quality.[20-22]

Analysis of berries

Frozen berries were allowed to partially thaw and the skin was carefully removed from the flesh using a scalpel, and without rupturing the hypodermal cells. The content of total soluble solids (TSS) was measured in ºBrix in the flesh, using a digital refractometer. The skins were immediately submerged in liquid nitrogen, weighed and lyophilized. Lyophilized berry skins were weighed and ground to obtain a homogeneous powder for each replicate. Then, all the samples were shipped to one laboratory for detailed analysis of metabolites.

For each analytical sample used for the analysis of phenolic compounds, 50 mg of skin powder was frozen in liquid nitrogen and ground again in a TissueLyser (Qiagen, Hilden, Germany). The total content of methanol-soluble phenolic compounds (MSPCs), mainly located in the vacuoles,[18] was measured by spectrophotometry. For this analysis, 2 ml of a mixture of methanol: water: 7M HCl (70:29:1 v:v:v) was added for extraction (24 h at 4ºC in the dark). The extract was centrifuged at 6000 g for 15 min and the supernatant was selected for spectrophotometry. The level of MSPCs was measured as the area under the absorbance curve in the wavelength intervals between 280-315 and 280-400 nm ($AUC_{280–315}$ and $AUC_{280–400}$ respectively) and normalised per unit of dry weight (DW),[23] using a λ35 spectrophotometer (Perkin-Elmer, Wilton, CT, USA). Individual phenolic compounds were analysed by ultra-performance liquid chromatography (UPLC) using a Waters Acquity UPLC system (Waters Corporation, Milford, MA, USA).[23] Solvents were: A, water/formic acid (0.1%), and B, acetonitrile with 0.1% formic acid. The gradient program employed was: 0-7 min, 99.5-80% A; 7-9 min, 80-50% A; 9-11.7 min, 50-0% A; 11.7-15 min, 0-99.5% A. The UPLC system was coupled to a micrOTOF II high-resolution mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with an Apollo II ESI/APCI multimode source and controlled by the Bruker Daltonics DataAnalysis software. The electrospray source was operated in positive or negative mode. The capillary potential was set to 4 kV; the drying gas temperature was 200 ºC and its flow 9 l min^{-1}; the nebulizer gas was set to 3.5 bar and 25 ºC. Spectra were acquired between *m/z* 120 and 1505 in positive mode for anthocyanins and in negative mode for the remaining phenolic compounds. The different phenolic compounds analysed were identified according to their order of elution and the retention times of the following pure compounds: myricetin, quercetin, catechin, epicatechin, astilbin, *trans*-resveratrol, *p*-coumaric acid, caffeic acid and ferulic acid (Sigma, St. Louis, MO, USA); kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-glucoside, syringetin-3-*O*-glucoside, procyanidin B1 and malvidin-3-*O*-glucoside (Extrasynthese, Genay, France); isorhamnetin, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucopyranoside, quercetin-3-*O*-glucuronide and quercetin-3-rutinoside (Fluka, Buchs, Germany). Quantification of compounds that were not commercially available was carried out using the calibration curves belonging to the most similar compound: myricetin for its glucosides; isorhamnetin for isorhamnetin-3-*O*-glucuronide; quercetin for quercetin-3-*O*-arabinoside; astilbin for taxifolin-3-*O*-glucoside; *trans*-resveratrol for its glucoside; *p*-coumaric acid for *p*-coumaroyl-tartaric acid; caffeic acid for *p*-caffeoyl-tartaric acid; ferulic acid for feruloyl-tartaric acid; and malvidin-3-*O*-glucoside for anthocyanins. Total contents of the different phenolic groups were obtained as the sum of the individual compounds. The ratios between trihydroxylated and dihydroxylated (3´,4´,5´-OH/3´,4´-OH) anthocyanins, and between trihydroxylated and monohydroxylated (3´,4´,5´-OH/4´-OH) and trihydroxylated and dihydroxylated (3´,4´,5´-OH/3´,4´-OH) flavonols, were also calculated.

For carotenoid and chlorophyll extraction,[24] 6 ml of a mixture of methanol, acetone, and hexane (1:1:1 v:v:v) was added to a glass tube containing 50 mg of lyophilized skin powder. The mixture was vortexed for 30 s and then stirred for 30 min at 4°C in the dark. After the addition of 2 ml of MilliQ water the tube was vigorously shaken for 1 min and then centrifuged for 1 min at 1500 g. The non-polar phase containing carotenoids and chlorophylls was recovered. The extraction was repeated by adding 2 ml of hexane to the remaining mixture. The two extracts were pooled and the volume reduced to 1 ml by vacuum evaporation. The extract was filtered through 0.2-µm filters and immediately subjected to high-performance liquid chromatography (HPLC) analysis as follows. Separation was performed at room temperature by a Spectra System P4000 HPLC, equipped with a UV 6000 LP photodiode array detector (Thermo Fisher Scientific, Waltham, MA, USA) using a Zorbax ODS column (5 µm particle size, 250 x 4.6 mm, Agilent Technologies, Santa Clara, CA, USA). HPLC separation was carried out at a flow rate of 0.8 ml min^{-1} using the following linear gradient: 0 min, 82% A (CH_3CN), 18% B (methanol/hexane/CH_2Cl_2 1:1:1 v:v:v); 20 min, 76% A, 24% B; 30 min, 58% A, 42% B; 40 min, 39% A, 61% B. The column was allowed to re-equilibrate in the starting solution (82% A, 18% B) for 5 min before the next injection. Different individual chlorophylls and carotenoids were detected by their absorbance at 445 nm.

The antioxidant capacity of berry skins was measured by generating the radical cation 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$).[25] The radical solution was diluted in ethanol to obtain an absorbance of 0.700 ± 0.020 at 734 nm (Perkin-Elmer λ35 spectrophotometer). After addition of 1 ml of diluted $ABTS^{\bullet+}$ solution to 100 µl of skin extract (250 µg of skin powder in 1 ml of a mixture of methanol: water: 7M HCl 70:29:1 v:v:v), the decrease in absorbance was monitored and compared to that of the Trolox standard (Sigma) exactly 4 min after initial mixing. Antioxidant capacity was expressed in terms of Trolox equivalent antioxidant capacity (TEAC) per g DW of skin.

DNA isolation from lyophilized berry skins was carried out using the ZenoGene40 Plant DNA Purifying Kit (Zenon Bio Kft., Szeged, Hungary). Concentration of the samples was measured with a Genova Nano Spectrophotometer (Jenway, Staffordshire, UK). DNA content per DW of berry skin (ng mg^{-1} DW) was calculated using the formula: mean of DNA concentration (ng μl^{-1}) multiplied by the volume of extraction (µl) and divided by the DW of the lyophilized sample (mg). This analysis served to calculate the metabolite concentrations on a DNA basis.

Statistical analysis

Pearson correlation coefficients (*r*) were used to examine the relationships between all the variables studied, both the environmental-geographical parameters and the traits analyzed in berry skins, including the total contents of the different groups of phenolic compounds. Correlations were considered significant when $p<0.05$. The sampling localities were ordinated by Principal Components Analysis (PCA), taking into account MSPCs and the total contents of the different groups of phenolic compounds. All the statistical procedures were performed with SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Variation in environmental variables

The latitudinal gradient used in this study was associated with substantial differences in several meteorological variables (Table 2). For the period from bud break to harvest, these differences were, amongst others, around 5ºC in mean daily temperature, 500 degree days, almost 300 mm in rainfall, almost 900 MJ m^{-2} in DSSF dose, and 241 kJ m^{-2} in T UVery dose. Interestingly, the parameters displaying the greatest differences were the DSSF and T UVery doses accumulated during the 10 days before harvest. For these variables, the differences between the maximum and the minimum values along the gradient were more than 80% of the maximum value. The highest and lowest values of temperature variables were usually recorded in Pécs and Rioja, respectively, except for the veraison-harvest period, in which they were recorded in Spanish localities (Jerez or Girona) and Lednice, respectively. The highest mean values of solar radiation (GGR, DSSF, T UVery) were always recorded in Jerez, and this included also the highest accumulated doses in the 5 or 10 days before veraison and before harvest. The highest accumulated doses over longer periods were recorded in Spanish localities (either Rioja, Girona or more rarely Jerez) or in Lednice, depending on the length of the period considered, because those periods were longer in Rioja, Girona or Lednice than in Jerez (see Table 1 for the length of the period bud break-harvest). The lowest values of radiation variables were generally recorded in Geisenheim or Lednice.

Variation in berries variables

Metabolite contents were obtained and normalized against both berry skin DW (Table 3) and DNA amount. The correlations between metabolites and environmental parameters were similar irrespective of the normalization approach, given that DNA amount and berry skin DW were significantly correlated (r = 0.79, p<0.01, n = 11). Therefore, results are only described on a per berry skin DW basis. MSPC values varied between 9.7 and 40.3 (as $AUC_{280\text{-}315}$ mg^{-1} DW) and between 17.1 and 74.3 (as $AUC_{280\text{-}400}$ mg^{-1} DW). Absorption levels in the two wavelength regions were strongly and positively correlated (Table S1). The highest and lowest MSPC values were found in Girona and Lednice, respectively. We quantified 29 phenolic compounds: 24 flavonoids

(14 flavonols, 5 anthocyanins, 3 flavanols –monomeric or dimeric tannins-, and 2 flavanonols) and 5 non-flavonoids (3 cinnamic acids and 2 stilbenes). Great differences in the concentrations of most groups of phenolic compounds were found between localities. Anthocyanins were the most abundant group, showing values between 18.9 (Bilje) and 110.1 (Girona) mg g^{-1} DW. In every locality, malvidin-3-*O*-glucoside was the major anthocyanin. Flavonols were the second most abundant group of flavonoids, ranging between 1.76 (Bilje) and 7.7 (Girona) mg g^{-1} DW. The major flavonol was quercetin 3-*O*-glucuronide. Flavanonols (between 0.18 and 1.14 mg g^{-1} DW, in Bilje and Jerez, respectively) and flavanols (between 0.21 and 0.99 mg g^{-1} DW, in Lednice and Bilje, respectively) were less abundant. Among non-flavonoids, cinnamic acids were the most abundant group, and also the group showing the greatest variability between localities, with values between 0.16 (Lednice) and 7.2 (Firenze) mg g^{-1} DW. Finally, the least abundant compounds were stilbenes, which also showed a great variability (between 14 and 928 µg g^{-1} DW, in Lednice and Girona, respectively).

The antioxidant capacity of berry skin extracts varied between 3592 (Lednice) and 9104 (Firenze) µM TE g^{-1} DW. Chlorophylls and all carotenoids showed the highest values in Rioja and the lowest in Pécs. β-Carotene was the most abundant carotenoid. The berry fresh weight varied between 1.1 (Girona and Bordeaux) and 2.1 g (Geisenheim), although most localities showed values between 1.1 and 1.3 g. TSS varied between 19.1 (Bilje) and 23.7 ºBrix (Jerez).

Correlations between variables

The correlations between all the environmental and plant response variables were determined (Table S1). Unless otherwise stated, the correlations mentioned in this text were significant ($p<0.05$) and positive. With respect to the correlations between berry skin variables, MSPCs were correlated with the contents of most phenolic compounds (except flavanols) and carotenoids. The total contents of flavonols, flavanonols, stilbenes and anthocyanins were correlated with one another, whereas the total content of cinnamic acids was only correlated with that of flavanonols. Total flavanol content was not correlated with the total content of any other phenolic group. The antioxidant capacity of berry skin extracts was correlated with anthocyanins, MSPCs, flavonols, the ratio 3´,4´,5´-OH/3´,4´-OH flavonols and, less significantly, with flavanonols, cinnamic acids, the ratio 3´,4´,5´-OH/4´-OH flavonols, and carotenoids. There was no correlation between the antioxidant capacity and contents of stilbenes or flavanols. Carotenoid and chlorophyll contents were correlated with each other, and carotenoid levels were also correlated with those of stilbenes.

Possible correlations between environmental-geographical parameters and berry skin variables were also explored. It was found that latitude was negatively correlated with MSPCs and the total contents of flavonols, flavanonols and stilbenes, but not flavanols, cinnamic acids, anthocyanins and carotenoids (Figure 2).

Correlations between temperature variables and berry variables were few for the periods bud break-veraison and bud break-harvest. The mean daily temperature and degree days in the period bud break-veraison (but not bud break-harvest) were correlated (negatively) with carotenoids, chlorophylls and TSS, only. Degree days in the period bud break-veraison were also correlated with flavanonols. No temperature variable in these two periods was correlated with the total content of any other phenolic group, although there were some correlations between temperature variables and individual compounds. For the period veraison-harvest, the mean daily temperature and degree days were correlated with MSPCs and the total contents of flavonols and flavanonols. In addition, the mean daily temperature was correlated with the ratios 3´,4´,5´-OH/4´-OH and 3´,4´,5´-OH/3´,4´-OH flavonols, and the degree days with the total content of anthocyanins.

Rainfall and aridity indices were hardly correlated with berry skin variables for the periods bud break-veraison and bud break-harvest. Only quercetin showed somewhat consistent (positive) correlations with rainfall, the Rainfall/ETP ratio and Gaussen Index (but only in the period bud break-harvest). For the period veraison-harvest, rainfall and aridity indices were negatively correlated with the total content of flavonols and flavanonols. In addition, Gaussen index was negatively correlated with MSPCs and the ratios 3´,4´,5´-OH/4´-OH and 3´,4´,5´-OH/3´,4´-OH flavonols.

Radiation variables, particularly DSSF and T UVery variables, correlated well with berry skin variables for the three periods considered. The daily means of DSSF and T UVery in the periods bud break-harvest and veraison-harvest, the DSSF doses in the 10 days before harvest, the daily mean of T UVery in the 5 and 10 days before veraison, and the T UVery doses in the 5 and 10 days before veraison were all correlated with MSPCs. The same variables, together with the T UVery doses in the 10 days before harvest and in the period bud break-harvest (in this last case, with a lower significance level), were correlated with the total contents of flavonols and flavanonols. Total stilbene content was only correlated with the DSSF and T UVery doses in the period bud break-harvest, and total cinnamic acid content only with the daily mean and the dose of T UVery in the 10 days before veraison. Total flavanol and anthocyanin contents were not correlated with any radiation variable. Regarding individual compounds, the strongest correlations were found between contents of several flavonols and flavanonols and the daily means of DSSF and T UVery in the periods bud break-harvest and veraison-harvest, as well as with the DSSF and T UVery doses in the periods of 5 or 10 days before veraison or harvest. Levels of two flavanols, one anthocyanin and the three cinnamic acids analyzed were also correlated with some of those T UVery expressions.

The ratio 3´,4´,5´-OH/3´,4´-OH anthocyanins was not correlated with any radiation or temperature variable. Yet, the ratios 3´,4´,5´-OH/4´-OH and 3´,4´,5´-OH/3´,4´-OH flavonols were the berry skin variables that displayed the strongest correlations with specific radiation variables, such as the daily means of DSSF and T UVery in the periods bud break-harvest and veraison-harvest, and the accumulated doses in the 10 days before veraison and harvest. This correlation did, however, not extend to the accumulated doses in longer periods, as Figure 3 shows for the period bud break-harvest. Finally, the number of days from bud break to harvest and from veraison to harvest were negatively correlated with total and several individual flavanols.

Principal Components Analysis

The localities studied were ordinated by PCA using MSPCs and the different groups of phenolic compounds. The accumulated variance by the first three axes was 94.0% (67.3% for axis I, 17.3% for axis II and 9.4% for axis III). The plot using the first two axes, together with the loading factors and their significance, is shown in Figure 4. The total contents of all the phenolic groups, except flavanols, were significant loading factors for the positive part of axis I, which broadly ordinated the localities on the basis of their latitude, with southernmost localities situated towards the positive part of the axis and the northernmost ones towards the negative part. Total flavanols and total cinnamic acids were the only significant loading factors for the positive part of axis II, which separated localities 4, 6, 9, 7 and 1 from the remaining ones. No significant loading factor was found for the negative part of axes I and II.

DISCUSSION

Environmental-geographical gradients, such as those related to latitude, can be exploited to explore and predict the physiological and/or biochemical responses of plants by using a space-for-time substitution.[6] This type of study cannot necessarily pinpoint the influence of one particular environmental parameter on a plant response, as can be done in controlled studies. However, the strength of latitudinal studies is that plant responses are studied under realistic conditions (i.e. commercial vineyards), where plants are exposed to a natural combination of ambient, environmental parameters. In this study a range of metabolites were measured in skins of Pinot Noir berries, originating from 11 vineyards along a latitudinal gradient of nearly 14º. The levels of the various metabolites measured in Pinot Noir berry skins were broadly in agreement with levels measured in other studies using this, or other cultivars.[12,18,23]

Radiation is an important determinant of berry skin metabolite profile

A key finding of this study is that the contents of MSPCs, flavonols, flavanonols and stilbenes in Pinot Noir berry skins increased with decreasing latitudes. Previously, similar results were found for MSPC contents in leaves of *Lolium perenne*,[7] but no comparative results existed for specific phenolic compounds nor for grapevine. It might be argued that negative correlations between latitude and the abovementioned phenolic groups are due to the longer berry maturation period at lower latitudes. However, we consider this unlikely because (1) latitude was not significantly correlated with the number of days from veraison to harvest, and (2) the latter variable was not correlated with the contents of those phenolic compounds. Rather, the correlations between latitude and contents of phenolic compounds were probably due to the negative correlation between latitude and radiation (both global and UV) variables. Radiation variables were strongly and positively correlated with the total contents of most phenolic groups, mainly flavonols and flavanonols, and to a lesser extent with stilbenes and cinnamic acids, together with MSPCs. The relationship between radiation levels and the content of these phenolic compounds had previously been reported for berry skins of several red grapevine varieties, such as Pinot Noir, Merlot, Malbec and Cabernet Sauvignon,[26-29] although not in relation with latitudinal gradients.

Rather than radiation in general, the means of DSSF and T UVery over long periods (bud break-veraison, bud break-harvest and veraison-harvest) and the means or doses in important development periods (5-10 days before veraison and harvest) were the variables best correlated with phenolic compounds, particularly flavonols, flavanonols and cinnamic acids. This is related to the fact that the periods around veraison and prior to harvest are important for the synthesis of phenolic compounds.[20-22] The stimulation of flavonol acumulation was expected because these compounds are radiation-reactive and concentrations are well known to increase with increasing levels of solar radiation (particularly UV-B) in grapevine berry skins.[13,18,27,29-33]

It is not simply total flavonol levels that correlate with radiation parameters, the ratios 3´,4´,5´-OH/4´-OH and 3´,4´,5´-OH/3´,4´-OH flavonols were the berry skin variables best correlated with specific radiation variables, such as the mean values or doses of DSSF and T UVery radiation in critical periods (5-10 days before veraison and harvest), but not with the accumulated doses over long periods (Figure 3). Thus, higher solar radiation values (both total and UV) in those critical periods might increase the B-ring hydroxylation level of flavonols in Pinot Noir berry skins. Previously, it was shown that the hydroxylation level depends on both the grape variety[12] and environmental factors, such as the radiation level. The effect of radiation, in turn, may depend again on the variety considered: the hydroxylation ratios increased with increasing total or UV radiation in Pinot Noir (this study), but decreased with increasing total or UV radiation in Sangiovese[22] and Tempranillo.[18,34] This complexity may be caused by the intricate regulation mechanism of the genes and enzymes involved in the synthesis of flavonols with different hydroxylation levels.[21,30,31] In petunia, the highest level of B-ring hydroxylation was caused by the specific effect of increased UV-B radiation.[35] The antioxidant activity of flavonoids strongly depends on the number of hydroxyl groups bound to the aromatic B-ring.[36] Given that the hydroxylation ratios were positively correlated with the antioxidant capacity in our study, flavonols may be important as both sunscreens and antioxidants in Pinot Noir berry skins, and their role as antioxidants would increase in those localities with higher radiation levels.

Flavanonols (dihydroflavonols) are bioactive compounds that contribute to tolerance to fungal infections and colour expression in some red wines.[37] Given that flavanonols comprise a relatively small fraction of total wine flavonoids, their regulation by, and responses to, radiation were not clear. However, the results in this paper show that flavanonol levels were positively correlated with radiation. This observation is consistent with a previous study that reported increases in flavanonols in Malbec berry skins following exposure to higher solar radiation levels due to cluster thinning.[37] Similarly, flavanonol levels were found to be elevated in berries exposed to ambient UV-B, in comparison with berries receiving no UV-B.[13]

The reported data indicate positive correlations of cinnamic acid levels with radiation. Consistently, higher values of caffeoyl-tartaric acid were found in skins of Pinot Noir berries exposed to solar radiation when compared with shaded berries.[26] However, not all studies show increases in cinnamic acids with increasing radiation. Coumaroyl-tartaric acid levels showed no response to solar UV-B radiation exposure in Malbec berry skins.[28] Probably, the synthesis of cinnamic acids in berries is more influenced by the radiation received prior to veraison, because contents are highest before berry maturation.[14] Besides, there is some debate on whether cinnamic acids are predominantly present in pulp, rather than skin. Furthermore, the response of cinnamic acid levels to variations in radiation appears to be influenced by the specific year,[38] and each specific cinnamic acid seems to react in a different way.[18]

In contrast to flavonol and flavanonol content, the levels of total stilbenes were only correlated with the global and UV radiation doses over long periods (bud break-harvest). Both stilbenes and flavonoids derive from coumaroyl-coenzyme A in the general phenylpropanoid metabolism, but stilbenes are synthesized by stilbene synthase instead of chalcone synthase. Stilbene synthase is found in berry skins during all stages of fruit development,[39] which could explain the correlation of total stilbene contents with global and UV doses over long periods. Yet, similar to flavonols, stilbenes (resveratrol) were also found to be UV-induced, as was demonstrated in studies using Malbec berry skins.[28]

It was found in this study that the total content of anthocyanins was not correlated with any radiation variable. This finding is congruent with previous findings on Pinot Noir berry skins, which showed that anthocyanin content was not affected by sun exposure.[26] The finding is also consistent with the fact that anthocyanin biosynthesis is controlled by a different system than that controlling flavonol biosynthesis.[40] In general, anthocyanins are accumulated under conditions of low temperature and high radiation levels,[8,41] but contradictory data have been reported in grape berries as a consequence of differences in cultivar, site, season, sampling and analytical techniques.[42] In addition, it has often been difficult to separate the effects of light and temperature.

The ratio 3´,4´,5´-OH/3´,4´-OH anthocyanins was also not correlated with any radiation variable (unlike the hydroxylation ratio of flavonols). Previous studies had shown that the hydroxylation ratio of anthocyanins may increase[43] or decrease[31,44] with increasing (total or UV) radiation in different grapevine varieties, and even the responses may vary depending on the year of study.[27,30] These diverse responses to radiation may be due not only to a complex regulation of the synthesis of differently hydroxylated anthocyanins in the different varieties (as occurred with respect to the hydroxylation ratios of flavonols), but also to the specific responses of each individual anthocyanin. For example, in our study the trisubstituted malvidin-3-*O*-glucoside was the only anthocyanin (positively) correlating with radiation variables, thus affecting the response of the ratio to radiation.

Total flavanol levels were not correlated with any radiation variable nor with levels of any other phenolic group. A likely explanation for this observation is that flavanols are synthesized during the early stages of berry development and that their levels remain fairly stable during subsequent berry growth. Several authors have reported that flavanol levels are stable, and show little responsiveness to changes in radiation or other environmental parameters.[14,44,45] Nevertheless, there is no consensus on this point, as solar UV exclusion has been reported to decrease flavanol content,[29] and responses to temperature and water availability have also been reported.[45-47]

Thus, it is concluded that radiation is strongly correlated with Pinot Noir berry skin phenolic profile. Radiation-related changes in phenolic profile are highly specific. Radiation appears to affect one class of metabolites, while other compounds are not affected. Such specific regulatory interactions offer scope to precision manipulation of berry skin metabolite profiles, in order to increase berry and wine quality.

Effects of temperature and water supply on berry skin metabolic profile

Along the latitudinal gradient studied, the effect of temperature on overall phenolic composition of Pinot Noir berry skins was weaker than the effect of radiation, because temperature variables were correlated with phenolic composition only when they were calculated for the period veraison-harvest. In this case, MSPCs, flavonols, flavanonols, anthocyanins, and the ratios 3´,4´,5´-OH/4´-OH and 3´,4´,5´-OH/3´,4´-OH flavonols, were positively correlated with the mean daily temperature and/or degree days. These correlations might be due to the fact that temperature and radiation variables were also correlated for that period (Table S1), and it may be difficult to differentiate radiation and temperature effects.[42] It may not be surprising that the effects of temperature were more clear in the most important period for berry maturity (veraison-harvest),[20] particularly in the case of anthocyanins, which increase strongly in that period.[20-22] Anthocyanins are known to be influenced by specific temperature conditions, such as ambient temperatures recorded after veraison.[27,41,47,48] Results are also congruent for flavonols because, although more influenced by radiation, these compounds can also respond to temperature.[4] Flavanols are known to be influenced by specific temperature conditions, but in this study effects of a limited range of temperatures were tested, and it is possible that more extreme temperatures are required to impact on these phenolics. With respect to cinnamic acids, their synthesis in the first stages of berry development and the strong decrease in concentrations after veraison[20] may mask the influence of temperature on their content at harvest, thus concealing any correlation between temperature parameters and cinnamic acid concentrations.

Rainfall and aridity indices showed a similar behavior as temperature variables, and were correlated with some phenolic compounds only when the period veraison-harvest was considered. In this period, water availability variables were correlated with temperature and radiation variables, and thus the individual effect of each variable could not be differentiated. Water availability typically shows strong relationships with different plant traits,[49] but direct effects on the contents of grape skin phenolic compounds are considered to be relatively minor.[50,51] This could be due to the fact that the effects of water availability on berry skin composition are mainly mediated by changes in berry size which subsequently affect the proportion of skin in relation to total berry, or by changes in photosynthesis rates modifying source-sink relationships.[42]

Nevertheless, changes in anthocyanins, flavonols and stilbenes caused by water deficit or excess have been described, sometimes in contradictory ways,[42,52] and drought conditions have been reported to increase the expression of different genes involved in the biosynthesis of phenolic compounds.[31,52] Overall, correlations between water availability and phenolic composition were not conclusive in our study.

In summary

PCA was used to summarize the results described above. Axis I mostly represented a latitude gradient, and was determined by nearly all different groups of phenolic compounds that are present in berry skins (flavonols, flavanonols, anthocyanins, stilbenes and cinnamic acids, together with MSPCs). Thus, Pinot Noir berry skins from southern localities were more enriched in most phenolics than those from northern latitudes. This is congruent with the general variation in phenolic compounds (except anthocyanins) with latitude.[4] Changes in phenolic composition can influence wine quality and will contribute to wine genuineness in each locality. Given that, in our study, latitude was more often correlated with radiation variables than with temperature or water availability variables, radiation appeared to be the most important factor contributing to the differentiation of berry skin composition at the localities studied. Nevertheless, in the most important period for phenolic ripeness (veraison-harvest), latitude and radiation, temperature and water availability variables were correlated with one another, and the effect of each type of variable was difficult to separate. Thus, apart from the effect of radiation in every period considered, the interaction of radiation, temperature and water availability in the period veraison-harvest was strongly correlated with the phenolic composition of berry skins along the latitudinal gradient considered. Flavanols and cinnamic acids were the only phenolic compounds that define axis II of the PCA, thus contributing to the differentiation of berry skins from some localities, in particular those situated to the positive part of the axis II, such as Bilje, Firenze, Retz, Potoče and Jerez.

Genetic and environmental factors (other than radiation, temperature and water availability) have not been considered in our study, but may also affect the metabolite composition of berry skins. In particular, a clone effect cannot be excluded. However, this effect has been demonstrated to be relatively minor and/or non-significant in previous studies using both Pinot Noir[48,53] and other grapevine cultivars.[54] On the other hand, additional environmental factors related to the so-called "terroir" and not analyzed in detail in our study, such as soil type or mineral nutrition, could have influenced metabolites composition,[54,55] although it is doubtful whether the impacts of such variables would have been correlated with latitude. Overall, in spite of having used different clones, plant ages and soils, a significant relationship between metabolites composition and the latitude-dependent environmental changes in radiation, temperature and water availability was found. It is likely that this environmental influence masked the possible effects of genetic factors and other non-considered environmental variables.

Particularly relevant is the finding that skin phenolic composition was correlated with the DSSF and T UVery means and doses in relatively short development periods (5-10 days before veraison and harvest). Thus, increasing the total and/or UV radiation received by the clusters in those periods through management practices, such as leaf removal or supplemental UV exposure, could promote the synthesis of valuable phenolic metabolites. This may eventually contribute to improved wine quality because of the notable contribution of phenolic compounds to wine flavor and also by increasing the amount of nutraceuticals and healthy antioxidants, such as flavonols, flavanonols, stilbenes and cinnamic acids.[10,11] Among others, UV radiation has been demonstrated to be an important factor correlated with berry skin composition in our study. Although some of the effects observed, such as the increase in MSPCs, flavonols and cinnamic acids, have been repeatedly attributed to UV (particularly UV-B) radiation,[13,18,29,31] more specific manipulative experiments are needed to prove the specific effects of this fraction of solar radiation across the latitudinal gradient considered.

It is concluded that radiation in several development periods, and an interaction between radiation, temperature and water availability in the period veraison-harvest, were the environmental factors most correlated with the phenolic composition of Pinot Noir berry skins along a latitudinal gradient in Europe. In addition, it was demonstrated that effects of environmental variables may be different for different compounds and that some compounds were more responsive (for example, flavonols) than others (flavanols).

ASSOCIATED CONTENT

Supporting information

Table S1. Correlation coefficients among environmental-geographic and berry variables. Significant correlations are indicated in different colours depending on the significance level: purple, $p<0.001$; fuchsia, $p<0.01$; pink, $p<0.05$. Bb, bud break; v, veraison; h, harvest; see the remaining abbreviations in Table 2 and 3 legends.

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NOTES

The authors declare no competing financial interest.

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FIGURE AND TABLE LEGENDS

Figure 1. Geographic location of the 11 European sampling localities used in this study. 1, Jerez de la Frontera (Spain); 2, Girona (Spain); 3, La Rioja (Spain); 4, Firenze (Italy); 5, Bordeaux (France); 6, Bilje (Slovenia); 7, Potoče (Slovenia); 8, Pécs (Hungary); 9, Retz (Austria); 10, Lednice (Czech Republic); 11, Geisenheim (Germany).

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Table 3. Values (means ± SE) of the variables analyzed in Pinot Noir berries in the 11 European sampling localities used in this study. MSPC, methanol-soluble phenolic compounds. AUC, area under curve. TSS, total soluble solids.

	sampling site	country	latitude (ºN)	longitude (ºE)	altitude (m)	days from bud break to harvest
1	Jerez de la Frontera	Spain	36.7	-6.2	40	141
2	Girona	Spain	41.8	2.6	150	174
3	La Rioja	Spain	42.5	-2.3	342	175
4	Firenze	Italy	43.9	11.2	280	131
5	Bordeaux	France	44.8	-0.6	22	176
6	Bilje	Slovenia	45.9	13.6	70	143
7	Potoče	Slovenia	45.9	13.8	120	140
8	Pécs	Hungary	46.1	18.1	200	152
9	Retz	Austria	48.8	15.9	324	172
10	Lednice	Czech Republic	48.8	16.8	176	183
11	Geisenheim	Germany	50.0	8.0	95	170

Table 1. Geographic location (latitude, longitude and altitude) of the 11 European sampling localities used in this study, together with the number of days from bud break to harvest.

	min	max
mean daily temperature (°C)	16.4 (3)	21.2 (8)
mean daily temperature (°C)	16.6 (10)	21.1 (8)
mean daily temperature (°C)	13.1 (10)	24.4 (1)
degree days (°C)	936 (3)	1367 (8)
degree days (°C)	1197 (3)	1703 (8)
degree days (°C)	113 (10)	381 (2)
rainfall (mm)	155 (4)	439 (5)
rainfall (mm)	196 (4)	481 (5)
rainfall (mm)	0 (1)	103 (10)
rainfall/ETP	0.31 (4)	0.80 (5)
rainfall/ETP	0.28 (1)	0.82 (9)
rainfall/ETP	0 (1)	0.9 (9,10)
Gaussen Index	4.0 (4)	12.8 (5)
Gaussen Index	4.9 (4)	13.7 (5)
Gaussen Index	0 (1)	4.7 (10)
GGR (mean) (MJ m^{-2} d^{-1})	12.7 (9)	24.2 (1)
GGR (mean) (MJ m^{-2} d^{-1})	11.2 (9)	24.9 (1)
GGR (mean) (MJ m^{-2} d^{-1})	8.1 (9)	28.6 (1)
GGR (dose) (MJ m^{-2})	1487 (9)	3035 (3)
GGR (dose) (MJ m^{-2})	1939 (9)	3718 (2)
GGR (dose) (MJ m^{-2})	370 (4)	759 (10)
DSSF (mean) (MJ m^{-2} d^{-1})	18.3 (11)	23.8 (1)
DSSF mean (MJ m^{-2} d^{-1})	15.9 (11)	24.5 (1)
DSSF mean (MJ m^{-2} d^{-1})	10.1 (11)	28.4 (1)
DSSF (dose) (MJ m^{-2})	2201 (11)	2908 (2)
DSSF (dose) (MJ m^{-2})	2684 (11)	3542 (2)
DSSF (dose) (MJ m^{-2})	384 (4)	695 (10)
T UVery (mean) (kJ m^{-2} d^{-1})	3.0 (11)	3.8 (1)
T UVery (mean) (kJ m^{-2} d^{-1})	2.4 (11)	4.0 (1)
T UVery (mean) (kJ m^{-2} d^{-1})	1.5 (11)	4.8 (1)
T UVery (dose) (kJ m^{-2})	254 (11)	483 (3)
T Uvery (dose) (kJ m^{-2})	329 (11)	570 (3)
T Uvery (dose) (kJ m^{-2})	49 (4)	114 (1)
DSSF (10-days-before-harvest dose) (MJ m^{-2})	56.6 (11)	284 (1)
T Uvery (5-days-before-veraison mean (kJ m^{-2} d^{-1})	2.0 (10,11)	5.1 (1)
T Uvery (10-days-before-veraison mean (kJ m^{-2} d^{-1})	2.4 (10,11)	5.0 (1)
T Uvery (5-days-before-veraison dose) (kJ m^{-2})	9.9 (10)	25.3 (1)
T Uvery (10-days-before-veraison dose) (kJ m^{-2})	23.8 (10)	50.2 (1)
T Uvery (10-days-before-harvest dose) (kJ m^{-2})	6.9 (11)	47.4 (1)

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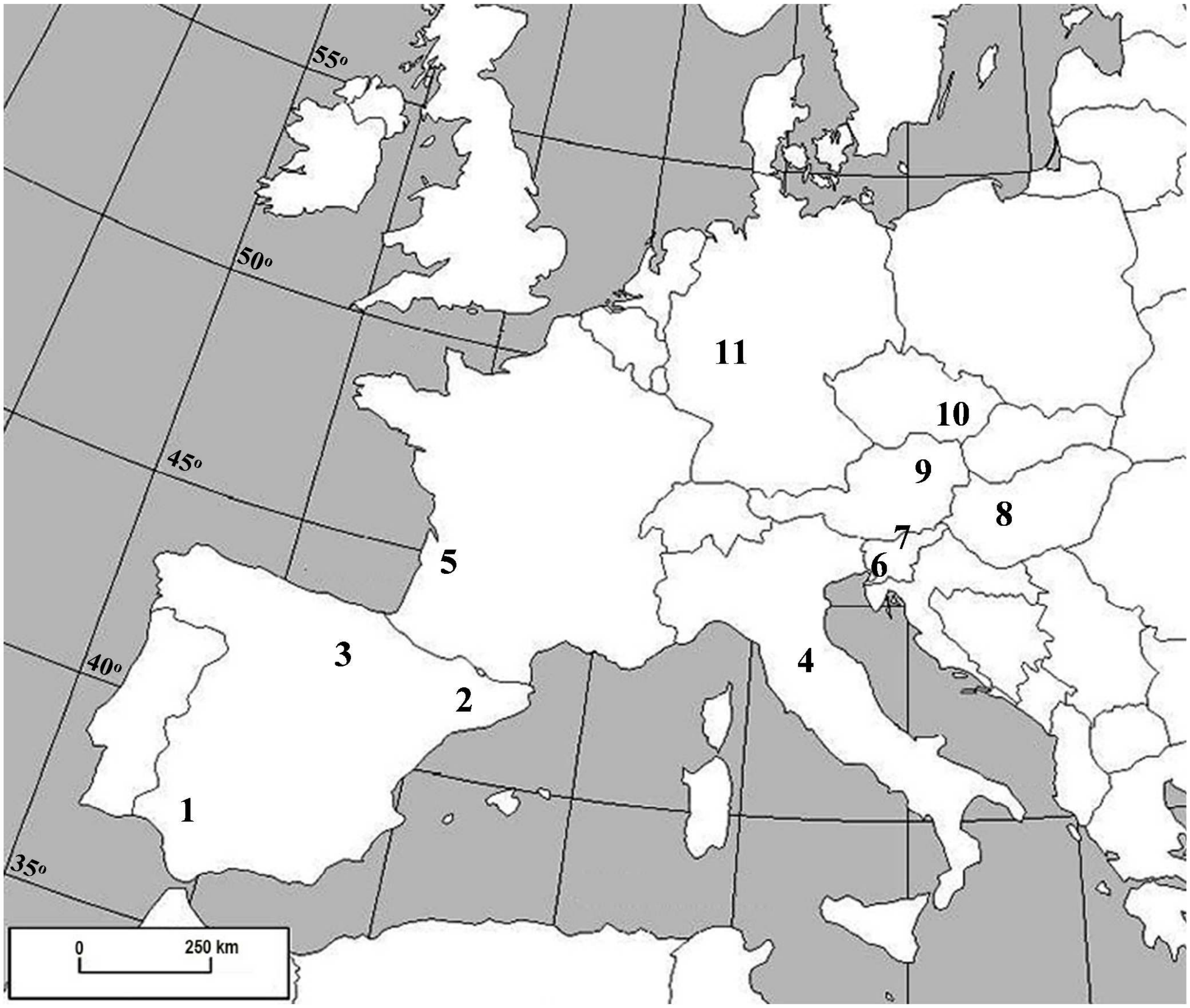
	Jerez	Girona	La Rioja	Firenze	Bordeaux	Bilje	Potoče	Pécs	Retz	Lednice	Geisenheim
total content of MSPC											
AUC$_{280-315}$ mg^{-1} DW	39.1 ± 1.5	40.3 ± 1.2	31.0 ± 3.0	32.3 ± 0.7	22.2 ± 1.3	14.7 ± 0.2	13.2 ± 0.4	32.3 ± 0.2	32.1 ± 5.3	9.7 ± 0.1	24.3 ± 1.2
AUC$_{280-400}$ mg^{-1} DW	71.2 ± 3.5	74.3 ± 3.0	54.5 ± 5.4	58.4 ± 1.7	41.0 ± 2.4	24.5 ± 0.1	22.7 ± 0.4	56.9 ± 0.6	56.1 ± 9.9	17.1 ± 0.4	40.7 ± 2.2
flavonols (µg g^{-1} DW)											
myricetin	139± 20	153± 8	112± 24	234± 27	38.7± 5.6	7.3± 2.8	13.2± 3.4	74.1± 9.2	164± 31	2.5± 0.8	15.3± 1.8
myricetin-3-*O*-glucoside	1066 ± 137	1041 ± 62	864 ± 86	918 ± 112	487 ± 37	157 ± 17	277 ± 45	473 ± 38	535 ± 92	61.2 ± 16.1	272 ± 30
myricetin-3-*O*-glucuronide	391 ± 50	355 ± 54	183 ± 32	368 ± 21	117 ± 11	62.5 ± 6.8	86.1 ± 7.5	267 ± 23	68.5 ± 9.1	22.2 ± 6.0	47.4 ± 8.6
kaempferol-3-*O*-glucoside	177 ± 37	273 ± 61	78.5 ± 9.9	109 ± 30	106 ± 7	21.6 ± 5.0	43.9 ± 8.2	40.7 ± 5.2	145 ± 36	48.1 ± 20.9	106 ± 36
isorhamnetin 3-*O*-glucoside	319 ± 31	433 ± 49	324 ± 33	274 ± 25	252 ± 16	84.4 ± 8.2	109 ± 11	234 ± 6	252 ± 27	138 ± 39	283 ± 21
isorhamnetin 3-*O*-glucuronide	72.9 ± 8.1	92.2 ± 6.5	41.8 ± 3.3	79.5 ± 6.3	50.4 ± 3.4	22.3 ± 4.6	28.2 ± 1.3	66.5 ± 1.8	27.3 ± 5.2	77.0 ± 15.2	51.6 ± 5.3
syringetin 3-*O*-glucoside	171 ± 26	130 ± 15	139 ± 16	87.8 ± 12.2	132 ± 8	62.1 ± 3.7	68.5 ± 4.9	156 ± 5	66.3 ± 8.3	57.2 ± 10.7	106 ± 7
quercetin	4.3 ± 0.4	5.6 ± 0.7	3.9 ± 0.7	2.8 ± 0.3	7.3 ± 3.2	1.3 ± 0.2	1.3 ± 0.1	3.5 ± 0.3	5.8 ± 2.4	2.3 ± 0.3	3.4 ± 0.5
quercetin 3-*O*-glucoside	105 ± 12	160 ± 21	159 ± 26	133 ± 13	50.9 ± 2.7	17.7 ± 2.2	22.9 ± 3.6	92.9 ± 9.0	181 ± 26	27.7 ± 5.0	94.3 ± 10.8
quercetin 3-*O*-galactoside	240 ± 33	400 ± 68	174 ± 11	228 ± 32	187 ± 14	39.5 ± 9.0	51.2 ± 3.1	106 ± 3	133 ± 30	50.8 ± 9.3	120 ± 24
quercetin-3-*O*-glucopyranoside	1075 ± 100	1361 ± 122	849 ± 47	973 ± 90	825 ± 45	260 ± 47	447 ± 41	629 ± 19	599 ± 107	300 ± 51	622 ± 100
quercetin-3-*O*-arabinoside	24.9 ± 3.0	22.1 ± 2.3	16.6 ± 1.6	15.3 ± 2.0	17.8 ± 2.1	3.6 ± 1.1	10.9 ± 1.7	8.6 ± 1.4	10.7 ± 2.0	5.7 ± 1.0	13.0 ± 2.2
quercetin 3-*O*-glucuronide	2726 ± 177	3121 ± 128	1951 ± 103	3014 ± 108	2119 ± 89	995 ± 132	1211 ± 19	2900 ± 44	1430 ± 253	1454 ± 259	1656 ± 156
quercetin-3-*O*-rutinoside	272 ± 35	170 ± 23	76.4 ± 9.8	279 ± 22	114 ± 10	28.3 ± 5.3	51.4 ± 3.2	144 ± 3	107 ± 38	48.7 ± 13.5	57.1 ± 5.9
flavanols (µg g^{-1} DW)											
catechin	126 ± 9	110 ± 8.7	111 ± 14	224 ± 19	81.9 ± 7.4	355 ± 25	188 ± 48	66.4 ± 1.8	162 ± 23	77.9 ± 5.7	102 ± 5
epicatechin	8.8 ± 1.3	5.1 ± 0.6	8.4 ± 0.7	13.3 ± 1.3	5.9 ± 0.7	7.2 ± 1.2	4.5 ± 0.6	3.3 ± 0.3	9.2 ± 1.0	1.8 ± 0.2	2.7 ± 0.1
procyanidin B1	331 ± 27	324 ± 35	266 ± 23	467 ± 40	208 ± 18	633 ± 40	384 ± 59	173 ± 7	323 ± 40	130 ± 6	168 ± 10
flavanonols (µg g^{-1} DW)											
astilbin	715 ± 61	591 ± 68	629 ± 59	511 ± 40	568 ± 45	163 ± 12	265 ± 35	476 ± 17	493 ± 43	299 ± 58	257 ± 43
taxifolin-3-*O*-glucoside	429 ± 64	114 ± 14	194 ± 37	250 ± 19	168 ± 38	21.8 ± 8.4	75.0 ± 19.1	138 ± 11	141 ± 21	10.7 ± 2.2	27.2 ± 6.0
stilbenes (µg g^{-1} DW)											
resveratrol	54.7 ± 6.7	123 ± 28	105 ± 29	34.1 ± 12.1	31.4 ± 5.1	21.7 ± 8.5	6.4 ± 1.4	41.4 ± 4.3	57.1 ± 19.2	11.8 ± 6.5	15.4 ± 0.9
resveratrol-3-*O*-glucoside	395 ± 62	805 ± 77	385 ± 52	117 ± 32	120 ± 27	53.9 ± 27.6	17.7 ± 5.5	243 ± 32	303 ± 19	2.2 ± 0.6	29.2 ± 8.3
cinnamic Acids (µg g^{-1} DW)											
coumaroyl-tartaric acid	876 ± 142	221 ± 14	215 ± 37	1016 ± 143	208 ± 54	72.6 ± 32.0	89.4 ± 24.9	72.0 ± 50.1	824 ± 114	14.8 ± 9.5	48.7 ± 14.9
caffeoyl-tartaric acid	4943 ± 716	2101 ± 427	1763 ± 214	6195 ± 809	1870 ± 497	894 ± 282	1047 ± 244	1597 ± 296	5855 ± 967	144 ± 108	947 ± 315
feruloyl-tartaric acid	5.7 ± 0.4	5.1 ± 0.6	2.3 ± 0.2	5.9 ± 0.7	5.8 ± 0.8	3.6 ± 0.4	5.0 ± 0.8	5.7 ± 2.4	4.0 ± 0.3	1.8 ± 0.3	2.1 ± 0.4
anthocyanins (mg g^{-1} DW)											
delphinidin-3-*O*-glucoside	1.7 ± 0.2	2.9 ± 0.3	3.0 ± 0.5	2.9 ± 0.2	0.8 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	1.4 ± 0.0	3.7 ± 0.5	0.3 ± 0.0	2.6 ± 0.3
cyanidin-3-*O*-glucoside	0.9 ± 0.1	4.4 ± 0.1	1.6 ± 0.2	1.0 ± 0.3	0.9 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	1.8 ± 0.3	1.7 ± 0.2	0.3 ± 0.0	1.5 ± 0.1
petunidin-3-*O*-glucoside	5.0 ± 0.9	6.4 ± 0.8	4.3 ± 0.0	5.7 ± 0.7	1.8 ± 0.3	0.7 ± 0.1	0.9 ± 0.1	2.7 ± 0.0	4.8 ± 2.0	1.0 ± 0.1	2.8 ± 0.2
peonidin-3-*O*-glucoside	14.9 ± 1.6	34.9 ± 1.0	20.9 ± 1.3	13.8 ± 1.4	11.7 ± 1.2	5.7 ± 0.8	3.2 ± 0.2	25.9 ± 0.3	16.7 ± 2.7	5.8 ± 0.8	8.1 ± 0.8
malvidin-3-*O*-glucoside	54.6 ± 1.1	61.5 ± 0.7	39.8 ± 3.6	44.2 ± 0.3	26.4 ± 3.6	12.3 ± 0.2	17.4 ± 0.1	39.8 ± 1.4	36.2 ± 3.9	13.3 ± 0.6	27.1 ± 0.1
other variables											
antioxidant capacity (µM TE g^{-1} DW)	8013 ± 942	8639 ± 408	8637 ± 216	9104 ± 212	5576 ± 654	4134 ± 308	5111 ± 600	6330 ± 730	8212 ± 902	3592 ± 685	8424 ± 595
lutein (µg g^{-1} DW)	66.2 ± 0.8	55.5 ± 5.2	67.7 ± 1.2	32.9 ± 1.6	32.3 ± 1.2	24.1 ± 1.0	31.8 ± 1.3	16.1 ± 1.3	48.4 ± 0.6	20.2 ± 1.6	52.0 ± 10.1
zeaxanthin (µg g^{-1} DW)	8.6 ± 0.4	8.4 ± 0.0	9.2 ± 0.7	3.7 ± 0.3	5.5 ± 0.4	3.7 ± 0.5	4.9 ± 0.3	2.1 ± 0.0	6.7 ± 0.4	2.6 ± 0.1	9.2 ± 0.4
β-carotene (µg g^{-1} DW)	171 ± 7	165 ± 6	195 ± 2	96.4 ± 3.8	112 ± 11	83.1 ± 3.5	68.8 ± 7.6	56.7 ± 5.0	129 ± 9	66.8 ± 4.8	148 ± 19

chlorophylls (*a+b*) (µg g^{-1} DW)	438 ± 22	424 ± 44	525 ± 14	227 ± 6	290 ± 32	188 ± 16	182 ± 9	117 ± 10	360 ± 16	135 ± 5	480 ± 51
fresh weight per berry (g)	1.4 ± 0.2	1.1 ± 0.1	1.3 ± 0.0	1.3 ± 0.1	1.1 ± 0.1	1.2 ± 0.2	1.2 ± 0.0	1.4 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	2.1 ± 0.0
TSS (°Brix)	23.7 ± 0.3	20.4 ± 0.4	22.3 ± 0.3	21.3 ± 0.0	21.1 ± 0.4	19.1 ± 0.1	20.1 ± 0.5	19.5 ± 2.0	23.2 ± 0.4	20.9 ± 0.1	22.0 ± 0.2

Table 3. Values (means ± SE) of the variables analyzed in Pinot Noir berries in the 11 European sampling localities used in this study. MSPC, methanol-soluble phenolic compounds. AUC, area under curve. TSS, total soluble solids.



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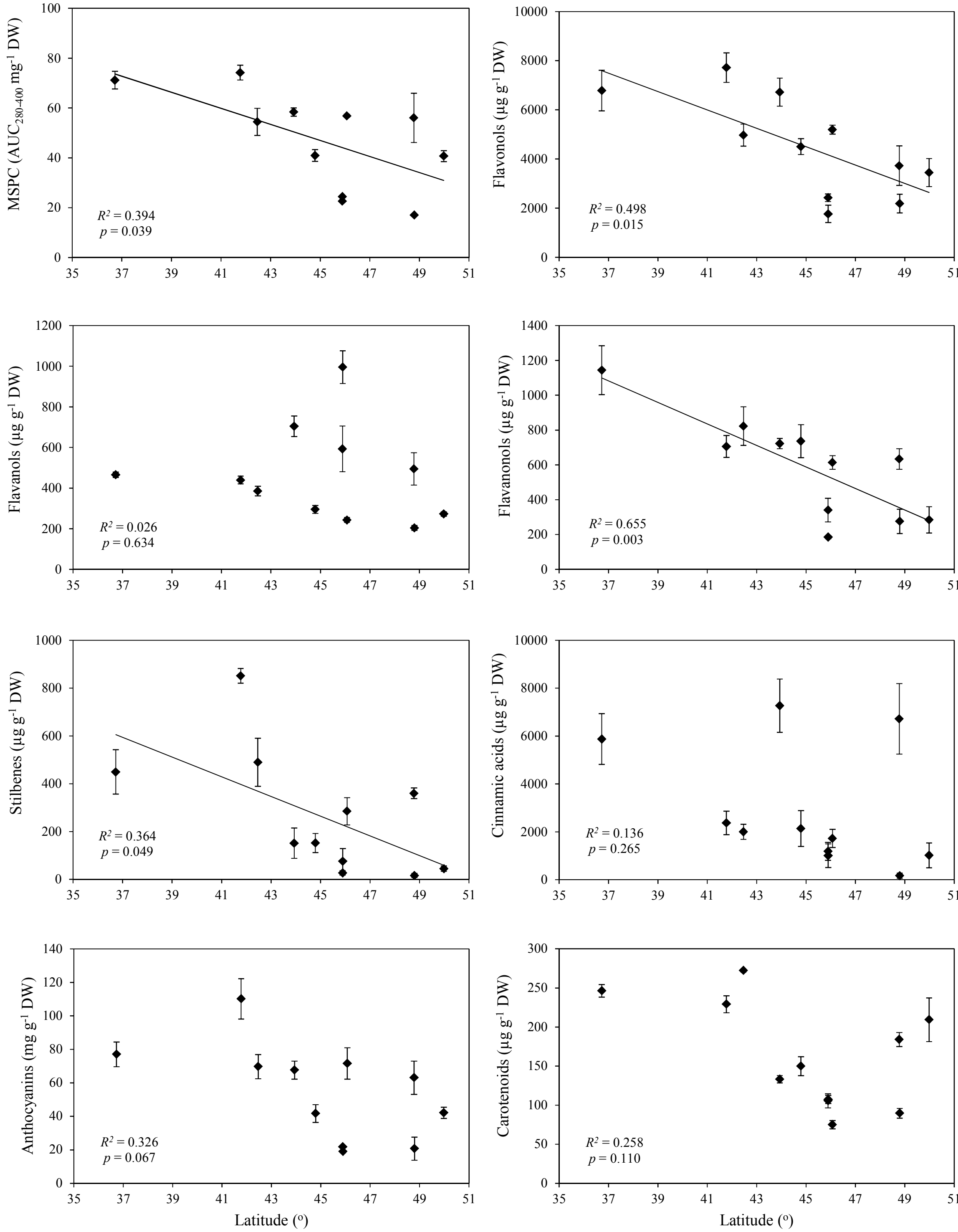

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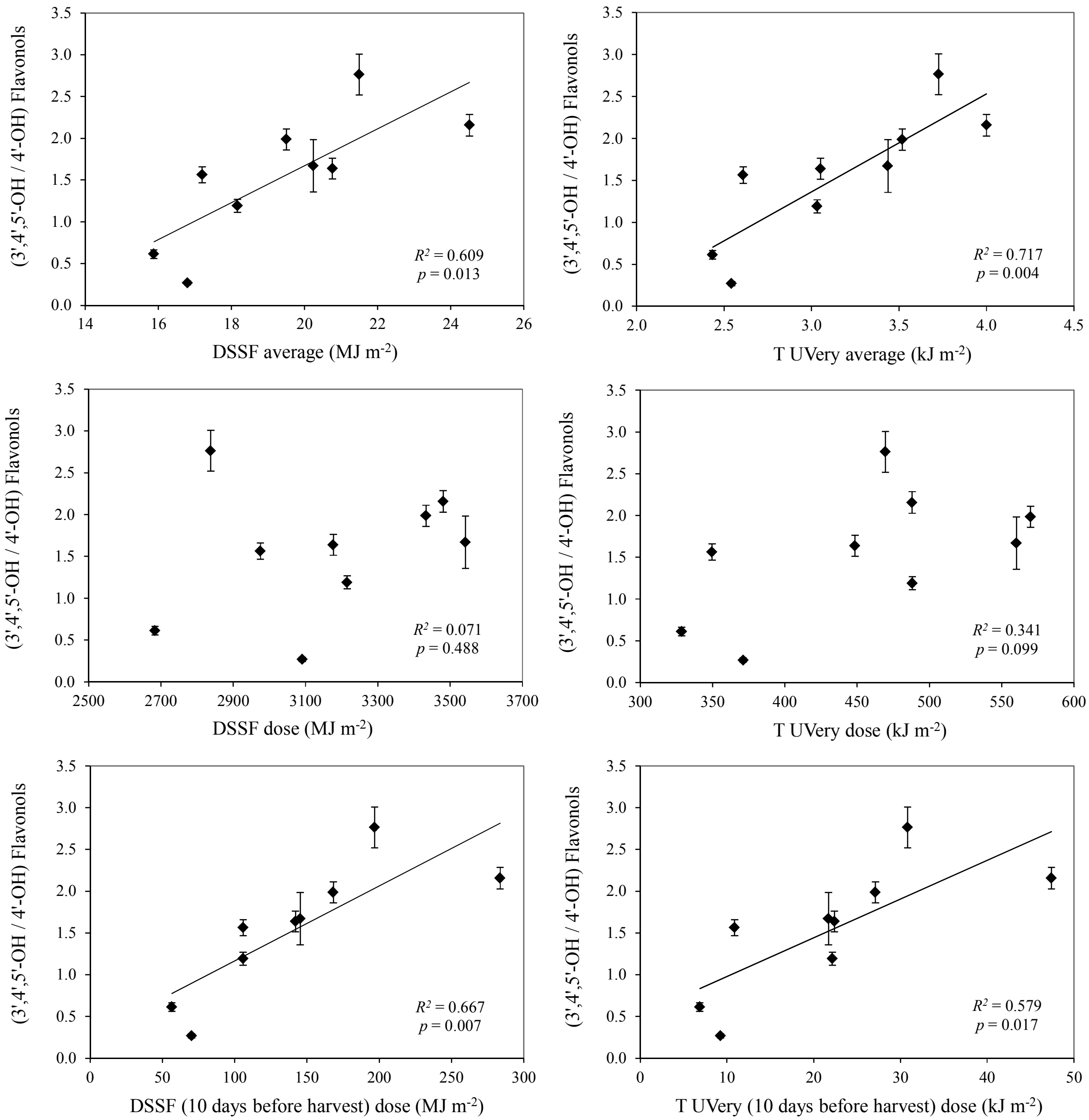

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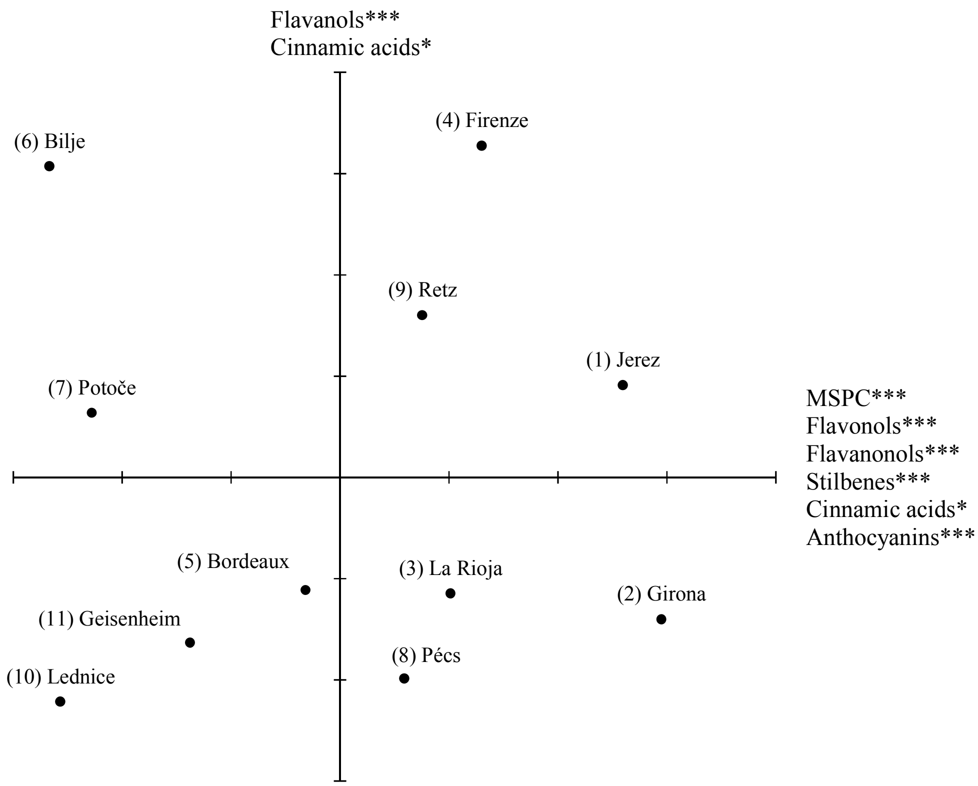

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