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Article

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

María Ángeles Del-Castillo-Alonso<sup>1</sup>, Antonella Castagna<sup>2</sup>, Kristóf Csepregi<sup>3</sup>, Éva Hideg<sup>3</sup>, Gabor Jakab<sup>3,11</sup>, Marcel A.K. Jansen<sup>4</sup>, Tjaša Jug<sup>5</sup>, Laura Llorens<sup>6</sup>, Anikó Máta<sup>3</sup>, Johann Martínez-Lüscher<sup>7</sup>, Laura Monforte<sup>1</sup>, Susanne Neugart<sup>8</sup>, Julie Olejnickova<sup>9</sup>, Annamaria Ranieri<sup>2</sup>, Katharina Schödl-Hummel<sup>10</sup>, Monika Schreiner<sup>8</sup>, Gonzalo Soriano<sup>1</sup>, Péter Teszlák<sup>11</sup>, Susanne Tittmann<sup>12</sup>, Otmar Urban<sup>9</sup>, Dolors Verdaguer<sup>6</sup>, Gaetano Zipoli<sup>13</sup>, Javier Martínez-Abaigar<sup>1</sup>, and Encarnación Núñez-Olivera<sup>1,\*</sup>

<sup>1</sup>Faculty of Science and Technology, University of La Rioja, Madre de Dios 53, 26006 Logroño (La Rioja), Spain

<sup>2</sup>Department of Agriculture - Food and Environment, and Interdepartmental Research Center Nutrafood “Nutraceuticals and Food for Health”, University of Pisa, via del Borghetto 80, 56124 Pisa, Italy

<sup>3</sup>Institute of Biology, University of Pécs, Ifjúság u. 6, 7624 Pécs, Hungary

<sup>4</sup>School of Biological, Environmental and Earth Sciences, University College Cork, College Road, Cork, Ireland

<sup>5</sup>Agricultural and Forestry Institute of Nova Gorica, Pri hrastu 18, 5270 Nova Gorica, Slovenia

<sup>6</sup>Department of Environmental Sciences, Faculty of Sciences, University of Girona, Campus Montilivi, Maria Aurèlia Capmany i Farnés 69, 17003 Girona, Spain

<sup>7</sup>UMR 1287 EGFV, Bordeaux Sciences Agro, INRA, Université de Bordeaux, ISVV, 33882 Villenave d'Ornon, France

<sup>8</sup>Department Plant Quality, Leibniz-Institute of Vegetable and Ornamental Crops Grossbeeren/Erfurt e.V., Theodor-Echtermeyer-Weg 1, 14979 Grossbeeren, Germany

<sup>9</sup>Global Change Research Institute CAS, v.v.i, Bělidla 986/4a, 60300 Brno, Czech Republic

<sup>10</sup>Department of Crop Sciences, BOKU - University of Natural Resources and Life Sciences, Konrad-Lorenz-Str. 24, 3430 Tulln, Austria

33 <sup>11</sup>Research Institute for Viticulture and Oenology, University of Pécs, Pázmány P. u. 4,  
34 7634 Pécs, Hungary

35 <sup>12</sup>Institute for General and Organic Viticulture, Geisenheim University, Von-Lade-  
36 Strasse 1, 65366 Geisenheim, Germany

37 <sup>13</sup>Institute of Biometeorology – National Research Council, Via Caproni 8, 50144  
38 Firenze, Italy

39

40 Corresponding Author

41 \*(Encarnación Núñez-Olivera) Phone: +34 941299755. Fax: +34 941299721. E-mail:  
42 encarnacion.nunez@unirioja.es

**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 erythemal 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.<sup>1-8</sup> 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,<sup>3</sup> ryegrass<sup>7</sup> and currant.<sup>8</sup> 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.<sup>9</sup>

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.<sup>3-5,7,8</sup> 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.<sup>10,11</sup> Berry skin is the main source of many of these phenolic compounds, including anthocyanins, flavonols and stilbenes.<sup>12-14</sup>

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

## 116 MATERIALS AND METHODS

117

### 118 Collection sites and environmental variables

119

120 Berries of Pinot Noir grapevines (*Vitis vinifera* L.) were collected in 2013 from 11  
121 localities in Spain, France, Italy, Hungary, Austria, Slovenia, the Czech Republic and  
122 Germany (Figure 1, Table 1). This represented a latitudinal gradient of almost 14° (36.7-  
123 50.0 °N) and a linear distance of around 1,500 km, covering most of the commercial  
124 Pinot Noir growing latitudes in the Northern Hemisphere (35-55°).<sup>16</sup> Vineyard age  
125 varied between 6 and 30 years, and vineyard soils were mostly calcareous and neutral-  
126 alkaline (pH between 7.0 and 8.5). No fertilization or irrigation had been applied to the  
127 vineyards.

128

129 In each locality, berry samples were collected from three separate plants (replicates) at  
130 commercial maturity, always around noon-time, and on a sunny day. Collection dates  
131 varied from 31 July to 22 October, depending on the location. Three clusters were  
132 collected for each replicate. As row orientation varied between vineyards, clusters were  
133 always picked from a SE-orientated shoot. In situ, every berry was separated from its  
134 cluster by cutting the pedicel. Subsequently, berry density was determined as  
135 floatability in a NaCl solution series, which allowed for harvesting berries of a similar  
136 ripeness using a non-destructive method.<sup>17,18</sup> To reduce the variability that is normally  
137 found within a cluster, berries with a density between 140-160 g NaCl l<sup>-1</sup> were selected,  
138 rinsed in distilled H<sub>2</sub>O and immediately transported to the laboratory in a portable  
139 icebox. In the laboratory, berries were frozen in liquid nitrogen and kept at -80°C until  
140 further analyses.

141

142 Relevant environmental data were obtained for each locality. Daily values of mean  
143 temperature, rainfall and ground-station global radiation (GGR) were obtained for the  
144 period bud break-harvest from the nearest meteorological observatory to each vineyard.  
145 For most vineyards, meteorological stations were located less than 200 m from the  
146 actual vineyards. Remaining stations were located less than 20 km away, except in the  
147 case of Lednice (Czech Republic) where the station for GGR measurement was located  
148 50 km from the vineyard. In the latter cases, it was ascertained that meteorological  
149 stations were located at a similar latitude and altitude as the respective vineyards, which

150 makes the assumption that data were homogeneous. Based on these data, two aridity  
151 indices were calculated: the ratio Rainfall/ETP, where ETP is the potential  
152 evapotranspiration computed according to Hargraves formula (based on solar global  
153 radiation and mean air temperature), and the Gaussen Index (the ratio between rainfall  
154 and twice the mean daily temperature). In addition, daily values of DSSF (Downward  
155 Surface Shortwave Flux) global radiation and TEMIS-derived erythemal UV radiation  
156 (T U<sub>Very</sub>) were obtained for the period bud break-harvest. Daily DSSF was calculated  
157 by integrating the 30 minutes of data downloaded from the LandSaf web page  
158 (<http://landsaf.meteo.pt>). The data in this archive take into account the differences in the  
159 day-length of the various locations. T U<sub>Very</sub> was downloaded from the ESA-TEMIS  
160 web page (<http://www.temis.nl>) and estimated on the basis of Meteosat data (to assess  
161 cloud cover), SCIAMACHY data (to assess O<sub>3</sub> column) and a radiative transfer  
162 model.<sup>19</sup> The degree days (using 10°C as base temperature) and the daily doses of GGR,  
163 DSSF and T U<sub>Very</sub> were integrated over three different periods: bud break-veraison,  
164 bud break-harvest, and veraison-harvest. Additionally, DSSF and T U<sub>Very</sub> doses were  
165 integrated for 5 and 10 days before veraison, and for 5 and 10 days before harvest,  
166 because the periods around veraison and prior to harvest are important for the synthesis  
167 of phenolic compounds in grapevine berries and, thus, for their commercial quality.<sup>20-22</sup>

168

### 169 **Analysis of berries**

170

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

178

179 For each analytical sample used for the analysis of phenolic compounds, 50 mg of skin  
180 powder was frozen in liquid nitrogen and ground again in a TissueLyser (Qiagen,  
181 Hilden, Germany). The total content of methanol-soluble phenolic compounds  
182 (MSPCs), mainly located in the vacuoles,<sup>18</sup> was measured by spectrophotometry. For  
183 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 4°C 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),<sup>23</sup> using a  $\lambda$ 35 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).<sup>23</sup> 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 °C and its flow 9 l min<sup>-1</sup>; the nebulizer gas was set to 3.5 bar and 25 °C. 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.

219

For carotenoid and chlorophyll extraction,<sup>24</sup> 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<sup>-1</sup> using the following linear gradient: 0 min, 82% A (CH<sub>3</sub>CN), 18% B (methanol/hexane/CH<sub>2</sub>Cl<sub>2</sub> 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.

238

The antioxidant capacity of berry skins was measured by generating the radical cation 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS<sup>•+</sup>).<sup>25</sup> 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<sup>•+</sup> 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.

247

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,

251 UK). DNA content per DW of berry skin ( $\text{ng mg}^{-1}$  DW) was calculated using the  
252 formula: mean of DNA concentration ( $\text{ng } \mu\text{L}^{-1}$ ) multiplied by the volume of extraction  
253 ( $\mu\text{L}$ ) and divided by the DW of the lyophilized sample (mg). This analysis served to  
254 calculate the metabolite concentrations on a DNA basis.

255

## 256 **Statistical analysis**

257

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

## 266 RESULTS

267

### 268 Variation in environmental variables

269

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

288

### 289 Variation in berries variables

290

291 Metabolite contents were obtained and normalized against both berry skin DW (Table  
292 3) and DNA amount. The correlations between metabolites and environmental  
293 parameters were similar irrespective of the normalization approach, given that DNA  
294 amount and berry skin DW were significantly correlated ( $r = 0.79$ ,  $p < 0.01$ ,  $n = 11$ ).  
295 Therefore, results are only described on a per berry skin DW basis. MSPC values varied  
296 between 9.7 and 40.3 (as AUC<sub>280-315</sub> mg<sup>-1</sup> DW) and between 17.1 and 74.3 (as AUC<sub>280-  
297 400</sub> mg<sup>-1</sup> DW). Absorption levels in the two wavelength regions were strongly and  
298 positively correlated (Table S1). The highest and lowest MSPC values were found in  
299 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<sup>-1</sup> 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<sup>-1</sup> DW. The major flavonol was quercetin 3-*O*-glucuronide. Flavanonols (between 0.18 and 1.14 mg g<sup>-1</sup> DW, in Bilje and Jerez, respectively) and flavanols (between 0.21 and 0.99 mg g<sup>-1</sup> 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<sup>-1</sup> DW. Finally, the least abundant compounds were stilbenes, which also showed a great variability (between 14 and 928 µg g<sup>-1</sup> DW, in Lednice and Girona, respectively).

The antioxidant capacity of berry skin extracts varied between 3592 (Lednice) and 9104 (Firenze) µM TE g<sup>-1</sup> 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 °Brix (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

334 acids, the ratio 3',4',5'-OH/4'-OH flavonols, and carotenoids. There was no correlation  
335 between the antioxidant capacity and contents of stilbenes or flavanols. Carotenoid and  
336 chlorophyll contents were correlated with each other, and carotenoid levels were also  
337 correlated with those of stilbenes.

338

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

343

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

356

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

364

365 Radiation variables, particularly DSSF and T UVery variables, correlated well with  
366 berry skin variables for the three periods considered. The daily means of DSSF and T  
367 UVery in the periods bud break-harvest and veraison-harvest, the DSSF doses in the 10

368 days before harvest, the daily mean of T UVery in the 5 and 10 days before veraison,  
369 and the T UVery doses in the 5 and 10 days before veraison were all correlated with  
370 MSPCs. The same variables, together with the T UVery doses in the 10 days before  
371 harvest and in the period bud break-harvest (in this last case, with a lower significance  
372 level), were correlated with the total contents of flavonols and flavanonols. Total  
373 stilbene content was only correlated with the DSSF and T UVery doses in the period  
374 bud break-harvest, and total cinnamic acid content only with the daily mean and the  
375 dose of T UVery in the 10 days before veraison. Total flavanol and anthocyanin  
376 contents were not correlated with any radiation variable. Regarding individual  
377 compounds, the strongest correlations were found between contents of several flavonols  
378 and flavanonols and the daily means of DSSF and T UVery in the periods bud break-  
379 harvest and veraison-harvest, as well as with the DSSF and T UVery doses in the  
380 periods of 5 or 10 days before veraison or harvest. Levels of two flavanols, one  
381 anthocyanin and the three cinnamic acids analyzed were also correlated with some of  
382 those T UVery expressions.

383

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

393

### 394 **Principal Components Analysis**

395

396 The localities studied were ordinated by PCA using MSPCs and the different groups of  
397 phenolic compounds. The accumulated variance by the first three axes was 94.0%  
398 (67.3% for axis I, 17.3% for axis II and 9.4% for axis III). The plot using the first two  
399 axes, together with the loading factors and their significance, is shown in Figure 4. The  
400 total contents of all the phenolic groups, except flavanols, were significant loading  
401 factors for the positive part of axis I, which broadly ordinated the localities on the basis

402 of their latitude, with southernmost localities situated towards the positive part of the  
403 axis and the northernmost ones towards the negative part. Total flavanols and total  
404 cinnamic acids were the only significant loading factors for the positive part of axis II,  
405 which separated localities 4, 6, 9, 7 and 1 from the remaining ones. No significant  
406 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.<sup>6</sup> 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 14°. 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.<sup>12,18,23</sup>

### 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*,<sup>7</sup> 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,<sup>26-29</sup> although not in relation with latitudinal gradients.

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

450

451 It is not simply total flavonol levels that correlate with radiation parameters, the ratios  
452 3',4',5'-OH/4'-OH and 3',4',5'-OH/3',4'-OH flavonols were the berry skin variables  
453 best correlated with specific radiation variables, such as the mean values or doses of  
454 DSSF and T UVery radiation in critical periods (5-10 days before veraison and harvest),  
455 but not with the accumulated doses over long periods (Figure 3). Thus, higher solar  
456 radiation values (both total and UV) in those critical periods might increase the B-ring  
457 hydroxylation level of flavonols in Pinot Noir berry skins. Previously, it was shown that  
458 the hydroxylation level depends on both the grape variety<sup>12</sup> and environmental factors,  
459 such as the radiation level. The effect of radiation, in turn, may depend again on the  
460 variety considered: the hydroxylation ratios increased with increasing total or UV  
461 radiation in Pinot Noir (this study), but decreased with increasing total or UV radiation  
462 in Sangiovese<sup>22</sup> and Tempranillo.<sup>18,34</sup> This complexity may be caused by the intricate  
463 regulation mechanism of the genes and enzymes involved in the synthesis of flavonols  
464 with different hydroxylation levels.<sup>21,30,31</sup> In petunia, the highest level of B-ring  
465 hydroxylation was caused by the specific effect of increased UV-B radiation.<sup>35</sup> The  
466 antioxidant activity of flavonoids strongly depends on the number of hydroxyl groups  
467 bound to the aromatic B-ring.<sup>36</sup> Given that the hydroxylation ratios were positively  
468 correlated with the antioxidant capacity in our study, flavonols may be important as  
469 both sunscreens and antioxidants in Pinot Noir berry skins, and their role as antioxidants  
470 would increase in those localities with higher radiation levels.

471

472 Flavanonols (dihydroflavonols) are bioactive compounds that contribute to tolerance to  
473 fungal infections and colour expression in some red wines.<sup>37</sup> Given that flavanonols  
474 comprise a relatively small fraction of total wine flavonoids, their regulation by, and

475 responses to, radiation were not clear. However, the results in this paper show that  
476 flavanone levels were positively correlated with radiation. This observation is  
477 consistent with a previous study that reported increases in flavanones in Malbec berry  
478 skins following exposure to higher solar radiation levels due to cluster thinning.<sup>37</sup>  
479 Similarly, flavanone levels were found to be elevated in berries exposed to ambient  
480 UV-B, in comparison with berries receiving no UV-B.<sup>13</sup>

481

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

493

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

503

504 It was found in this study that the total content of anthocyanins was not correlated with  
505 any radiation variable. This finding is congruent with previous findings on Pinot Noir  
506 berry skins, which showed that anthocyanin content was not affected by sun exposure.<sup>26</sup>  
507 The finding is also consistent with the fact that anthocyanin biosynthesis is controlled  
508 by a different system than that controlling flavonol biosynthesis.<sup>40</sup> In general,

anthocyanins are accumulated under conditions of low temperature and high radiation levels,<sup>8,41</sup> but contradictory data have been reported in grape berries as a consequence of differences in cultivar, site, season, sampling and analytical techniques.<sup>42</sup> In addition, it has often been difficult to separate the effects of light and temperature.

513

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<sup>43</sup> or decrease<sup>31,44</sup> with increasing (total or UV) radiation in different grapevine varieties, and even the responses may vary depending on the year of study.<sup>27,30</sup> 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.

525

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.<sup>14,44,45</sup> Nevertheless, there is no consensus on this point, as solar UV exclusion has been reported to decrease flavanol content,<sup>29</sup> and responses to temperature and water availability have also been reported.<sup>45-47</sup>

534

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.

540

541

542

## 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.<sup>42</sup> It may not be surprising that the effects of temperature were more clear in the most important period for berry maturity (veraison-harvest),<sup>20</sup> particularly in the case of anthocyanins, which increase strongly in that period.<sup>20-22</sup> Anthocyanins are known to be influenced by specific temperature conditions, such as ambient temperatures recorded after veraison.<sup>27,41,47,48</sup> Results are also congruent for flavonols because, although more influenced by radiation, these compounds can also respond to temperature.<sup>4</sup> 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<sup>20</sup> 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,<sup>49</sup> but direct effects on the contents of grape skin phenolic compounds are considered to be relatively minor.<sup>50,51</sup> 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.<sup>42</sup>

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

582

583

#### 584 **In summary**

585

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

607

608 Genetic and environmental factors (other than radiation, temperature and water  
609 availability) have not been considered in our study, but may also affect the metabolite  
610 composition of berry skins. In particular, a clone effect cannot be excluded. However,

611 this effect has been demonstrated to be relatively minor and/or non-significant in  
612 previous studies using both Pinot Noir<sup>48,53</sup> and other grapevine cultivars.<sup>54</sup> On the other  
613 hand, additional environmental factors related to the so-called “terroir” and not analyzed  
614 in detail in our study, such as soil type or mineral nutrition, could have influenced  
615 metabolites composition,<sup>54,55</sup> although it is doubtful whether the impacts of such  
616 variables would have been correlated with latitude. Overall, in spite of having used  
617 different clones, plant ages and soils, a significant relationship between metabolites  
618 composition and the latitude-dependent environmental changes in radiation, temperature  
619 and water availability was found. It is likely that this environmental influence masked  
620 the possible effects of genetic factors and other non-considered environmental variables.

621

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

636

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

644

**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.

**AUTHOR INFORMATION****Corresponding Author**

\*(E.N.-O.) Phone: +34-941-941299755. Fax: +34-941-299721. E-mail: encarnacion.nunez@unirioja.es.

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680

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871 **FIGURE AND TABLE LEGENDS**

872

873 **Figure 1.** Geographic location of the 11 European sampling localities used in this study.

874 1, Jerez de la Frontera (Spain); 2, Girona (Spain); 3, La Rioja (Spain); 4, Firenze (Italy);

875 5, Bordeaux (France); 6, Bilje (Slovenia); 7, Potoče (Slovenia); 8, Pécs (Hungary); 9,

876 Retz (Austria); 10, Lednice (Czech Republic); 11, Geisenheim (Germany).

877

878 **Figure 2.** Regressions between selected berry variables, including carotenoids and the879 different groups of phenolic compounds, and latitude. Determination coefficients ( $R^2$ )880 and  $p$  values are shown.

881

882 **Figure 3.** Regressions between the ratio trihydroxylated / monohydroxylated flavonols

883 and selected radiation variables. DSSF, Downward Surface Shortwave Flux. T UVery,

884 TEMIS-derived erythemal UV. For both variables, the daily mean in the period bud

885 break-harvest, and the accumulated dose in the same period and in the 10 days before

886 harvest, were used for calculations. Determination coefficients ( $R^2$ ) and  $p$  values are

887 shown.

888

889 **Figure 4.** Ordination, through Principal Components Analysis (PCA), of the 11

890 sampling localities used in this study, taking into account the total content of methanol-

891 soluble phenolic compounds (MSPC) and the total contents of the different groups of

892 phenolic compounds. Significant loading factors for the positive and negative parts of

893 each axis, together with their corresponding significance levels, are shown (\*\*\*,

894  $p < 0.001$ ; \*\*,  $p < 0.01$ ; \*,  $p < 0.05$ ). Axis 1 is the horizontal one, and axis 2 is the vertical

895 one. Each mark on the axes represents 0.5 units.

896

897 **Table 1.** Geographic location (latitude, longitude and altitude) of the 11 European

898 sampling localities used in this study, together with the number of days from bud break

899 to harvest.

900

901 **Table 2.** Ranges of the environmental variables in the 11 European sampling localities

902 used in this study, together with the localities in which each extreme value was recorded

903 (between brackets). ETP, potential evapotranspiration. GGR, Ground-station Global

904 Radiation. DSSF, Downward Surface Shortwave Flux. T UVery, TEMIS-derived

905 erythematic UV. The different variables were calculated along three periods: bud break-  
906 veraison (white background), bud break-harvest (light grey background) and veraison-  
907 harvest (dark grey background). In addition, DSSF doses were calculated in the 10 days  
908 before harvest, and T UVery (mean values and total doses) in different periods.

909

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

|    | sampling site        | country        | latitude (°N) | longitude (°E) | 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.

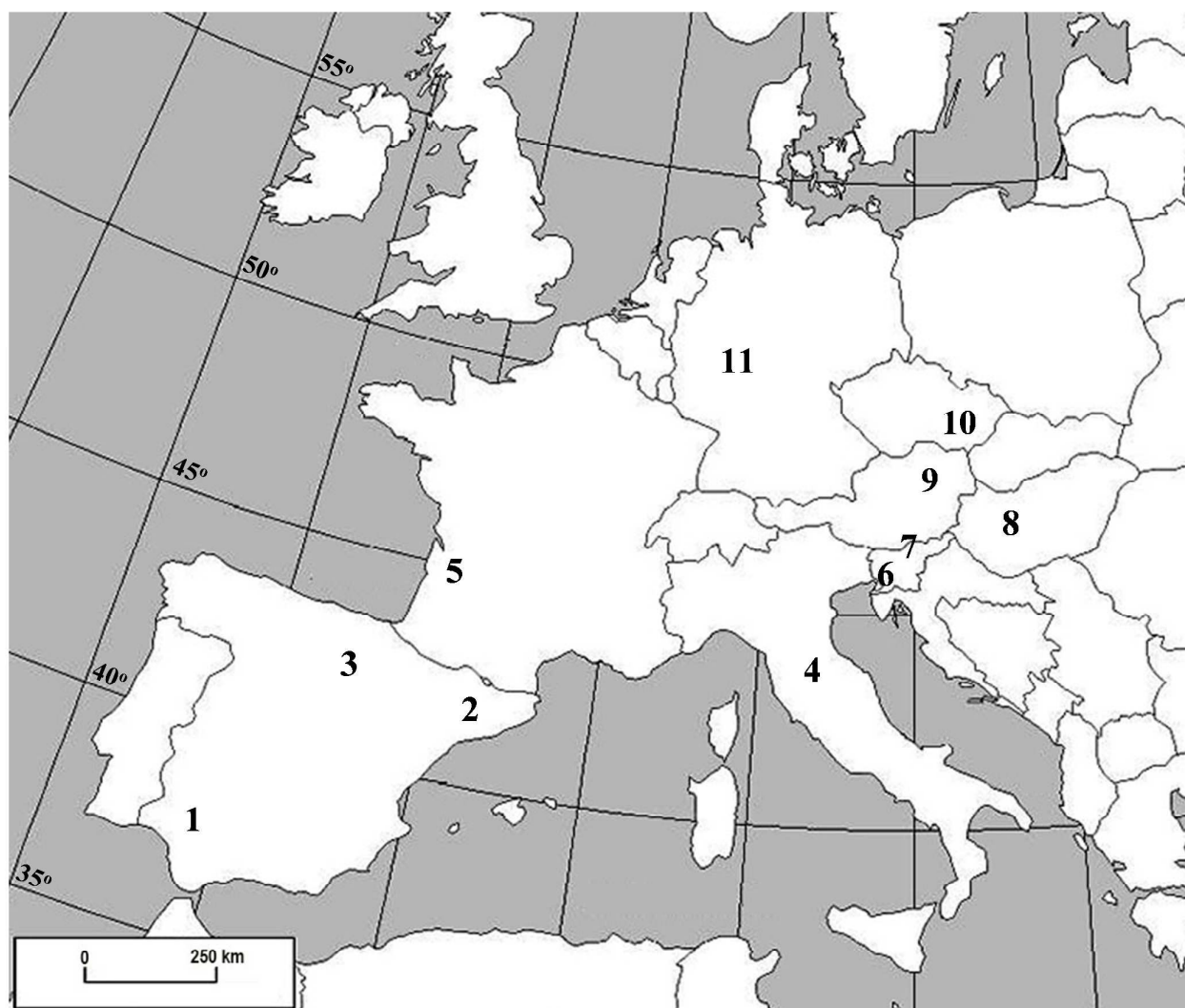
|  | min         | max        |
|--|-------------|------------|
| mean daily temperature (°C)  | 16.4 (3)    | 21.2 (8)   |
| mean daily temperature (°C)  | 16.6 (10)   | 21.1 (8)   |
| mean daily temperature (°C)  | 13.1 (10)   | 24.4 (1)   |
| degree days (°C)   | 936 (3)     | 1367 (8)   |
| degree 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 <sup>-2</sup> d <sup>-1</sup> )                             | 12.7 (9)    | 24.2 (1)   |
| GGR (mean) (MJ m <sup>-2</sup> d <sup>-1</sup> )                             | 11.2 (9)    | 24.9 (1)   |
| GGR (mean) (MJ m <sup>-2</sup> d <sup>-1</sup> )                             | 8.1 (9)     | 28.6 (1)   |
| GGR (dose) (MJ m <sup>-2</sup> )   | 1487 (9)    | 3035 (3)   |
| GGR (dose) (MJ m <sup>-2</sup> )   | 1939 (9)    | 3718 (2)   |
| GGR (dose) (MJ m <sup>-2</sup> )   | 370 (4)     | 759 (10)   |
| DSSF (mean) (MJ m <sup>-2</sup> d <sup>-1</sup> )                            | 18.3 (11)   | 23.8 (1)   |
| DSSF mean (MJ m <sup>-2</sup> d <sup>-1</sup> )                              | 15.9 (11)   | 24.5 (1)   |
| DSSF mean (MJ m <sup>-2</sup> d <sup>-1</sup> )                              | 10.1 (11)   | 28.4 (1)   |
| DSSF (dose) (MJ m <sup>-2</sup> )  | 2201 (11)   | 2908 (2)   |
| DSSF (dose) (MJ m <sup>-2</sup> )  | 2684 (11)   | 3542 (2)   |
| DSSF (dose) (MJ m <sup>-2</sup> )  | 384 (4)     | 695 (10)   |
| T UVery (mean) (kJ m <sup>-2</sup> d <sup>-1</sup> )                         | 3.0 (11)    | 3.8 (1)    |
| T UVery (mean) (kJ m <sup>-2</sup> d <sup>-1</sup> )                         | 2.4 (11)    | 4.0 (1)    |
| T UVery (mean) (kJ m <sup>-2</sup> d <sup>-1</sup> )                         | 1.5 (11)    | 4.8 (1)    |
| T UVery (dose) (kJ m <sup>-2</sup> )   | 254 (11)    | 483 (3)    |
| T UVery (dose) (kJ m <sup>-2</sup> )   | 329 (11)    | 570 (3)    |
| T UVery (dose) (kJ m <sup>-2</sup> )   | 49 (4)      | 114 (1)    |
| DSSF (10-days-before-harvest dose) (MJ m <sup>-2</sup> )                     | 56.6 (11)   | 284 (1)    |
| T UVery (5-days-before-veraison mean) (kJ m <sup>-2</sup> d <sup>-1</sup> )  | 2.0 (10,11) | 5.1 (1)    |
| T UVery (10-days-before-veraison mean) (kJ m <sup>-2</sup> d <sup>-1</sup> ) | 2.4 (10,11) | 5.0 (1)    |
| T UVery (5-days-before-veraison dose) (kJ m <sup>-2</sup> )                  | 9.9 (10)    | 25.3 (1)   |
| T UVery (10-days-before-veraison dose) (kJ m <sup>-2</sup> )                 | 23.8 (10)   | 50.2 (1)   |
| T UVery (10-days-before-harvest dose) (kJ m <sup>-2</sup> )                  | 6.9 (11)    | 47.4 (1)   |

**Table 2.** Ranges of the environmental variables in the 11 European sampling localities used in this study, together with the localities in which each extreme value was recorded (between brackets). ETP, potential evapotranspiration. GGR, Ground-station Global Radiation. DSSF, Downward Surface Shortwave Flux. T UVery, TEMIS-derived erythematic UV. The different variables were calculated along three periods: bud break-veraison (white background), bud break-harvest (light grey background) and veraison-harvest (dark grey background). In addition, DSSF doses were calculated in the 10 days before harvest, and T UVery (mean values and total doses) in different periods.

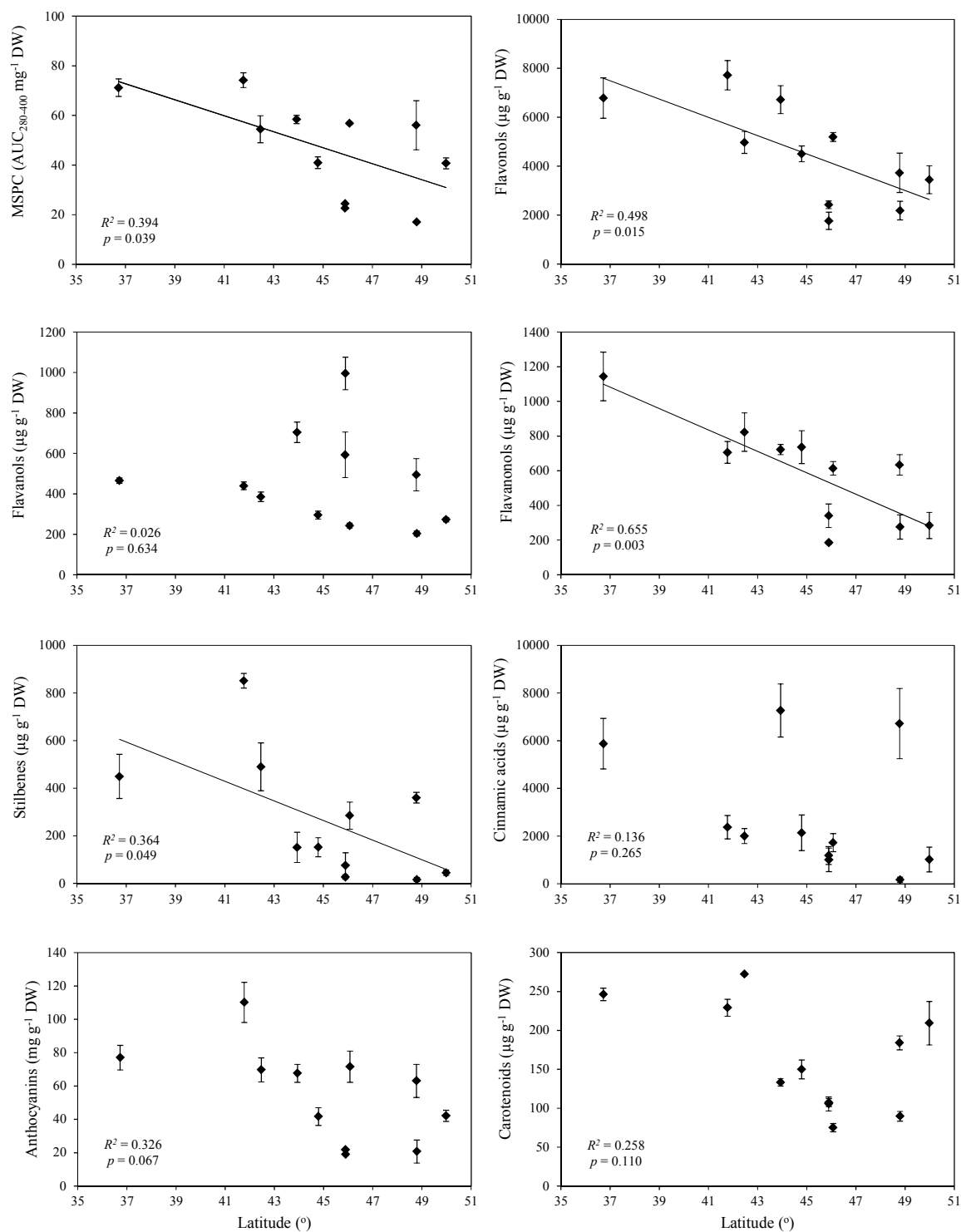
|   | Jerez      | Girona     | La Rioja   | Firenze     | Bordeaux   | Bilje       | Potoče      | Pécs        | Retz        | Lednice     | Geisenheim  |
|---|------------|------------|------------|-------------|------------|-------------|-------------|-------------|-------------|-------------|-------------|
| <b>total content of MSPC</b>                    |            |            |            |             |            |             |             |             |             |             |             |
| AUC <sub>280-315</sub> mg <sup>-1</sup> 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 <sub>280-400</sub> mg <sup>-1</sup> 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  |
| <b>flavonols (μg g<sup>-1</sup> DW)</b>         |            |            |            |             |            |             |             |             |             |             |             |
| 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- <i>O</i> -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- <i>O</i> -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- <i>O</i> -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- <i>O</i> -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- <i>O</i> -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- <i>O</i> -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- <i>O</i> -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- <i>O</i> -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- <i>O</i> -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- <i>O</i> -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- <i>O</i> -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- <i>O</i> -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  |
| <b>flavanols (μg g<sup>-1</sup> DW)</b>         |            |            |            |             |            |             |             |             |             |             |             |
| 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    |
| <b>flavanonols (μg g<sup>-1</sup> DW)</b>       |            |            |            |             |            |             |             |             |             |             |             |
| 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- <i>O</i> -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  |
| <b>stilbenes (μg g<sup>-1</sup> DW)</b>         |            |            |            |             |            |             |             |             |             |             |             |
| 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- <i>O</i> -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  |
| <b>cinnamic Acids (μg g<sup>-1</sup> DW)</b>    |            |            |            |             |            |             |             |             |             |             |             |
| 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   |
| <b>anthocyanins (mg g<sup>-1</sup> DW)</b>      |            |            |            |             |            |             |             |             |             |             |             |
| delphinidin-3- <i>O</i> -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- <i>O</i> -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- <i>O</i> -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- <i>O</i> -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- <i>O</i> -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  |
| <b>other variables</b>                          |            |            |            |             |            |             |             |             |             |             |             |
| antioxidant capacity (μM TE g <sup>-1</sup> 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 <sup>-1</sup> 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 <sup>-1</sup> 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 <sup>-1</sup> 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 ( <i>a+b</i> ) (μg g <sup>-1</sup> 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 |

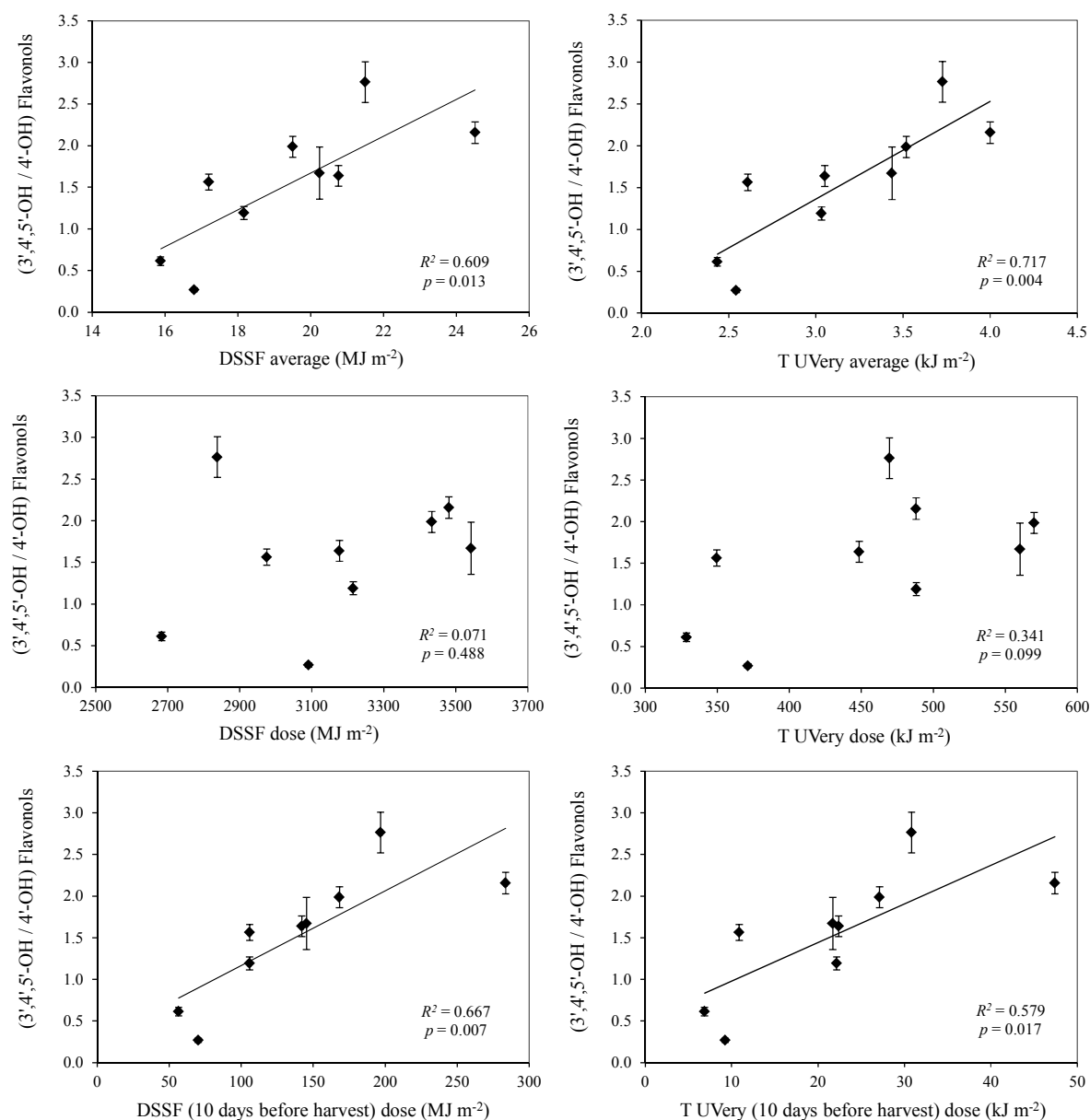
**Table 3.** Values (means ± SE) of the variables analyzed in Pinot Noir berries in the 11 European sampling localities used in this study. MSPC, methanol-soluble phenolic compounds. AUC, area under curve. TSS, total soluble solids.



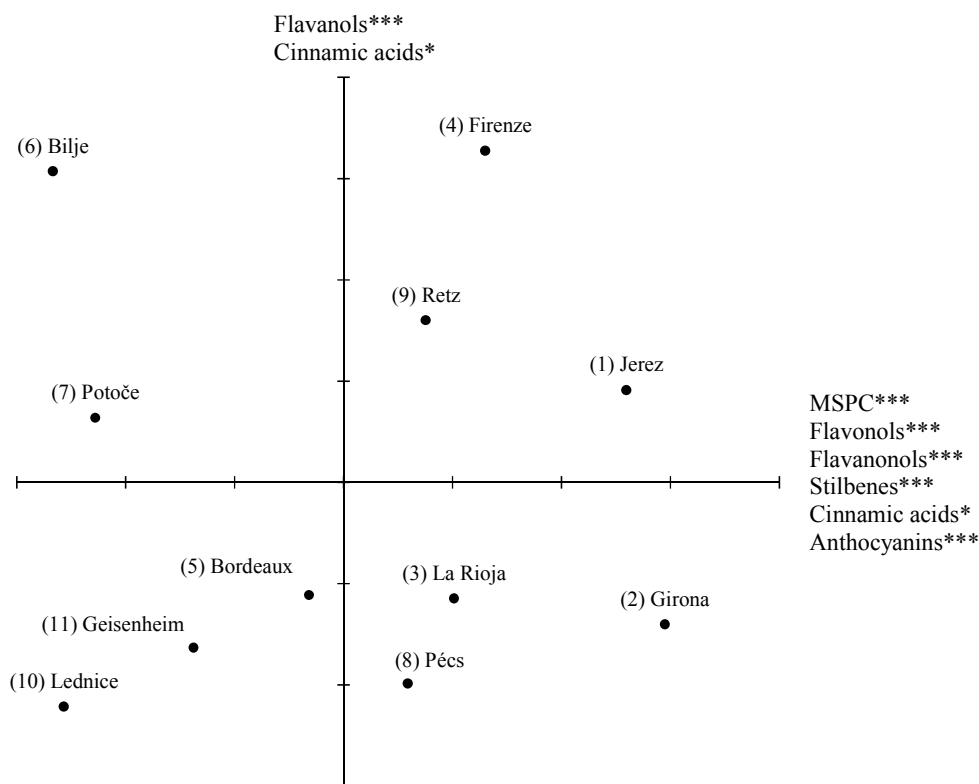
**Figure 1.** Geographic location of the 11 European sampling localities used in this study. 1, Jerez de la Frontera (Spain); 2, Girona (Spain); 3, La Rioja (Spain); 4, Firenze (Italy); 5, Bordeaux (France); 6, Bilje (Slovenia); 7, Potoče (Slovenia); 8, Pécs (Hungary); 9, Retz (Austria); 10, Lednice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



**Figure 2.** Regressions between selected berry variables, including carotenoids and the different groups of phenolic compounds, and latitude. Determination coefficients ( $R^2$ ) and  $p$  values are shown.



**Figure 3.** Regressions between the ratio trihydroxylated / monohydroxylated flavonols and selected radiation variables. DSSF, Downward Surface Shortwave Flux. T UVery, TEMIS-derived erythemal UV. For both variables, the daily mean in the period budbreak-harvest, and the accumulated dose in the same period and in the 10 days before harvest, were used for calculations. Determination coefficients ( $R^2$ ) and  $p$  values are shown.



**Figure 4.** Ordination, through Principal Components Analysis (PCA), of the 11 sampling localities used in this study, taking into account the total content of methanol-soluble phenolic compounds (MSPC) and the total concentrations of the different groups of phenolic compounds. Significant loading factors for the positive and negative parts of each axis, together with their corresponding significance levels, are shown (\*\*\*,  $p<0.001$ ; \*\*,  $p<0.01$ ; \*,  $p<0.05$ ). Axis 1 is the horizontal one, and axis 2 is the vertical one. Each mark on the axes represents 0.5 units.

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