



<b>Title</b>	Environmental factors correlated with the metabolite profile of <i>Vitis vinifera</i> cv. Pinot Noir berry skins along a European latitudinal gradient
<b>Author(s)</b>	Del-Castillo-Alonso, María Ángeles; Castagna, Antonella; Csepregi, Kristóf; Hideg, Éva; Jakab, Gabor; Jansen, Marcel A. K.; Jug, Tjaša; Llorens, Laura; Mátai, Anikó; Martínez-Lüscher, Johann; Monforte, Laura; Neugart, Susanne; Olejnickova, Julie; Ranieri, Annamaria; Schödl-Hummel, Katharina; Schreiner, Monika; Soriano, Gonzalo; Teszlák, Péter; Tittmann, Susanne; Urban, Otmar; Verdaguer, Dolors; Zipoli, Gaetano; Martínez-Abaigar, Javier; Núñez-Olivera, Encarnación
<b>Publication date</b>	2016-10-31
<b>Original citation</b>	Del-Castillo-Alonso, M. Á., Castagna, A., Csepregi, K., Hideg, É., Jakab, G., Jansen, M. A. K., Jug, T., Llorens, L., Mátai, A., Martínez-Lüscher, J., Monforte, L., Neugart, S., Olejnickova, J., Ranieri, A., Schödl-Hummel, K., Schreiner, M., Soriano, G., Teszlák, P., Tittmann, S., Urban, O., Verdaguer, D., Zipoli, G., Martínez-Abaigar, J. and Núñez-Olivera, E. (2016) 'Environmental Factors Correlated with the Metabolite Profile of <i>Vitis vinifera</i> cv. Pinot Noir Berry Skins along a European Latitudinal Gradient', <i>Journal of Agricultural and Food Chemistry</i> , 64(46), pp. 8722-8734. doi: 10.1021/acs.jafc.6b03272
<b>Type of publication</b>	Article (peer-reviewed)
<b>Link to publisher's version</b>	<a href="http://dx.doi.org/10.1021/acs.jafc.6b03272">http://dx.doi.org/10.1021/acs.jafc.6b03272</a> Access to the full text of the published version may require a subscription.
<b>Rights</b>	© 2016 American Chemical Society. This document is the Accepted Manuscript version of a Published Work that appeared in final form in <i>Journal of Agricultural and Food Chemistry</i> , copyright © American Chemical Society after peer review and technical editing by the publisher. To access the final edited and published work see <a href="http://pubs.acs.org/doi/abs/10.1021/acs.jafc.6b03272">http://pubs.acs.org/doi/abs/10.1021/acs.jafc.6b03272</a>
<b>Embargo information</b>	Access to this item is restricted until 12 months after publication by the request of the publisher
<b>Embargo lift date</b>	2017-10-31
<b>Item downloaded from</b>	<a href="http://hdl.handle.net/10468/3276">http://hdl.handle.net/10468/3276</a>



Downloaded on 2018-08-23T18:24:03Z



Article

## 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, Antonella Castagna, Kristóf Csepregi, Éva Hideg, Gabor Jakab, Marcel A.K. Jansen, Tjaša Jug, Laura Llorens, Anikó Máтай, Johann Martínez-Lüscher, Laura Monforte, Susanne Neugart, Julie Olejnickova, Annamaria Ranieri, Katharina Schoedl-Hummel, Monika Schreiner, Gonzalo Soriano, Péter Teszlák, Susanne Tittmann, Otmar Urban, Dolores Verdaguer, Gaetano Zipoli, Javier Martínez-Abaigar, and Encarnacion Núñez-Olivera

*J. Agric. Food Chem.*, **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.6b03272 • Publication Date (Web): 31 Oct 2016

Downloaded from <http://pubs.acs.org> on November 7, 2016

### Just Accepted

“Just Accepted” manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides “Just Accepted” as a free service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. “Just Accepted” manuscripts appear in full in PDF format accompanied by an HTML abstract. “Just Accepted” manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are accessible to all readers and citable by the Digital Object Identifier (DOI®). “Just Accepted” is an optional service offered to authors. Therefore, the “Just Accepted” Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these “Just Accepted” manuscripts.

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

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

12  
13 <sup>1</sup>Faculty of Science and Technology, University of La Rioja, Madre de Dios 53, 26006  
14 Logroño (La Rioja), Spain

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

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

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

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

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

25 <sup>7</sup>UMR 1287 EGFV, Bordeaux Sciences Agro, INRA, Université de Bordeaux, ISVV,  
26 33882 Villenave d'Ornon, France

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

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

31 <sup>10</sup>Department of Crop Sciences, BOKU - University of Natural Resources and Life  
32 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

43 **ABSTRACT**

44

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

65

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

68

69 **INTRODUCTION**

70

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

93

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

102

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 Gausse 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 UVery) 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 UVery 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 UVery were integrated over three different periods: bud break-veraison,  
164 bud break-harvest, and veraison-harvest. Additionally, DSSF and T UVery 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

184 for extraction (24 h at 4°C in the dark). The extract was centrifuged at 6000 g for 15 min  
185 and the supernatant was selected for spectrophotometry. The level of MSPCs was  
186 measured as the area under the absorbance curve in the wavelength intervals between  
187 280-315 and 280-400 nm (AUC<sub>280-315</sub> and AUC<sub>280-400</sub> respectively) and normalised per  
188 unit of dry weight (DW),<sup>23</sup> using a  $\lambda$ 35 spectrophotometer (Perkin-Elmer, Wilton, CT,  
189 USA). Individual phenolic compounds were analysed by ultra-performance liquid  
190 chromatography (UPLC) using a Waters Acquity UPLC system (Waters Corporation,  
191 Milford, MA, USA).<sup>23</sup> Solvents were: A, water/formic acid (0.1%), and B, acetonitrile  
192 with 0.1% formic acid. The gradient program employed was: 0-7 min, 99.5-80% A; 7-9  
193 min, 80-50% A; 9-11.7 min, 50-0% A; 11.7-15 min, 0-99.5% A. The UPLC system was  
194 coupled to a micrOTOF II high-resolution mass spectrometer (Bruker Daltonics,  
195 Bremen, Germany) equipped with an Apollo II ESI/APCI multimode source and  
196 controlled by the Bruker Daltonics DataAnalysis software. The electrospray source was  
197 operated in positive or negative mode. The capillary potential was set to 4 kV; the  
198 drying gas temperature was 200 °C and its flow 9 l min<sup>-1</sup>; the nebulizer gas was set to  
199 3.5 bar and 25 °C. Spectra were acquired between *m/z* 120 and 1505 in positive mode  
200 for anthocyanins and in negative mode for the remaining phenolic compounds. The  
201 different phenolic compounds analysed were identified according to their order of  
202 elution and the retention times of the following pure compounds: myricetin, quercetin,  
203 catechin, epicatechin, astilbin, *trans*-resveratrol, *p*-coumaric acid, caffeic acid and  
204 ferulic acid (Sigma, St. Louis, MO, USA); kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-  
205 *O*-glucoside, syringetin-3-*O*-glucoside, procyanidin B1 and malvidin-3-*O*-glucoside  
206 (Extrasynthese, Genay, France); isorhamnetin, quercetin-3-*O*-glucoside, quercetin-3-*O*-  
207 galactoside, quercetin-3-*O*-glucopyranoside, quercetin-3-*O*-glucuronide and quercetin-  
208 3-rutinoside (Fluka, Buchs, Germany). Quantification of compounds that were not  
209 commercially available was carried out using the calibration curves belonging to the  
210 most similar compound: myricetin for its glucosides; isorhamnetin for isorhamnetin-3-*O*-  
211 *O*-glucuronide; quercetin for quercetin-3-*O*-arabinoside; astilbin for taxifolin-3-*O*-  
212 glucoside; *trans*-resveratrol for its glucoside; *p*-coumaric acid for *p*-coumaroyl-tartaric  
213 acid; caffeic acid for *p*-caffeoyl-tartaric acid; ferulic acid for feruloyl-tartaric acid; and  
214 malvidin-3-*O*-glucoside for anthocyanins. Total contents of the different phenolic  
215 groups were obtained as the sum of the individual compounds. The ratios between  
216 trihydroxylated and dihydroxylated (3',4',5'-OH/3',4'-OH) anthocyanins, and between

217 trihydroxylated and monohydroxylated (3',4',5'-OH/4'-OH) and trihydroxylated and  
218 dihydroxylated (3',4',5'-OH/3',4'-OH) flavonols, were also calculated.

219

220 For carotenoid and chlorophyll extraction,<sup>24</sup> 6 ml of a mixture of methanol, acetone, and  
221 hexane (1:1:1 v:v:v) was added to a glass tube containing 50 mg of lyophilized skin  
222 powder. The mixture was vortexed for 30 s and then stirred for 30 min at 4°C in the  
223 dark. After the addition of 2 ml of MilliQ water the tube was vigorously shaken for 1  
224 min and then centrifuged for 1 min at 1500 g. The non-polar phase containing  
225 carotenoids and chlorophylls was recovered. The extraction was repeated by adding 2  
226 ml of hexane to the remaining mixture. The two extracts were pooled and the volume  
227 reduced to 1 ml by vacuum evaporation. The extract was filtered through 0.2- $\mu$ m filters  
228 and immediately subjected to high-performance liquid chromatography (HPLC)  
229 analysis as follows. Separation was performed at room temperature by a Spectra System  
230 P4000 HPLC, equipped with a UV 6000 LP photodiode array detector (Thermo Fisher  
231 Scientific, Waltham, MA, USA) using a Zorbax ODS column (5  $\mu$ m particle size, 250 x  
232 4.6 mm, Agilent Technologies, Santa Clara, CA, USA). HPLC separation was carried  
233 out at a flow rate of 0.8 ml min<sup>-1</sup> using the following linear gradient: 0 min, 82% A  
234 (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  
235 min, 58% A, 42% B; 40 min, 39% A, 61% B. The column was allowed to re-equilibrate  
236 in the starting solution (82% A, 18% B) for 5 min before the next injection. Different  
237 individual chlorophylls and carotenoids were detected by their absorbance at 445 nm.

238

239 The antioxidant capacity of berry skins was measured by generating the radical cation  
240 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS<sup>•+</sup>).<sup>25</sup> The radical solution  
241 was diluted in ethanol to obtain an absorbance of 0.700  $\pm$  0.020 at 734 nm (Perkin-  
242 Elmer  $\lambda$ 35 spectrophotometer). After addition of 1 ml of diluted ABTS<sup>•+</sup> solution to 100  
243  $\mu$ l of skin extract (250  $\mu$ g of skin powder in 1 ml of a mixture of methanol: water: 7M  
244 HCl 70:29:1 v:v:v), the decrease in absorbance was monitored and compared to that of  
245 the Trolox standard (Sigma) exactly 4 min after initial mixing. Antioxidant capacity was  
246 expressed in terms of Trolox equivalent antioxidant capacity (TEAC) per g DW of skin.

247

248 DNA isolation from lyophilized berry skins was carried out using the ZenoGene40  
249 Plant DNA Purifying Kit (Zenon Bio Kft., Szeged, Hungary). Concentration of the  
250 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-</sub>  
297 <sub>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

300 (14 flavonols, 5 anthocyanins, 3 flavanols –monomeric or dimeric tannins-, and 2  
301 flavanonols) and 5 non-flavonoids (3 cinnamic acids and 2 stilbenes). Great differences  
302 in the concentrations of most groups of phenolic compounds were found between  
303 localities. Anthocyanins were the most abundant group, showing values between 18.9  
304 (Bilje) and 110.1 (Girona) mg g<sup>-1</sup> DW. In every locality, malvidin-3-*O*-glucoside was  
305 the major anthocyanin. Flavonols were the second most abundant group of flavonoids,  
306 ranging between 1.76 (Bilje) and 7.7 (Girona) mg g<sup>-1</sup> DW. The major flavonol was  
307 quercetin 3-*O*-glucuronide. Flavanonols (between 0.18 and 1.14 mg g<sup>-1</sup> DW, in Bilje  
308 and Jerez, respectively) and flavanols (between 0.21 and 0.99 mg g<sup>-1</sup> DW, in Lednice  
309 and Bilje, respectively) were less abundant. Among non-flavonoids, cinnamic acids  
310 were the most abundant group, and also the group showing the greatest variability  
311 between localities, with values between 0.16 (Lednice) and 7.2 (Firenze) mg g<sup>-1</sup> DW.  
312 Finally, the least abundant compounds were stilbenes, which also showed a great  
313 variability (between 14 and 928 µg g<sup>-1</sup> DW, in Lednice and Girona, respectively).

314

315 The antioxidant capacity of berry skin extracts varied between 3592 (Lednice) and 9104  
316 (Firenze) µM TE g<sup>-1</sup> DW. Chlorophylls and all carotenoids showed the highest values in  
317 Rioja and the lowest in Pécs. β-Carotene was the most abundant carotenoid. The berry  
318 fresh weight varied between 1.1 (Girona and Bordeaux) and 2.1 g (Geisenheim),  
319 although most localities showed values between 1.1 and 1.3 g. TSS varied between 19.1  
320 (Bilje) and 23.7 °Brix (Jerez).

321

### 322 **Correlations between variables**

323

324 The correlations between all the environmental and plant response variables were  
325 determined (Table S1). Unless otherwise stated, the correlations mentioned in this text  
326 were significant ( $p < 0.05$ ) and positive. With respect to the correlations between berry  
327 skin variables, MSPCs were correlated with the contents of most phenolic compounds  
328 (except flavanols) and carotenoids. The total contents of flavonols, flavanonols,  
329 stilbenes and anthocyanins were correlated with one another, whereas the total content  
330 of cinnamic acids was only correlated with that of flavanonols. Total flavanol content  
331 was not correlated with the total content of any other phenolic group. The antioxidant  
332 capacity of berry skin extracts was correlated with anthocyanins, MSPCs, flavonols, the  
333 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, flavanols 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 flavanols. 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 flavanols. 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 flavanols. 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.

**407 DISCUSSION**

408

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

420

**421 Radiation is an important determinant of berry skin metabolite profile**

422

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

440

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,

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

513

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

525

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

534

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

540

541

542

**543 Effects of temperature and water supply on berry skin metabolic profile**

544

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

566

567 Rainfall and aridity indices showed a similar behavior as temperature variables, and  
568 were correlated with some phenolic compounds only when the period veraison-harvest  
569 was considered. In this period, water availability variables were correlated with  
570 temperature and radiation variables, and thus the individual effect of each variable could  
571 not be differentiated. Water availability typically shows strong relationships with  
572 different plant traits,<sup>49</sup> but direct effects on the contents of grape skin phenolic  
573 compounds are considered to be relatively minor.<sup>50,51</sup> This could be due to the fact that  
574 the effects of water availability on berry skin composition are mainly mediated by  
575 changes in berry size which subsequently affect the proportion of skin in relation to  
576 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, flavanols, 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

645 **ASSOCIATED CONTENT**

646

647 **Supporting information**

648

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

653

654 **AUTHOR INFORMATION**

655

656 Corresponding Author

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

659

660 **FUNDING**

661 ENO and JMA are grateful to the Ministerio de Economía y Competitividad of Spain  
662 and FEDER funds (Project CGL2014-54127-P) for financial support. MADCA and GS  
663 benefited from grants of the Universidad de La Rioja (Plan Propio 2014 and 2013,  
664 respectively). AM and GJ were supported by the grant OTKA K101430 of the  
665 Hungarian Scientific Research Foundation. This work was supported by COST  
666 (European Cooperation in Science and Technology) Action FA0906 of the European  
667 Union “UV-B radiation: a specific regulator of plant growth and food quality in a  
668 changing climate”.

669

670 **NOTES**

671 The authors declare no competing financial interest.

672

673 **ACKNOWLEDGEMENTS**

674 This manuscript is the result of joint work that was part of the activities of COST  
675 Action FA0906 “UV4growth” ([http://www.cost.eu/COST\\_Actions/fa/FA0906](http://www.cost.eu/COST_Actions/fa/FA0906)) of the  
676 European Union. We thank Enrique García-Escudero (ICVV, CSIC - Gobierno de La  
677 Rioja – Universidad de La Rioja), M<sup>a</sup> José Serrano and Belén Puertas (IFAPA-Centro

678 Rancho de la Merced, Junta de Andalucía) and Josep Trallero (Bodega Serrat de  
679 Montsoriu) for support to collect grapes.  
680

681 **REFERENCES**

682

683 (1) Núñez-Olivera, E.; Martínez-Abaigar, J.; Escudero, J. C. Adaptability of  
684 leaves of *Cistus ladanifer* to widely varying environmental conditions. *Funct. Ecol.*  
685 **1996**, *10*, 636-646.

686 (2) Llorens, L.; Peñuelas, J.; Beier, C.; Emmett, B.; Estiarte, M.; Tietema, A.  
687 Effects of an Experimental Increase of Temperature and Drought on the Photosynthetic  
688 Performance of Two Ericaceous Shrub Species Along a North–South European  
689 Gradient. *Ecosystems* **2004**, *7*, 613-624.

690 (3) Martz, F.; Peltola, R.; Fontanay, S.; Duval, R. L. E.; Julkunen-Tiitto, R.; Stark,  
691 S. Effect of Latitude and Altitude on the Terpenoid and Soluble Phenolic Composition  
692 of Juniper (*Juniperus communis*) Needles and Evaluation of Their Antibacterial  
693 Activity in the Boreal Zone. *J. Agr. Food Chem.* **2009**, *57*, 9575–9584.

694 (4) Jaakola, L.; Hohtola, A. Effect of latitude on flavonoid biosynthesis in plants.  
695 *Plant Cell Environ.* **2010**, *33*, 1239-1247.

696 (5) Jansen, M. A. K.; Le Martret, B.; Koornneef, M. Variations in constitutive and  
697 inducible UV-B tolerance; dissecting photosystem II protection in *Arabidopsis thaliana*  
698 accessions. *Physiol. Plant.* **2010**, *138*, 22-34.

699 (6) De Frenne, P.; Brunet, J.; Shevtsova, A.; Kolb, A.; Graae, B. J.; Chabrierie, O.;  
700 Cousins, S. A. O.; Decocq, G.; Schrijver, A. D.; Diekmann, M.; Gruwez, R.; Heinken,  
701 T.; Hermy, M.; Nilsson, C.; Stanton, S.; Tack, W.; Willaert, J.; Verheyen, K.  
702 Temperature effects on forest herbs assessed by warming and transplant experiments  
703 along a latitudinal gradient. *Global Change Biol.* **2011**, *17*, 3240-3253.

704 (7) Comont, D.; Martinez-Abaigar, J.; Albert, A.; Aphalo, P.; Causton, D. R.;  
705 Figueroa, F. L.; Gaberscik, A.; Llorens, L.; Hauser, M. T.; Jansen, M. A. K.; Kardefelt,  
706 M.; De la Coba Luque, P.; Neubert, S.; Núñez-Olivera, E.; Olsen, J.; Robson, M.;  
707 Schreiner, M.; Sommaruga, R.; Strid, A.; Torre, S.; Turunen, M.; Veljovic-Jovanovic,  
708 S.; Verdaguer, D.; Vidovic, M.; Wagner, J.; Barbro Winkler, J.; Zipoli, G.; Gwynn-  
709 Jones, D. UV-responses of *Lolium perenne* raised along a latitudinal gradient across  
710 Europe: A filtration study. *Physiol. Plant.* **2012**, *145*, 604-618.

711 (8) Yang, B.; Zheng, J.; Laaksonen, O.; Tahvonen, R.; Kallio, H. Effects of  
712 Latitude and Weather Conditions on Phenolic Compounds in Currant (*Ribes spp.*)  
713 Cultivars. *J. Agr. Food Chem.* **2013**, *61*, 3517–3532.

- 714 (9) Kenny, G. J.; Shao, J. An assessment of a latitude-temperature index for  
715 predicting climate suitability for grapes in Europe. *J. Hort. Sci.* **1992**, *67*, 239-246.
- 716 (10) Sun, T.; Chen, Q. Y.; Wu, L. J.; Yao, X. M.; Sun, X. J. Antitumor and  
717 antimetastatic activities of grape skin polyphenols in a murine model of breast cancer.  
718 *Food Chem. Toxicol.* **2012**, *50*, 3462–3467.
- 719 (11) Calabriso, N.; Scoditti, E.; Massaro, M.; Pellegrino, M.; Storelli, C.;  
720 Ingrosso, I.; Giovinazzo, G.; Carluccio, M. A. Multiple anti-inflammatory and anti-  
721 atherosclerotic properties of red wine polyphenolic extracts: differential role of  
722 hydroxycinnamic acids, flavonols and stilbenes on endothelial inflammatory gene  
723 expression. *Eur. J. Nutr.* **2016**, *55*, 477-489.
- 724 (12) Mattivi, F.; Guzzon, R.; Vrhovsek, U.; Stefanini, M.; Velasco, R. Metabolite  
725 Profiling of Grape: Flavonols and Anthocyanins. *J. Agr. Food Chem.* **2006**, *54*, 7692-  
726 7702.
- 727 (13) Berli, F. J.; Fanzone, M.; Piccoli, P.; Bottini, R. Solar UV-B and ABA Are  
728 Involved in Phenol Metabolism of *Vitis vinifera* L. Increasing Biosynthesis of Berry  
729 Skin Polyphenols. *J. Agr. Food Chem.* **2011**, *59*, 4874-4884.
- 730 (14) Sternad Lemut, M.; Sivilotti, P.; Franceschi, P.; Wehrens, R.; Vrhovsek, U.  
731 Use of Metabolic Profiling To Study Grape Skin Polyphenol Behavior as a Result of  
732 Canopy Microclimate Manipulation in a ‘Pinot noir’ Vineyard. *J. Agr. Food Chem.*  
733 **2013**, *61*, 8976–8986.
- 734 (15) Anderson, K. *Which winegrape varieties are grown where? A Global*  
735 *Empirical Picture*; University of Adelaide Press: Adelaide, **2013**.
- 736 (16) Clarke, O.; Rand, M. *Grapes & Wines: A Comprehensive Guide to Varieties*  
737 *and Flavours*; Pavilion Books: London, **2015**.
- 738 (17) Rolle, L.; Segade, S. R.; Torchio, F.; Giacosa, S.; Cagnasso, E.; Marengo, F.;  
739 Gerbi, V. Influence of Grape Density and Harvest Date on Changes in Phenolic  
740 Composition, Phenol Extractability Indices, and Instrumental Texture Properties during  
741 Ripening. *J. Agr. Food Chem.* **2011**, *59*, 8796-8805.
- 742 (18) Carbonell-Bejerano, P.; Diago, M. P.; Martínez-Abaigar, J.; Martínez-  
743 Zapater, J. M.; Tardáguila, J.; Núñez-Olivera, E. Solar ultraviolet radiation is necessary  
744 to enhance grapevine fruit ripening transcriptional and phenolic responses. *BMC Plant*  
745 *Biol.* **2014**, *14*, 183-198.

- 746 (19) Allaart, M.; Van Weele, M.; Fortuin, P.; Kelder, H. An Empirical model to  
747 predict the UV-index based on Solar Zenith Angle and Total Ozone. *Meteorol. Appl.*  
748 **2004**, *11*, 59-65.
- 749 (20) Downey, M. O.; Harvey, J. S.; Robinson, S. P. Synthesis of flavonols and  
750 expression of flavonol synthase genes in the developing grape berries of Shiraz and  
751 Chardonnay (*Vitis vinifera* L.). *Aust. J. Grape Wine Res.* **2003**, *9*, 110-121.
- 752 (21) Bogs, J.; Ebadi, A.; McDavid, D.; Robinson, S. P. Identification of the  
753 Flavonoid Hydroxylases from Grapevine and Their Regulation during Fruit  
754 Development. *Plant Physiol.* **2006**, *140*, 279-291.
- 755 (22) Pastore, C.; Zenoni, S.; Fasoli, M.; Pezzotti, M.; Tornielli, G. B.; Filippetti, I.  
756 Selective defoliation affects plant growth, fruit transcriptional ripening program and  
757 flavonoid metabolism in grapevine. *BMC Plant Biol.* **2013**, *13*, 30-45.
- 758 (23) Del-Castillo-Alonso, M. A.; Diago, M. P.; Monforte, L.; Tardáguila, J.;  
759 Martínez-Abaigar, J.; Núñez-Olivera, E. Effects of UV exclusion on the physiology and  
760 phenolic composition of leaves and berries of *Vitis vinifera* cv. Graciano. *J. Sci. Food*  
761 *Agr.* **2015**, *95*, 409-416.
- 762 (24) Heredia, A.; Peinado, I.; Rosa, E.; Andrés, A. Effect of osmotic pre-treatment  
763 and microwave heating on lycopene degradation and isomerization in cherry tomato.  
764 *Food Chem.* **2010**, *123*, 92-98.
- 765 (25) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans,  
766 C. Antioxidant activity applying an improved ABTS radical cation decolorization assay.  
767 *Free Radic. Biol. Med.* **1999**, *26*, 1231-1237.
- 768 (26) Price, S. F.; Breen, P. J.; Valladao, M.; Watson, B. T. Cluster Sun Exposure  
769 and Quercetin in Pinot noir Grapes and Wine. *Am. J. Enol. Vitic.* **1995**, *46*, 187-194.
- 770 (27) Spayd, S. E.; Tarara, J. M.; Mee, D. L.; Ferguson, J. C. Separation of  
771 Sunlight and Temperature Effects on the Composition of *Vitis vinifera* cv. Merlot  
772 Berries. *Am. J. Enol. Vitic.* **2002**, *53*, 171-182.
- 773 (28) Berli, F.; D'Angelo, J.; Cavagnaro, B.; Bottini, R.; Wuilloud, R.; Silva, M. F.  
774 Phenolic composition in grape (*Vitis vinifera* L. cv. Malbec) ripened with different solar  
775 UV-B radiation levels by capillary zone electrophoresis. *J. Agr. Food Chem.* **2008**, *56*,  
776 2892-2898.
- 777 (29) Koyama, K.; Ikeda, H.; Poudel, P. R.; Goto-Yamamoto, N. Light quality  
778 affects flavonoid biosynthesis in young berries of Cabernet Sauvignon grape.  
779 *Phytochemistry* **2012**, *78*, 54-64.

- 780 (30) Downey, M. O.; Harvey, J. S.; Robinson, S. P. The effect of bunch shading  
781 on berry development and flavonoid accumulation in Shiraz grapes. *Aust. J. Grape*  
782 *Wine Res.* **2004**, *10*, 55-73.
- 783 (31) Martínez-Lüscher, J.; Sánchez-Díaz, M.; Delrot, S.; Aguirreolea, J.; Pascual,  
784 I.; Gomès, E. Ultraviolet-B Radiation and Water Deficit Interact to Alter Flavonol and  
785 Anthocyanin Profiles in Grapevine Berries through Transcriptomic Regulation. *Plant*  
786 *Cell Physiol.* **2014**, *55*, 1925–1936.
- 787 (32) Liu, L. L.; Gregan, S.; Winefield, C.; Jordan, B. From UVR8 to flavonol  
788 synthase: UV-B-induced gene expression in Sauvignon blanc grape berry. *Plant Cell*  
789 *Environ.* **2015**, *38*, 905-919.
- 790 (33) Malacarne, G.; Costantini, L.; Collier, E.; Battilana, J.; Velasco, R.;  
791 Vrhovsek, U.; Grando, M. S.; Moser, C. Regulation of flavonol content and  
792 composition in (Syrah×Pinot Noir) mature grapes: integration of transcriptional  
793 profiling and metabolic quantitative trait locus analyses. *J. Exp. Bot.* **2015**, *66*, 4441–  
794 4453.
- 795 (34) Martínez-Lüscher, J.; Torres, N.; Hilbert, G.; Richard, T.; Sánchez-Díaz, M.;  
796 Delrot, S.; Aguirreolea, J.; Pascual, I.; Gomès, E. Ultraviolet-B radiation modifies the  
797 quantitative and qualitative profile of flavonoids and amino acids in grape berries.  
798 *Phytochemistry* **2014**, *102*, 106-114.
- 799 (35) Ryan, K. G.; Swinny, E. E.; Markham, K. R.; Winefield, C. Flavonoid gene  
800 expression and UV photoprotection in transgenic and mutant *Petunia* leaves.  
801 *Phytochemistry* **2002**, *59*, 23-32.
- 802 (36) Sroka, Z. Antioxidative and antiradical properties of plant phenolics. *Z. Nat.*  
803 *Sect. C J. Biosci.* **2005**, *60*, 833-843.
- 804 (37) Fanzone, M.; Zamora, F.; Jofré, V.; Assof, M.; Peña-Neira, Á. Phenolic  
805 Composition of Malbec Grape Skins and Seeds from Valle de Uco (Mendoza,  
806 Argentina) during Ripening. Effect of Cluster Thinning. *J. Agr. Food Chem.* **2011**, *59*,  
807 6120–6136.
- 808 (38) Feng, H.; Yuan, F.; Skinkis, P. A.; Qian, M. C. Influence of cluster zone leaf  
809 removal on Pinot noir grape chemical and volatile composition. *Food Chem.* **2015**, *173*,  
810 414-423.
- 811 (39) Fornara, V.; Onelli, E.; Sparvoli, F.; Rossoni, M.; Aina, R.; Marino, G.;  
812 Citterio, S. Localization of stilbene synthase in *Vitis vinifera* L. during berry  
813 development. *Protoplasma* **2008**, *233*, 83–93.

- 814 (40) Fujita, A.; Goto-Yamamoto, N.; Aramaki, I.; Hashizume, K. Organ-Specific  
815 Transcription of Putative Flavonol Synthase Genes of Grapevine and Effects of Plant  
816 Hormones and Shading on Flavonol Biosynthesis in Grape Berry Skins. *Biosci.*  
817 *Biotechnol. Biochem.* **2006**, *70*, 632-638.
- 818 (41) Azuma, A.; Yakushiji, H.; Koshita, Y.; Kobayashi, S. Flavonoid  
819 biosynthesis-related genes in grape skin are differentially regulated by temperature and  
820 light conditions. *Planta* **2012**, *236*, 1067-1080.
- 821 (42) Downey, M. O.; Dokoozlian, N. K.; Krstic, M. P. Cultural Practice and  
822 Environmental Impacts on the Flavonoid Composition of Grapes and Wine: A Review  
823 of Recent Research. *Am. J. Enol. Vitic.* **2006**, *57*, 257-268.
- 824 (43) Guan, L.; Dai, Z.; Wu, B. H.; Wu, J.; Merlin, I.; Hilbert, G.; Renaud, C.;  
825 Gomes, E.; Edwards, E.; Li, S. H.; Delrot, S. Anthocyanin biosynthesis is differentially  
826 regulated by light in the skin and flesh of white-fleshed and teinturier grape berries.  
827 *Planta* **2016**, *243*, 23-41.
- 828 (44) Cortell, J. M.; Kennedy, J. A. Effect of Shading on Accumulation of  
829 Flavonoid Compounds in (*Vitis vinifera* L.) Pinot Noir Fruit and Extraction in a Model  
830 System. *J. Agr. Food Chem.* **2006**, *54*, 8510-8520.
- 831 (45) Hanlin, R. L.; Downey, M. O. Condensed Tannin Accumulation and  
832 Composition in Skin of Shiraz and Cabernet Sauvignon Grapes during Berry  
833 Development. *Am. J. Enol. Vitic.* **2009**, *60*, 13-23.
- 834 (46) Pastor del Rio, J. L.; Kennedy, J. A. Development of Proanthocyanidins in  
835 *Vitis vinifera* L. cv. Pinot noir Grapes and Extraction into Wine. *Am. J. Enol. Vitic.*  
836 **2006**, *57*, 125-132.
- 837 (47) Cohen, S. D.; Tarara, J. M.; Gambetta, G. A.; Matthews, M. A.; Kennedy, J.  
838 A. Impact of diurnal temperature variation on grape berry development,  
839 proanthocyanidin accumulation, and the expression of flavonoid pathway genes. *J. Exp.*  
840 *Bot.* **2012**, *63*, 2655-2665.
- 841 (48) Nicholas, K. A.; Matthews, M. A.; Lobell, D. B.; Willits, N. H.; Field, C. B.  
842 Effect of vineyard-scale climate variability on Pinot noir phenolic composition. *Agr.*  
843 *Forest Meteorol.* **2011**, *151*, 1556-1567.
- 844 (49) Moles, A. T.; Perkins, S. E.; Laffan, S. W.; Flores-Moreno, H.; Awasthy, M.;  
845 Tindall, M. L.; Sack, L.; Pitman, A.; Kattge, J.; Aarssen, L. W.; Anand, M.; Bahn, M.;  
846 Blonder, B.; Cavender-Bares, J.; Hans, J.; Cornelissen, C.; Cornwell, W. K.; Díaz, S.;  
847 Dickie, J. B.; Freschet, G. T.; Griffiths, J. G.; Gutierrez, A. G.; Hemmings, F. A.;



848 Hickler, T.; Hitchcock, T. D.; Keighery, M.; Kleyer, M.; Kurokawa, H.; Leishman, M.  
849 R.; Liu, K.; Niinemets, U.; Onipchenko, V.; Onoda, Y.; Peñuelas, J.; Pillar, V. D.;  
850 Reich, P. B.; Shiodera, S.; Siefert, A.; Sosinski Jr, E. E.; Soudzilovskaia, N. A.; Swaine,  
851 E. K.; Swenson, N. G.; Van Bodegom, P. M.; Warman, L.; Weiher, E.; Wright, I. J.;  
852 Zhang, H.; Zobel, M.; Bonser, S. P. Which is a better predictor of plant traits:  
853 temperature or precipitation? *J. Veg. Sci.* **2014**, *25*, 1167–1180.

854 (50) Kennedy, J. A.; Matthews, M. A.; Waterhouse, A. L. Effect of Maturity and  
855 Vine Water Status on Grape Skin and Wine Flavonoids. *Am. J. Enol. Vitic.* **2002**, *53*,  
856 268-274.

857 (51) Cadot, Y.; Chevalier, M.; Barbeau, G. Evolution of the localisation and  
858 composition of phenolics in grape skin between veraison and maturity in relation to  
859 water availability and some climatic conditions. *J. Sci. Food Agr.* **2011**, *91*, 1963–1976.

860 (52) Kuhn, N.; Guan, L.; Dai, Z. W.; Wu, B. H.; Lauvergeat, V.; Gomès, E.; Li, S.  
861 H.; Godoy, F.; Arce-Johnson, P.; Delrot, S. Berry ripening: recently heard through the  
862 grapevine. *J. Exp. Bot.* **2014**, *65*, 4543–4559.

863 (53) Lee, J.; Skinkis, P. A. Oregon ‘Pinot noir’ grape anthocyanin enhancement  
864 by early leaf removal. *Food Chem.* **2013**, *139*, 893-901.

865 (54) Van Leeuwen, C.; Friant, P.; Choné, X.; Tregoat, O.; Koundouras, S.;  
866 Dubourdieu, D. Influence of Climate, Soil, and Cultivar on Terroir. *Am. J. Enol. Vitic.*  
867 **2004**, *55*, 207-217.

868 (55) Schreiner, R. P.; Scagel, C. F.; Lee, J. N, P, and K Supply to Pinot noir  
869 Grapevines: Impact on Berry Phenolics and Free Amino Acids. *Am. J. Enol. Vitic.* **2014**,  
870 *65*, 43-49.

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)

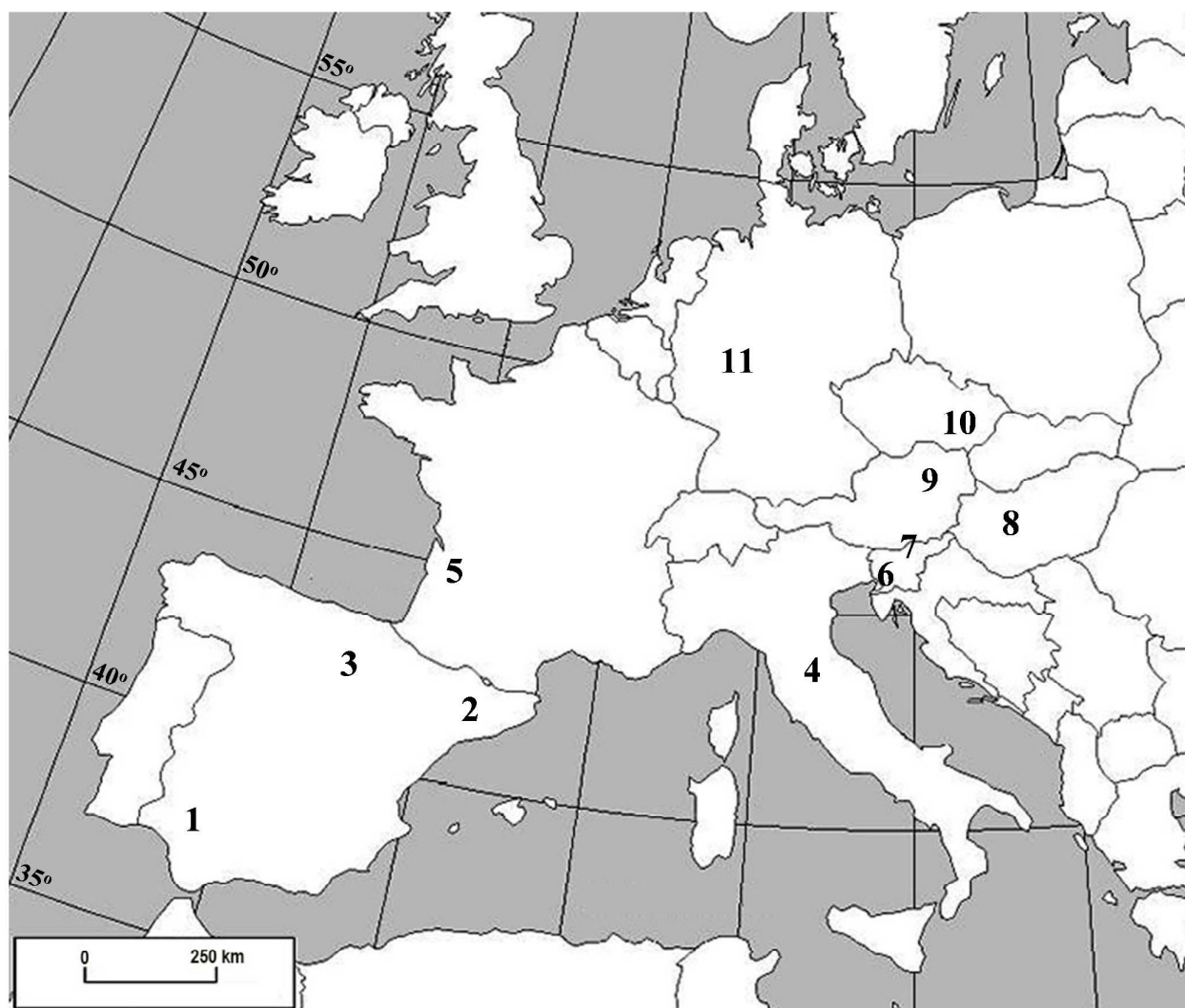
**Table 2.** Ranges of the environmental variables in the 11 European sampling localities used in this study, together with the localities in which each extreme value was recorded (between brackets). ETP, potential evapotranspiration. GGR, Ground-station Global Radiation. DSSF, Downward Surface Shortwave 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

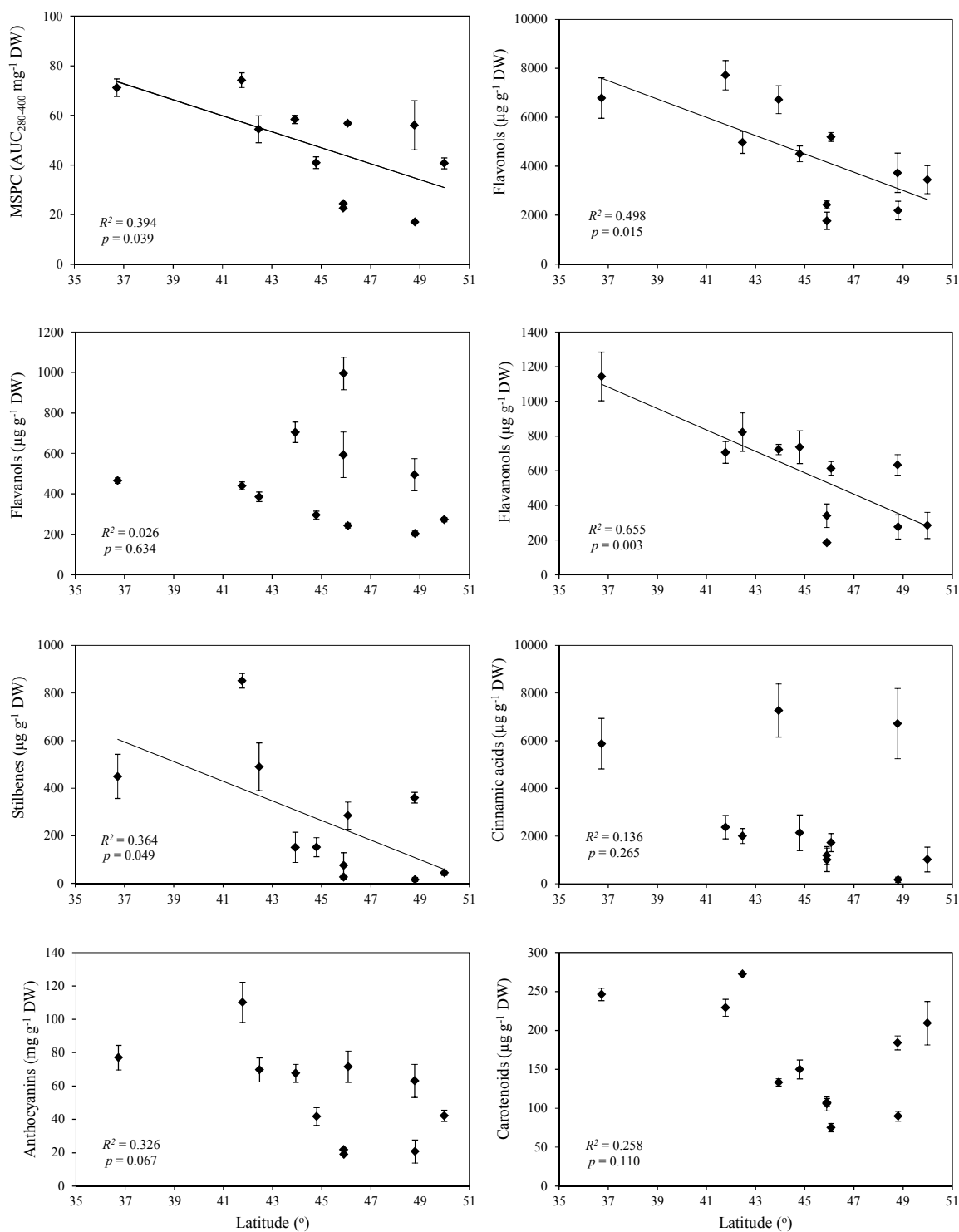
chlorophylls ( <i>a+b</i> ) ( $\mu\text{g g}^{-1}$ DW)	438 $\pm$ 22	424 $\pm$ 44	525 $\pm$ 14	227 $\pm$ 6	290 $\pm$ 32	188 $\pm$ 16	182 $\pm$ 9	117 $\pm$ 10	360 $\pm$ 16	135 $\pm$ 5	480 $\pm$ 51
fresh weight per berry (g)	1.4 $\pm$ 0.2	1.1 $\pm$ 0.1	1.3 $\pm$ 0.0	1.3 $\pm$ 0.1	1.1 $\pm$ 0.1	1.2 $\pm$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

**Table 3.** Values (means  $\pm$  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.

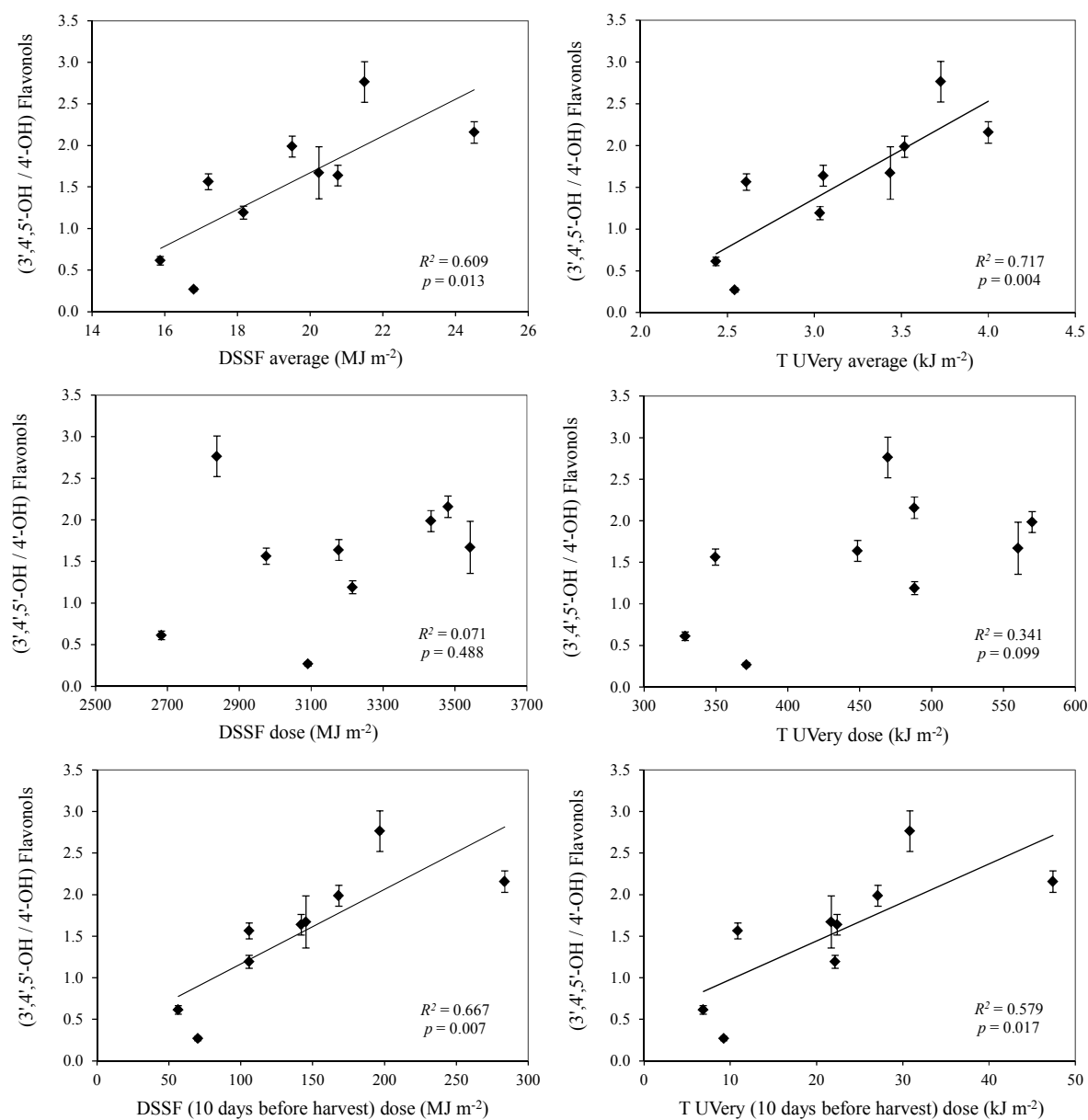




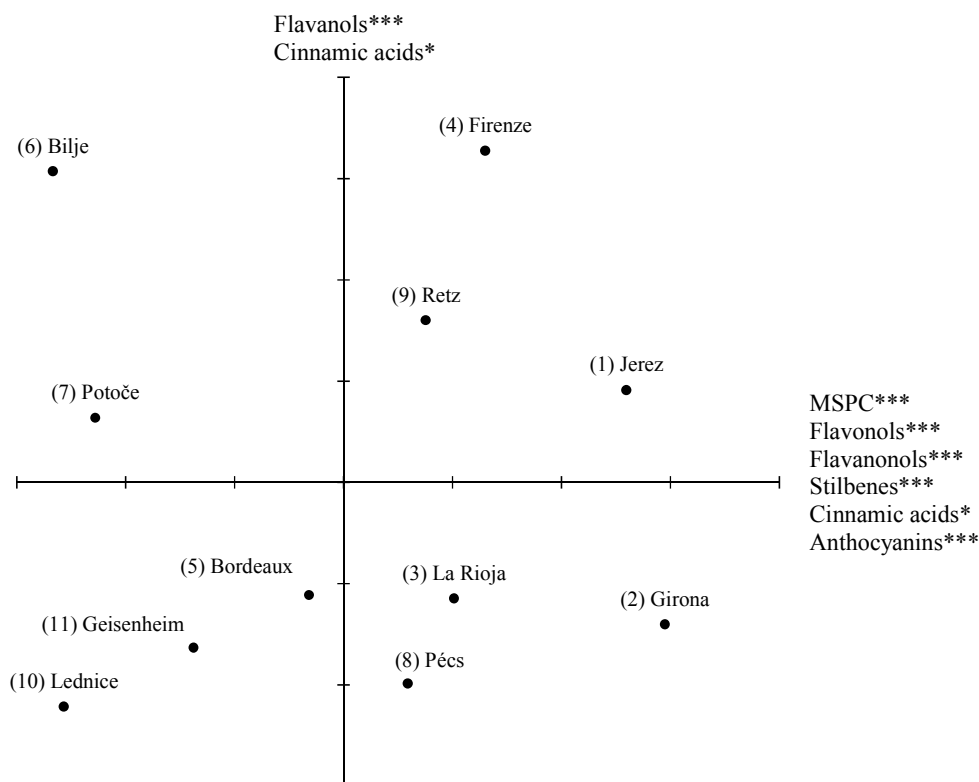
**Figure 1.** Geographic location of the 11 European sampling localities used in this study. 1, Jerez de la Frontera (Spain); 2, Girona (Spain); 3, La Rioja (Spain); 4, Firenze (Italy); 5, Bordeaux (France); 6, Bilje (Slovenia); 7, Potoče (Slovenia); 8, Pécs (Hungary); 9, Retz (Austria); 10, Lednice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

## GRAPHIC FOR TABLE OF CONTENTS

