Environmental plasticity of Pinot noir grapevine leaves; a trans-European study of morphological and biochemical changes along a 1500 km latitudinal climatic gradient

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Environment Keywords:	UV radiation, light quality

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Other Keywords:	latitude, grapevine, adaptation, phenolic compounds

Abstract:	A two-year study explored metabolic and phenotypic plasticity of sun acclimated Vitis vinifera cv. Pinot noir leaves collected from twelve locations across a 36.69°N – 49.98°N latitudinal gradient. Leaf morphological and biochemical parameters were analysed in the context of meteorological parameters and the latitudinal gradient. We found that leaf fresh weight and area were negatively correlated with both global and UV radiation; cumulated global radiation being a stronger correlator. Cumulative UV radiation (sumUVR) was the strongest correlator with most leaf metabolites and pigments. Leaf UV absorbing pigments, total antioxidant capacities and phenolic compounds increased with increasing sumUVR, while total carotenoids and xanthophylls decreased. Despite of this re-allocation of metabolic resources from carotenoids to phenolics, an increase in xanthophyll cycle pigments (VAZ) with increasing sumUVR indicates active, dynamic protection for the photosynthetic apparatus. In addition, increased amounts of flavonoids (quercetin-glycosides) and constitutive β-carotene and α-tocopherol pools provide antioxidant protection against ROS. However, rather than a continuum of plant acclimation responses, principal component analysis indicates clusters of metabolic states across the explored 1500 km long latitudinal gradient. This study emphasizes the physiological component of plant responses to latitudinal gradients, and reveals the physiological plasticity that may act to complement genetic adaptations.

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Environmental plasticity of Pinot noir grapevine leaves; a trans-European study of morphological and biochemical changes along a 1500 km latitudinal climatic gradient

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1

2 **ABSTRACT**

3 A two-year study explored metabolic and phenotypic plasticity of sun acclimated *Vitis vinifera* cv.
4 Pinot noir leaves collected from twelve locations across a 36.69°N – 49.98°N latitudinal gradient.
5 Leaf morphological and biochemical parameters were analysed in the context of meteorological
6 parameters and the latitudinal gradient. We found that leaf fresh weight and area were negatively
7 correlated with both global and UV radiation; cumulated global radiation being a stronger correlator.
8 Cumulative UV radiation (sumUVR) was the strongest correlator with most leaf metabolites and
9 pigments. Leaf UV absorbing pigments, total antioxidant capacities and phenolic compounds
10 increased with increasing sumUVR, while total carotenoids and xanthophylls decreased. Despite of
11 this re-allocation of metabolic resources from carotenoids to phenolics, an increase in xanthophyll
12 cycle pigments (VAZ) with increasing sumUVR indicates active, dynamic protection for the
13 photosynthetic apparatus. In addition, increased amounts of flavonoids (quercetin-glycosides) and
14 constitutive β-carotene and α-tocopherol pools provide antioxidant protection against ROS.
15 However, rather than a continuum of plant acclimation responses, principal component analysis
16 indicates clusters of metabolic states across the explored 1500 km long latitudinal gradient. This
17 study emphasises the physiological component of plant responses to latitudinal gradients, and
18 reveals the physiological plasticity that may act to complement genetic adaptations.

19

20 **KEY WORDS**

21 grapevine, plasticity, climate, ultraviolet radiation, global radiation, latitude, morphology, phenolic
22 compounds, carotenoids, alpha-tocopherol

23

24 **INTRODUCTION**

25 Latitudinal climatic gradients are important determinants of plant growth, metabolism and
26 development (Willin et al. 2003, Hulshof et al. 2013). Conversely, climatic variation along latitudinal

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1 and altitudinal gradients provides an excellent and natural experimental set-up for investigating the
2 impacts of climatic variables on terrestrial organisms and ecosystems (Hillebrand 2004, Körner 2007,
3 De Frenne et al. 2013, Del-Castillo-Alonso et al. 2016). Climatic variables along the latitudinal
4 gradient represent typical growth conditions of crops and/or wild species, and therefore studies are
5 not subjected to the potential flaws of experimental manipulation that may arise under controlled
6 conditions. Rather, plants are exposed to a "realistic" environment where changes in any particular
7 climatic factor will co-occur with changes in other climatic factors. This is particularly important as in
8 a natural environment different climatic factors can interact with each other, while crosstalk
9 between multiple plant response pathways may further modify effects on plant growth, metabolism
10 and development.

11 In the northern extratropical hemisphere there is a substantial decrease in mean annual
12 temperature of -0.73°C per degree of latitude (De Frenne et al. 2013), while mean annual
13 precipitation tends to increase by 4.04 mm per degree of latitude. Moreover, solar radiation
14 characteristics vary along the latitudinal gradient, and this variation includes an increase in the
15 difference between the shortest and the longest photo-period, and decreases in both total solar
16 energy and solar ultraviolet radiation amount towards higher latitudes (Caldwell et al. 1982). It has
17 been extensively demonstrated that the light environment has a great influence on plant growth and
18 development, through effects on photosynthesis, specific photoreceptors, and/or by causing
19 photoinhibition-type damage. Indeed, gradients of decreasing solar energy supply towards the
20 higher latitudes have been proposed as crucial drivers of environmental plasticity in plant traits
21 (Currie 1991, Allen et al. 2002). Currently, little is known about the impact of the latitudinal UV-
22 gradient on plant morphology and metabolism. It has been demonstrated that UV, especially UV-B
23 (280-315 nm), stimulates the biosynthesis and accumulation of plant flavonoids and other secondary
24 plant metabolites (reviewed in Schreiner et al. 2012) under controlled conditions. These biochemical
25 effects are paralleled by morphological effects, including changes in cell division, elongation and/or
26 differentiation leading to thicker leaves, shorter petioles and stems, increased auxiliary branching

1 and altered root-to-shoot ratios (reviewed in Robson et al. 2015). Yet, it is not known whether small
2 changes in UV-exposure, associated with a latitudinal gradient, will have a significant impact on
3 plants when these are simultaneously exposed to multiple other latitude-associated changes in the
4 environmental variables.

5 Best studied are effects of latitudinal gradients on phenological phenomena. For example,
6 both emergence time and flowering phenology of *Anemone nemorosa* and *Milium effusum* were
7 found to be delayed with increasing latitude (De Frenne et al. 2013). However, latitudinal climate
8 gradients are also associated with a broad range of other plant physiological responses. For example,
9 intraspecific leaf composition (N-P ratio) and seed mass decrease significantly with latitude in
10 natural populations (De Frenne et al. 2013). Plant metabolic make up is also associated with
11 latitudinal climatic gradients. Yang et al. (2013) showed that the total content of phenolic
12 compounds was 10–19% higher in currant berries (*Ribes spp.*) grown at a northerly latitude,
13 compared to those grown further south. Higher total hydroxycinnamic acid contents were also
14 reported for berries from northerly latitudes, but this finding was cultivar specific. In juniper
15 (*Juniperus communis*) needles, increasing latitude was associated with increased content of a broad
16 range of phenolic compounds (flavonols, proanthocyanins and monoterpenes) (Martz et al. 2009),
17 while white birch (*Betula pubescens*) leaves contained higher levels of quercetin derivatives (but not
18 total flavonoids) at higher latitudes (Stark et al. 2008). A study of 179 bilberry (*Vaccinium myrtillus*)
19 clones (Lätti et al. 2008) revealed that populations from lower latitudes contained significantly less
20 total anthocyanins. However, concentrations of the anthocyanins delphinidin glycoside and
21 petunidin glycoside were higher in plants from higher latitudes. Thus, the limited available data do
22 indicate an important correlation between latitudinal climate gradients and plant metabolite
23 profiles. Unavoidably, many of these latitudinal climatic effects on plant growth and biochemistry
24 will, in turn, impact on other plant-environment interactions. For example, there is good evidence
25 that latitudinal climatic gradients impact on trophic interactions and biodiversity (Hillebrand 2004,
26 Proulx et al. 2015). The latitude associated changes in metabolic profile can also be hypothesised to

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1 impact on the nutritional quality of fruits and vegetables. Thus, the study of the impact of latitudinal
2 climatic gradients on plants is relevant in the context of fundamental plant biology, plant eco-
3 geography, nutritional biology and climate change.

4 Plant responses to latitudinal climatic gradients comprise two major components, short term
5 adjustments of physiology in response to imposed climate factors, and long-term-adaptive
6 responses. Much of the published variation in plant responses observed along latitudinal climatic
7 gradients is due to a combination of acclimation and adaptation, with most studies not attempting
8 to separate this complex response mixture. Testing under standardised conditions of plant material
9 collected along a latitudinal gradient can visualise the genetic adaptations associated with such a
10 gradient (Li et al. 1998, Stenøien et al. 2002, Biswas & Jansen 2012, Comont et al. 2012). Fewer
11 studies have focussed on analysing physiological responses (environmental plasticity) of genetically-
12 similar material when grown along a latitudinal climatic gradient. Here, we analysed phenotypic and
13 metabolic plasticity of *Vitis vinifera* cv. Pinot noir leaves across a latitudinal climatic gradient that
14 includes most of the commercial growing areas of this wine grape in Europe. This grapevine variety
15 is popular among growers due to its stable yield performance, early ripening characteristic and high
16 wine quality parameters. This traditional wine cultivar is present in all European viticulture regions,
17 from the Mediterranean to continental cool-climate vine growing areas (Kenny & Harrison 1992).
18 Thus, the widespread use of the cultivar enabled coordinated field experiments across European
19 vineyards located at latitudes between 36.69°N and 49.99°N. Moreover, local climatic factors in
20 vineyards are routinely registered. Grapevine as a perennial and economically important fruit crop is
21 a suitable model plant because its leaves contain a diverse range of secondary plant metabolites,
22 especially flavonoids and carotenoids. From a practical perspective, the blue colouration of the
23 berries of Pinot noir during ripening makes it easy to determine the onset of ripening (veraison), and
24 this phenophase was chosen as sampling time in all participating vineyards. Although veraison is
25 determined by developmental events, it is also influenced by environmental constrains. Up to

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1 veraison, leaves are in the phase of extensive growth and the metabolite status of leaves is
2 representative for the entire plant.

3 In this study, the hypothesis tested was that Pinot noir grapevine leaves show considerable
4 phenotypic and metabolic plasticity when grown along a latitudinal climatic gradient that includes
5 most of the commercial growing areas of this wine grape. To test this hypothesis, morphological and
6 biochemical parameters (flavonoid and carotenoid composition, non-enzymatic leaf total antioxidant
7 capacity and UV absorbing pigment content) were measured and related to latitude as well as
8 climate parameters. Within the overall aim of the study, it was explored to what extent solar UV
9 radiation affects metabolite composition and morphology relative to other climate parameters, such
10 as global solar radiation, or temperature. In addition, associations between different metabolite
11 groups were explored, as was their relative contribution to leaf antioxidant capacity. The study
12 generates new insights in how climatic gradients can drive environmental plasticity.
13

14 **MATERIALS AND METHODS**

15 **A latitudinal gradient of vineyards**

16 For this study, 12 vineyards were selected across Europe. Vineyards were located in Spain,
17 France, Italy, Hungary, Austria, Slovenia, the Czech Republic and Germany (Table 1). The selected
18 vineyards represent a latitudinal gradient of almost 14° (36.69 - 49.99°N) and a linear distance of
19 around 1,500 km, covering most of the commercial Pinot noir growing latitudes in the northern
20 hemisphere (35-55°). Vineyard age varied between 6 and 30 years, and vineyard soils were mostly
21 calcareous and neutral-alkaline (pH between 7.0 and 8.5).

22 **Meteorological Parameters**

23 Dates of bud break and veraison, and air temperature data for the period between these
24 two dates were locally collected for each site. Air temperature data obtained from meteorological
25 ground-based stations close to each location were used to compute the daily average (°C) and the
26 degree day integral (DD, ∑ °C) parameters. The degree day integral was calculated over the period

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1 between bud break and veraison using 10°C as base temperature. For most vineyards,
2 meteorological stations were located less than 200 m from the actual vineyards. Remaining stations
3 were located less than 20 km away, except in the case of Lednice (Czech Republic) where the station
4 was located 50 km from the vineyard. In the latter cases, it was ascertained that meteorological
5 stations were located at a similar latitude and altitude as the vineyard.

6 Daily values of DSSF (Downward Surface Shortwave Flux) solar global radiation were
7 calculated by integrating the 30 minutes of data obtained from the Land Surface Analysis Satellite
8 Applications Facility web page (http://landsaf.meteo.pt). Daily erythemal UV radiation data were
9 downloaded from the ESA-TEMIS web page (http://www.temis.nl) for the period bud break to leaf
10 sampling at veraison. For this study we used erythemal UV-data, which are widely available (i.e. ESA-
11 TEMIS) as a proxy for plant UV-exposure. The erythemal spectrum is not the same as most
12 commonly used “plant response spectra” (Flint & Caldwell 1996). Yet, it is very likely that the two
13 spectra are positively correlated with one another, thus not affecting conclusions in this paper.
14 Cumulated doses (sumGR) and average values (avgGR) of DSSF global radiation (MJ m^{-2} and MJ m^{-2} d^{-1}, respectively), as well as cumulated doses (sumUVR) and average (avgUVR) UV radiation (kJ m^{-2} and
16 kJ m^{-2} d^{-1}, respectively) were calculated for the period from bud break to veraison for each location.
17 In addition, the effects of cumulated UV radiation during the last 10 days before veraison (10d-
18 sumUVR) have been evaluated.

19 **Plant Material and Morphological Parameters**

20 Grapevine (*Vitis vinifera* cv. Pinot noir) leaves were collected from 7 different vineyards in 2012. In
21 2013, a further 5 locations were added, giving a total of 12 vineyards (Table 1, Figure 1). At each
22 location, 5 fully developed (5-7th leaf from shoot tip) sun exposed leaves were selected from each of
23 3 different plants at mid-veraison. Leaves were collected during the extended noon period (11:00-
24 13:00 LMT) of sunny days when photosynthetically active radiation (PAR) was above 1000 μmol m^{-2}
25 s^{-1} at all locations. Detached leaves were photographed with digital cameras including a size scale in
26 all images, fresh weights were measured, and samples were stored in liquid nitrogen until

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1 lyophilisation. Leaves were later ground into fine powder for laboratory analyses, and leaves
2 collected from the same plant in the same year were mixed to form one sample. Lyophilized and
3 powdered leaf samples were distributed among participating laboratories.
4 In order to assess morphology parameters leaf circumference, area and the average lobe to
5 indentation ratio were quantified based on leaf images using IMAGEJ software (Abràmoff et al.
6 2004)
7 **Flavonoids**
8 Flavonol glycosides and phenolic acids were analysed according to Schmidt et al. (2010) with
9 a modification. Lyophilized leaf powder (20 mg) was subjected to an extraction using 600 µL of 60%
10 aqueous methanol on a magnetic stirrer plate for 40 min at 20°C. The extract was centrifuged at
11 2200 x g for 10 min at room temperature (Function Line, Thermo Fischer Scientific, Waltham USA),
12 and the supernatant was collected in a reaction tube. This process was repeated twice with 300 µL
13 of 60% aqueous methanol and an extraction time of 15 minutes. The three supernatants per sample
14 were subsequently combined. The combined extract was evaporated until dryness and was re-
15 suspended in 200 µL of 10% aqueous methanol. The re-suspended extract was centrifuged at 1100 x
16 g for 5 min at 20°C through a Corning® Costar® Spin-X® plastic centrifuge tube filter (Sigma Aldrich
17 Chemical Co., St. Louis, MO, USA) for HPLC analysis. Each extraction was carried out in duplicate.
18 Flavonol glycosides and phenolic acids and concentrations were determined using a series 1100
19 HPLC (Agilent Technologies, Waldbronn, Germany) equipped with a degasser, binary pump, auto-
20 sampler, column oven, and photodiode array detector. An Ascentis® Express F5 column (150 mm ×
21 4.6 mm, 5 µm, Supelco/Sigma-Aldrich, Bellefonte, PA, USA) was used to separate the compounds at
22 a temperature of 25°C. Eluent A was 0.5% acetic acid, and eluent B was 100% acetonitrile. The
23 gradient used for eluent B was 5-12% (0-3 min), 12-25% (3-46 min), 25-90% (46-49.5 min), 90%
24 isocratic (49.5-52 min), 90-5% (52-52.7 min), and 5% isocratic (52.7-59 min). The separation was
25 conducted at a flow rate of 0.85 mL min^{-1} and a detection wavelength of 320 nm and 370 nm for
26 phenolic acids, and flavonol glycosides, respectively. The phenolic acids and glycosides of flavonols

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1 were identified as deprotonated molecular ions and characteristic mass fragment ions according to
2 Schmidt et al. (2010) by HPLC-DAD-ESI-MS^n using an Agilent series 1100 ion trap mass spectrometer
3 in negative ionization mode. Nitrogen was used as the dry gas (10 L min^{-1}, 325°C) and the nebulizer
4 gas (40 psi) with a capillary voltage of -3500 V. Helium was used as the collision gas in the ion trap.
5 The mass optimization for the ion optics of the mass spectrometer for quercetin was performed at
6 *m/z* 301 or arbitrarily at *m/z* 1000. The MS^n experiments were performed in auto mode up to HPLC-
7 DAD-ESI-MS^3 in a scan from *m/z* 200-2000. Standards (chlorogenic acid, quercertin 3-glucoside, and
8 kaempferol 3-glucoside; Roth, Karlsruhe, Germany) were used for external calibration curves.
9 Results are presented as mg g^{-1} dry weight (DW).

10 **Carotenoids**

11 Carotenoids and total chlorophyll were analysed according to the method reported by
12 Castagna et al. (2001), with slight modifications. Lyophilized leaf samples (20 mg) were ground in a
13 mortar with 3 mL of 80% aqueous HPLC-grade acetone in the presence of sodium ascorbate under
14 dimmed light. Samples were filtered through 0.2-μm filters (Sartorius Stedim Biotech, Göttingen,
15 Germany). Carotenoid separation was achieved by HPLC analysis using a Spectra System P4000
16 HPLC, equipped with a UV 6000 LP photodiode array detector (Thermo Fisher Scientific, Waltham,
17 MA) and a Zorbax ODS column (SA, 5μm particle size, 250 mm×4.6 mm; Phenomenex, Castel
18 Maggiore, Italy). Elution was performed using solvent-A (acetonitrile/methanol, 75/25, v/v) and
19 solvent-B (methanol/ethylacetate, 68/32, v/v). The gradient used was as follows: 100 % solvent-A for
20 the first 15 min, followed by a 2.5-min linear gradient to 100 % solvent-B, which continued
21 isocratically until the end of the cycle (32 min). The column was allowed to re-equilibrate in 100 %
22 solvent-A for 10 min before the next injection. Carotenoids were detected by their absorbance at
23 445 nm at a flow rate of 1 mL min^{-1}. Known concentrations of pure standards (Sigma Aldrich
24 Chemical Co., USA) were injected into the HPLC system and used to quantify the pigment content.
25 Results are presented as mg g^{-1} dry weight. The sum of the amounts of three xanthophylls (VAZ =

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1 violaxanthin + antheraxanthin + zeaxanthin) was also used to characterize the xanthophyll cycle
2 (Bilger et al. 1995).

3 **Alpha-Tocopherol Content**

4 Alpha-tocopherol was analysed according to Melchert et al. (2002) with modification.
5 Lyophilized and powdered Pinot noir leaves (dry weight 60-70 mg) were used for the step-wise
6 extraction of tocopherols in 1.5 mL methanol (Merck, Kenilworth, NJ, USA). Extracts were
7 subsequently derivatized with 50 µL of pyridine (Sigma Aldrich Chemical Co., USA) and 70 µL of N,O-
8 bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS**,** Sigma Aldrich
9 Chemical Co., USA) for 30 min at 60 °C. After derivatization, samples were analysed using a Trace GC
10 Ultra gas chromatograph (Thermo Fisher Scientific, Waltham MA USA) interfaced with a TSQ
11 Quantum XLS triple Quadrupole (Thermo Scientific, Waltham MA USA), and using a Zebron DB5-MS
12 capillary column (Phenomenex, Torrance CA USA; length 30 m, internal diameter 0.25 mm, film
13 thickness 0.25 µm). The oven temperature was held at 220 °C for 1 min followed by a gradual
14 increase to 290 °C at the rate of 6 °C min^{-1} and kept isothermally for at least 2.5 minutes. The
15 samples were injected in splitless mode at a temperature 290 °C, the flow rate of the carrier gas
16 (helium) was 1 mL min^{-1}. The temperature of the MS Transfer line and an ion source was set at 250
17 °C. The m/z of 502 and 458 were used for the identification of TMS α-tocopherol and TMS
18 cholesterol, respectively. The cholesterol (Sigma Aldrich Chemical Co., USA) was used as an internal
19 standard. Data are presented as µg g^{-1} DW.

20 **Total Antioxidant and UV Absorbing Capacities**

21 To measure total antioxidant capacities, lyophilized grapevine leaves were extracted into
22 30/70 water/ethanol (v/v) as described in Csepregi et al. (2016). Trolox equivalent antioxidant
23 potential (TEAC) measurements were based on the reduction of 2,2'-azino-bis-3-ethyl-
24 benzothiazoline-6-sulfonic cation radical ($ABTS^{\bullet+}$) that was performed according to the method of Re
25 et al. (1999) as described earlier (Majer & Hideg 2012). $ABTS^{\bullet+}$ was prepared by mixing 0.1 mM ABTS,
26 0.0125 mM horse radish peroxidase and 1 mM H_2O_2 in a 50 mM phosphate buffer (pH 6.0). After 15

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1 min, 10 µL diluted leaf extract was added to 190 µL $ABTS^{\bullet+}$ solution and conversion of the cation
2 radical into colourless ABTS was followed as decrease in absorption at 651 nm using a plate reader.
3 TEAC of leaves were given as µM 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox)
4 equivalent mg^{-1} DW.

5 Ferric reducing antioxidant potential (FRAP) was quantified as the capacity of leaf extracts to
6 reduce ferric ions, which is measured as an absorbance change of ferrous 2,4,6-tripyridin-2-yl-1,3,5-
7 triazine (TPTZ) complex (Szőllősi & Szőllősi-Varga 2002). FRAP reagent was prepared by mixing 12.5
8 mL of acetate buffer (300 mM, pH 3.6), 1.25 mL TPTZ solution (10 mM TPTZ in 40 mM HCl) and 1.25
9 mL of $FeCl_3$ (20 mM in water solution). For each sample, 10 µL diluted leaf extract or test compound
10 was added to 190 µL freshly mixed FRAP reagent. Samples were incubated in microplate wells at
11 room temperature for 30 min before measuring OD at 620 nm in plate reader. FRAP data of
12 grapevine leaf extracts were expressed as µM ascorbate equivalents mg^{-1} DW.

13 UV absorbing capacities of leaf extracts were determined in acidified ethanol (1/29/70;
14 HCl/water/ethanol, v/v/v) solution in a UV-1800 spectrophotometer (Shimadzu, Tokyo Japan).
15 Absorption spectra were integrated between 280-315 nm and 315-400 nm to get UV-B and UV-A
16 absorbing capacities, respectively. Adding these two parameters gave total, 280-400 nm UV
17 absorbing capacities corresponding to the area under the whole UV absorption curve.

18 **Statistics**

19 A total of fifteen leaves were collected at each site in each year. Leaves were collected from
20 three different plants (i.e. 5 leaves each). For all laboratory analyses, the 5 leaves collected from the
21 same plant in the same year were pooled into one sample and thus biological variability was
22 represented by an average of data from 3 mixed samples from three plants. Pooling of the leaves
23 from one plant was necessary to ensure that all analytical measurements could be performed on the
24 same sample. Given the variation in size and shape of leaves from the same plant, for morphological
25 analysis 15 measurements were averaged to form one data point. Parameter sets (including data
26 from all sites) were compared pair wise by calculating Pearson's correlation coefficient (R).

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1 Significance of R calculated from the samples was determined by testing the null hypothesis of no
2 correlation present in the population against the alternative that there is correlation present. Two
3 data sets were regarded as strongly correlated when this test gave $P<0.01$ and correlated for
4 $0.01<P<0.05$. Selected parameter pairs showing strong correlation were also tested using linear
5 regression. A straight line was fitted to these data sets and the null hypothesis of the slope being
6 equal to zero in the population was tested against the alternative that the slope is different from
7 zero. Significant ($P<0.05$) linear correlations were also characterized by the coefficient of
8 determination R^2. Principal component and cluster analysis were done using PAST (Hammer et al.
9 2001). All other calculations were carried out using Excel Analysis ToolPack (Version 2007, Microsoft
10 Corporation, Redmond WA USA), and XLStat2006 (Addinsoft, New York NY USA). Graphs were
11 prepared using SigmaPlot (Systat Software Inc., San Jose CA USA).
12

13 **RESULTS AND DISCUSSION**

14 **Meteorology – UV radiation changes with latitude**

15 A range of climatic parameters were assessed along a 1500 km latitudinal gradient ranging
16 from 36.69 to 49.99 °N. Daily average temperatures ranged between 16.1 °C (Villenave d'Ornon-
17 2012) and 22.1 °C (Florence-2012). The temperature (degree day) integral varied between 1366.9
18 (Pécs-2013) and 727.8 (Geisenheim-2013). Along this gradient, neither daily averaged temperatures,
19 nor the temperature integral were significantly correlated with latitude, indicating that during the
20 period of leaf development southern locations were not warmer than northern ones. Indeed, the
21 number of days between bud break and veraison (ranging between 93 and 143 days in Vilajuïga and
22 La Rioja, respectively) was not significantly different for different locations along the latitudinal
23 gradient. In contrast, both avgGR (ranging between 12.78 MJ m^{-2} d^{-1} in Retz-2012 and 23.78 MJ m^{-2} d^{-1}
24 Jerez-2013) and avgUVR (ranging between 2.96 kJ m^{-2} d^{-1} in Geisenheim-2013 and 3.98 kJ m^{-2} d^{-1} in
25 Florence-2012) displayed strong negative correlations with latitude (Table 2, Fig. 2). The latter data
26 are in agreement with those generated by Häder et al. (2007) who found a negative linear latitudinal

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1 dependence of annual UV-B exposure using terrestrial dosimeters. Parameters avgGR and avgUVR
2 showed strong positive correlations. The sumUVR parameter was also negatively correlated with
3 latitude. However, sumGR was not significantly correlated with either sumUVR or avgUVR. Global
4 radiation is measured using pyranometers operating between 295-300 to 2800 nm, and correlations
5 between GR and UVR are not necessarily expected as only a few percent of incident global radiation
6 is in the UV part of the solar spectrum (Aphalo et al. 2012). UV wavelengths may be differently
7 absorbed, reflected and scattered than visible wavelengths due to latitudinal differences in, for
8 example, cloud cover. Furthermore, while the intensity of radiation is decreasing with increasing
9 latitude (Table 2), day length during the summer growing season is increasing with increasing
10 latitude (Jaakola & Hohtola 2010), and this may further contribute to the lack of correlation between
11 sumGR and sumUVR. Finally, the lack of correlation between sumGR and sumUVR (P=0.070) despite
12 the observed correlation between averages (avgGR and avgUVR, P=0.009) can be due to larger
13 fluctuations among doses (14.2% and 16.6% relative standard deviation for sumGR and sumUVR,
14 respectively) compared to averages (10.0% and 12.1% for avgGR and avgUVR, respectively).

15 **Leaf morphological characteristics are correlated with latitude and solar irradiation**

16 Data on measured leaf morphological characteristics showed significant positive correlations
17 between latitude and fresh weight, area and circumference of leaves (Table 3). Leaf sizes varied
18 between 98.8 and 244.2 cm^2 in, respectively Jerez and Geisenheim, with larger leaves being
19 associated with higher latitudes. Previously, leaf size had been positively associated with
20 precipitation, humidity, and soil fertility, but was found to decrease with increasing irradiance
21 (Givnish 1987). Here we found that the decrease in leaf area at lower latitudes was strongly
22 correlated with the increase in global solar radiation, consistent with published literature (Givnish
23 1987). The increase in leaf area at higher latitudes was also (but to a lesser extent) associated with a
24 decrease in UV-radiation. Exposure to UV-B radiation has been reported to result in decreases in leaf
25 area (Hectors et al. 2010). Thus, observed increases in leaf area along a latitudinal climatic gradient
26 are consistent with well-studied responses of plants to decreases in global solar radiation and UV-

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1 radiation. Increases in specific leaf area with increasing latitude have been noted by several authors
2 (Hulshof et al. 2013). For example, Tian et al. (2016) showed a substantial increase in Specific Leaf
3 Area with increasing latitude, along a 4200 km transect, and across 99 tree species. In this study no
4 latitudinal effects on the ratio between leaf fresh weight and leaf area were observed (P=0.082),
5 presumably reflecting the similar increases in each of these two parameters with increasing latitude.
6 Leaf fresh weights varied between 1.83 and 5.67 g in, respectively, Jerez and Geisenheim, the most
7 southern and northern location in our study. In all, leaf fresh weight increased by almost 60% across
8 the latitudinal gradient of decreasing global radiation (Figure 3). Our analysis also revealed that the
9 shape of leaves, and specifically the extent of indentation varied substantially across locations.
10 Previously, it was reported that leaves are more indented in colder climates (Peppe et al. 2011),
11 however, our single species study did not reveal a significant correlation between indentation and
12 latitude or any other climatic parameter. It is tempting to relate changes in leaf morphology to
13 observed changes in metabolites. Previously some studies have showed a negative correlation
14 between phenolic (flavonol) accumulation and aspects of leaf development (Klem et al. 2012). In this
15 study, we noted that total phenolic content decreased and leaf weight and area increased, with
16 increasing latitude (see below). Similarly, total phenolic concentration increased and leaf weight and
17 area decreased, with increasing sumUVR (see below). Yet, it remains to be shown whether there is
18 any mechanistic relationship between leaf morphology and plant biochemistry across a latitudinal
19 gradient.

20 **Associations between a latitudinal climatic gradient and plant metabolite levels**

21 In this study, we measured concentrations of specific flavonoids, and carotenoids, as well as
22 total UV-absorption of methanolic extracts, and total antioxidant activity. Global radiation
23 parameters (either sumGR or avgGR) were not significantly correlated with the metabolites
24 measured in our data set (Supporting Information Table S2). The daily average temperature was
25 positively associated with UV-B absorption and negatively with the total amount of xanthophyll cycle
26 pigments (VAZ) (Table 4). The most extensive number of positive correlations was, however, found

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1 for the various UV parameters, including sumUVR, which positively correlated with leaf UV
2 absorbing capacity, total antioxidant capacity measured as FRAP and the total phenolic content; but
3 negatively with total xanthophylls and carotenoid contents (Table 4).

4 ***Phenolic compounds***

5 Pinot noir leaves contained both phenolic acids and flavonoids at relatively high amounts.
6 The most abundant phenolic acid was caftaric acid which occurred at contents up to 1.6 mg g^{-1} DW
7 (Fig.4). Flavonoids were present as flavonols, the most abundant compounds being glycosylated
8 quercetins in concentrations ranging up to 4.7 mg g^{-1} DW. Quercetin-3-*O*-glucuronide comprised
9 approximately 70% of flavonol glycosides in the leaf, while quercetin-3-*O*-glucoside made up a
10 further 20% of flavonol glycosides followed by quercetin-3-*O*-rutinoside. Pinot noir leaves also
11 contained traces of the corresponding kaempferol glycosides. In this study, leaf phenolic compounds
12 were characterized as: (i) total phenolic content, (ii) total flavonoid content (iii) total phenolic acid
13 content and (iv) the concentration of the most abundant compound, quercetin-3-*O*-glucuronide.

14 Flavonoids were present in all samples, yet the abundance of particular compounds, as well
15 as the total flavonoid content varied substantially between sites (Fig. 4). All the above four
16 parameters reflecting phenolic compound metabolism were significantly and positively correlated
17 with sumUVR (Table 4). A study on *Juniperus* leaves collected from different locations between
18 59.97°N and 69.63°N in Finland showed strong correlations between total phenolics and latitude,
19 but correlations between latitude and specific compound classes varied (Martz et al. 2009). A similar
20 study on birch leaves collected from locations between 60°N and 70°N in Finland showed that the
21 content of quercetin glycosides increased with increasing latitude, while apigenin glycosides
22 decreased, and kaempferol glycosides and total flavonoid content were not affected (Stark et al.
23 2008). In the current study we observed the opposite, an increase in quercetin-3-*O*-glucuronide and
24 also in total flavonoid content with increasing sumUVR (which in turn was negatively correlated with
25 latitude). In the approach taken by Stark et al. (2008), differences in phenolic-profile are likely to
26 represent a complex mixture of environmental plasticity and genetic adaptation. In contrast, in the

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1 current study genetically closely related clones of a horticultural cultivar were being used. Thus, the
2 distinct responses of wild species and horticultural clones grown along a latitudinal climate gradient
3 may relate to the relative importance of environmental plasticity and genetic adaptation across
4 latitudes. Both Stark et al. (2008) and Martz et al. (2009) named temperature and radiation
5 (corresponding to sumGR in this study) as potential factors influencing the accumulation of
6 phenolics. Our study revealed a correlation between cumulative UV (sumUVR) and leaf phenolic
7 contents but showed no significant effect of temperature (Table 4) or sumGR (Supporting
8 Information Table S2) indicating a dominating UV effect on the phenolic biosynthesis.

9 Exposure to natural sunlight has already been shown to result in an increase in poly-
10 hydroxylated flavonoids (Agati et al. 2011, Majer et al. 2014). The same was found in studies where
11 enhanced UV-B intensities were applied (Jansen et al. 2008, Hectors et al. 2012, Neugart et al. 2012,
12 Jaakola & Hohtola 2010, Morales et al. 2010, Bidel et al. 2007). Such observations are consistent
13 with the positive correlation between sumUVR and quercetin-3-*O*-glucuronide identified in the
14 present study (Table 4). Discrepancies with the findings of Stark et al. (2008) and Martz et al. (2009)
15 with respect to the effect of global radiation can be explained by differences in locations and
16 latitude. This study included a more southern and approximately twice larger range (36.69°-49.99°N)
17 than the two studies carried out in the Boreal zone of Finland (60°-70°N, Stark et al. 2008, Martz et
18 al. 2009). Thus, in this study plants were exposed to a larger decrease in intensity of radiation with
19 increasing latitude (Table 2), as well as a larger increase in day length with increasing latitude during
20 the growing season (Jaakola & Hohtola 2010).

21 ***Carotenoids***

22 Carotenoid profiles of Pinot noir leaves from various locations are shown in Figure 5. The
23 most abundant carotenoids were found to be lutein and β-carotene, which made up on average 43%
24 and 40% of total carotenoids. Contents of lutein and β-carotene ranged between 32-66 and 19-49
25 mg g^{-1} DW, respectively. While lutein was negatively affected by both sumUVR and 10d-sumUVR, β-
26 carotene remained unaffected.

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1 Xanthophylls are known to have a specific role in protection of plants against photooxidative
2 stress (Demmig-Adams & Adams 2006). Accordingly, we hypothesised an increase in neoxanthin and
3 VAZ contents with increasing radiation intensities. However, total xanthophylls, neoxanthin and
4 violaxanthin were negatively correlated to sumUVR (Table 4). Although VAZ significantly decreased
5 with increasing latitude, no significant correlations with global radiation conditions (sumGR or
6 avgGR) were found (Supporting Information Table S2). However, correlation analysis revealed that
7 VAZ, a measure of photoprotective xanthophyll-cycle pool size (Demmig-Adams & Adams 2006), was
8 positively associated with 10d-sumUVR (Fig. 6), but negatively with DD (Table 4). Since the leaves
9 were collected during the noon hours of sunny days, de-epoxided forms of xanthophylls prevailed
10 (zeaxanthin was present at 3-14-times higher contents than violaxanthin). A similar temperature
11 effect was observed in needles of *Pinus strobus*; colder spring temperatures led to higher VAZ and
12 zeaxanthin accumulation than observed under warmer temperatures (Fréchette et al. 2015). Thus
13 not only radiation intensity, but also temperature is involved in the modulation of xanthophyll-cycle
14 pool size along the climatic gradient. Our data can be thus interpreted as that long term acclimation
15 of grapevine leaves to local solar conditions decreases the total amount of xanthophyll pigments
16 without compromising their photoprotection. This finding is consistent with a study by Klem et al.
17 (2015) on effects of UV-radiation on spring barley, and confirms that the UV component of sunlight
18 may stimulate defence against excess PAR.

19 ***UV-absorbing pigments and antioxidant capacity***

20 UV-absorbing and antioxidant capacities of leaf extracts were found to differ between sites.
21 Leaves collected in Florence in 2012 had the highest total UV absorbing pigment levels,
22 approximately 2.3-times higher than samples from Geisenheim (2013) which showed the lowest
23 values. Total antioxidant capacities were assessed as TEAC and FRAP and the samples with highest
24 capacities were from Villenave d'Ornon (2.76 μM Trolox equivalent mg^{-1} DW) and Pécs (2.71 μM
25 ascorbate equivalent mg^{-1} DW), respectively, both in 2013. Leaves with the lowest TEAC and FRAP
26 values were collected in Lednice in 2013, and were characterized by 1.11 μM Trolox equivalent and

1 0.94 μM ascorbate equivalent mg^{-1} DW, respectively. Although latitude and sumUVR were also
2 correlated (Table 2), the latter was the significant factor affecting the above capacities. It was the
3 total UV exposure of leaves (sumUVR) and not the average (avgUVR) during development which had
4 a significant positive effect on total phenolic contents, UV absorbing capacity and the total
5 antioxidant capacity assessed as FRAP (Table 4). Leaf UV absorbing capacities are known to increase
6 in response to UV (Jansen et al. 2008) and our results show that, among the studied climate factors,
7 sumUVR was the main driving force of this effect. Both UV-B and UV-A absorption of methanolic
8 extracts were positively correlated with sumUVR. This is most likely explained by the up-regulation
9 of polyphenol biosynthesis by both UV-B and UV-A (Morales et al. 2010) and the strong UV
10 absorbing properties of phenolic compounds (Hernández et al. 2009). We also identified a weak
11 (R=0.473, P=0.047) positive correlation between average temperature and leaf UV-B absorption
12 capacity. This may be explained by the stimulating effect of higher growth temperature on the
13 biosynthesis of UV-absorbing flavonoids, as observed in grapevine berry skins (Mori et al. 2007),
14 broccoli (Mølmann et al. 2015) and turnip greens (Francisco et al. 2009), although other studies have
15 reported increasing flavonoid glycoside formation with decreasing temperature (Neugart et al. 2013,
16 Neugart et al. 2014). In the present study, there was no significant correlation between average
17 temperature and UV-A or total UV absorption which implies that temperature is associated with a
18 subset of phenolic compounds that absorbs predominantly in the UV-B part of the spectrum,
19 although this was not confirmed by any correlation between temperature and phenolic contents
20 (Table 4). Correlations were also observed between the total antioxidant capacity parameters and
21 the UV absorbing capacities of leaf extracts (Table 5A), which indicates the dual (UV screening and
22 antioxidant) role of phenolic compounds in acclimation to environmental UV radiation (Hernández
23 et al. 2009, Agati & Tattini 2010).

24 **Alpha-tocopherol**

25 Leaf α-tocopherol contents were also measured, due to the significance of this lipophilic
26 antioxidant in stress responses (Munné-Bosch 2005), and especially in scavenging of lipid peroxy

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1 radicals (Buettner 1993, Eugeni Piller et al. 2014), and singlet oxygen (Fahrenholtz et al. 1974, Sattler
2 et al. 2003, Rastogi et al. 2014). The importance of α-tocopherol relates to its role in maintaining the
3 integrity of biological membranes under stress-conditions (Kruk et al. 2000), and the
4 interdependence between α-tocopherol and biosynthesis of terpenes (Munné-Bosch & Alegre
5 2002). Leaves in our study contained 1.83-4.35 mg α-tocopherol g^{-1} DW, the lowest and highest
6 contents were found in samples from Sant Feliu de Buixalleu and Villenave d'Ornon, respectively,
7 both collected in 2012. We found a positive correlation between leaf α-tocopherol contents and the
8 number of days from bud break to veraison (Table 4), showing that the longer leaves developed the
9 higher their α-tocopherol contents were. This is in accordance with the regulatory role of α-
10 tocopherol on the concentration of plant hormones, such as jasmonic acid, which control both the
11 growth and development of plants (Munné-Bosch 2005). Previously, induction of α-tocopherol was
12 demonstrated in UV-B exposed Arabidopsis leaves; contents of α-tocopherol were rapidly
13 upregulated following UV-exposure, and remained high for the duration of the experiment (Hectors
14 et al. 2014). In the current study a negative correlation was observed between α-tocopherol and
15 avgUVR while no correlation was found with sumUVR. It is possible that irreversible degradation of
16 α-tocopherol occurred in UV-exposed leaves (a process suggested by Szarka et al. 2012), thus
17 explaining the negative association with avgUVR, but not with sumUVR.

18 **Interdependence between metabolic responses**

19 Previous studies have started to reveal the complex interactions between various classes of
20 metabolites in responding to environmental conditions. For example, α-tocopherol was found to
21 rapidly increase in UV-acclimated Arabidopsis. Similarly, polyamines also accumulated rapidly, but
22 this was a transient accumulation, whereby the levels of various polyamines were again decreasing
23 at the time that phenolics were slowly accumulating (Hectors et al. 2014). The present study of
24 various classes of metabolites, as well as leaf morphology, creates an opportunity to analyse possible
25 connections between various metabolite groups in sun acclimated grapevine leaves. Correlations
26 were analysed pair-wise and results are shown in Tables 5A and 5B.

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1 ***Phenolic compounds, antioxidant capacities and UV-absorption***

2 The total phenolic content of grapevine leaves showed a very strong positive correlation
3 with the UV-A and UV-B absorption of leaf extracts ($P<10^{-6}$, Table 5A and Supporting Information
4 Table S3A). This is largely due to flavonols and especially the dominant quercetin-3-*O*-glucuronide
5 absorbing in both wavelength regions (Table 5A, Supporting Information Table S3A). This result is
6 consistent with the proposed contribution of phenolic compounds in shielding against UV radiation
7 (Bidel et al. 2007, Barnes et al. 2016), and the relatively higher absorption by phenolic acids in the
8 UV-B compared to the UV-A wavelength range (Zhang et al. 2013).

9 Leaf phenolic compounds also act as antioxidants (Larson 1988, Csepregi et al. 2016), which
10 is reflected in a positive correlation between total phenolic content and total antioxidant capacities
11 assessed as either TEAC or FRAP (Table 5A). Phenolic acid contents correlated with both TEAC and
12 FRAP, but flavonoid contents correlated only with FRAP. It has been documented that different
13 assays for antioxidant capacity respond differently to specific antioxidants. For example, quercetin-
14 3-*O*-glucuronide (corresponding to 70% of the total flavonoid content of our samples) displays a
15 stronger reaction with the chromophore of the FRAP assay compared to with TEAC (Csepregi et al.
16 2016), which explains the data reported here. Another antioxidant present in the grapevine leaves is
17 α-tocopherol. This potent antioxidant was present in all samples and its amount correlated positively
18 with the observed antioxidant capacities (Table 5A).

19 ***Phenolic compounds, α-tocopherol and carotenoids***

20 The total amount of phenolic acids was positively correlated with α-tocopherol content
21 (Table 5A). Biochemical studies have shown that caffeic acid is capable of recycling oxidized α-
22 tocopherol and thus extend its antioxidant capacity (Laranjinha et al. 1995, Kadoma et al. 2006). The
23 dominant phenolic acid in Pinot noir leaves is caftaric acid (Csepregi et al. 2016), consisting of a
24 caffeic acid and a tartaric acid moiety, and the observed correlation between phenolic acids and α-
25 tocopherol content may indicate a similar protective role in plants, too. Ascorbate has been known
26 to have a similar α-tocopherol regenerating function in leaves, but assuming an accessory role for

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1 more lipophilic phenolic compounds is feasible and supported by our data. Flavonoids with a
2 catechol structure, such as quercetin, have also been suggested to contribute to the role of α-
3 tocopherol in preventing lipid peroxidation in phospholipid bilayers (Terao et al. 1994). However,
4 neither total flavonoid nor quercetin-3-*O*-glucuronide contents were significantly correlated with α-
5 tocopherol contents in our study (Supporting Information Table S3A) indicating that in Pinot noir
6 leaves the potential α-tocopherol regenerating protective molecule is caftaric acid (also featuring a
7 cathechol group) rather than flavonoids.

8 The amounts of total leaf phenolics, the two sub-groups phenolic acids and flavonoids, and
9 the main flavonoid quercetin-3-*O*-glucuronide were all positively correlated with each other (Table
10 5A), and this may be due to their partly shared biosynthetic pathway (Zoratti et al. 2014). Similarly,
11 positive correlations exist between total carotenoid content and the amount of the two dominant
12 carotenoids, lutein and β-carotene (Table 5B). Leaf β-carotene can contribute to ca. 40% of total leaf
13 carotenoids (Fig. 5), but was not affected by UV, or by any of the meteorological parameters
14 monitored in this study. However, lutein, an α-carotene-derived major carotenoid, significantly
15 decreased with increasing sumUVR or 10d-sumUVR (Table 4). Lutein is present in the photosynthetic
16 antenna, and a study on lutein-deficient Arabidopsis mutants showed its role in photo-protecting
17 reaction centres via energy dissipation, similarly to zeaxanthin (Dall'Osto et al. 2006). The
18 contribution of lutein to the rapid photoprotection response, rather than to long term acclimation,
19 may explain why lutein and β-carotene display different correlations with sumUVR and latitude.
20 SumUVR had also an opposite effect on total phenolic compounds and carotenoids, i.e. while all the
21 parameters characterizing phenolic contents were positively correlated with sumUVR, total
22 xanthophylls and total carotenoid contents were both negatively correlated with the same
23 meteorological parameter (Table 4). The negative correlation between xanthophyll and phenolic
24 content (Fig. 7) might be interpreted as a preferential enhancement of defence against UV at the
25 expense of defence against high PAR. Such a model was recently suggested to explain the increase in
26 flavonoids and parallel decrease in carotenoids in two *Oleaceae* species exposed to natural UV (Guidi

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1 et al. 2016). The authors showed that UV caused flavonoid accumulation, while simultaneously
2 decreasing the capacity of thermal dissipation of excess energy. Yet, a thorough investigation of a
3 range of mutants and transgenic lines showed that perturbations in carotenoid biosynthesis do not
4 generally alter phenolic or flavonoid content significantly even when devoid of carotenoids.
5 Similarly, the down-regulation of ferulate 5-hydroxylase had no effect on carotenoid content (Long
6 et al. 2006). Indeed, Klem et al. (2015) showed that phenolics and xanthophylls (VAZ) are
7 simultaneously induced in barley exposed to UV-B and high PAR. Therefore, it is unlikely that the
8 observed negative correlation between carotenoids and phenolics in grapevine leaves reflects an
9 incompatibility between accumulation responses of the two types of metabolites. Rather, it may be
10 argued that carotenoids and phenolics are differentially regulated by the environment. Consistently,
11 10d-sumUVR was not correlated with total UV-absorbance, nor with total phenolics, flavonoids or
12 quercetin-3-O-glucuronide. In contrast, 10d-sumUVR had a clear positive effect on zeaxanthin and
13 VAZ (Table 4, Supporting Information Table S2). Thus, while carotenoids may constitute a rapid
14 environmental response system for the plant, and hence mirror weather conditions, total phenolics
15 may respond slower to alterations in climate and their concentrations may mirror a much longer
16 period.

17

18 **CONCLUSIONS**

19 Principal component analysis (Fig. 8) revealed that metabolite content was mostly correlated
20 with sumUVR rather than sumGR. Yet, due to the limited variation explanation (about 60%) of
21 metabolite responses by PCA it must be assumed that other environmental factors also contribute to
22 plant metabolite content. More detailed analysis of measured responses indicates two kinds of
23 acclimation strategies in response to sumUVR. One is an increase in UV absorbing and antioxidant
24 phenolic compounds and the second is a decrease in total carotenoids, lutein and total xanthophylls
25 but not β-carotene. These responses suggest a re-allocation of metabolic resources from
26 carotenoids to phenolics as a consequence of environmental UV variation. Despite of a negative

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1 effect of sumUVR on xanthophyll concentrations, the increase in VAZ indicates active, dynamic
2 protection for the photosynthetic apparatus lessening ROS formation. In addition, increased
3 amounts of flavonoids (quercetin-glycosides) and maintained β-carotene and α-tocopherol pools
4 provide antioxidant protection against ROS in sun acclimated Pinot noir leaves. However, rather than
5 a continuum of plant acclimation responses along a latitudinal gradient, principal component (Fig. 8)
6 and cluster (Supporting Information Fig. S1) analyses identified three main functional groups which
7 are determined by different latitude ranges: group-1 from 36 to 42°N (various locations in Spain) is
8 marked mostly by the xanthophyll cycle (VAZ and especially zeaxanthin) and radiation parameters;
9 group-2 from 43 to46°N (France, Italy, Southern Slovenia and Hungary) is characterized mainly by
10 antioxidant compounds (α-tocopherol and various phenolic compounds) and their corresponding
11 scavenging (TEAC, FRAP) and UV shielding capacities; and group-3 from 46 to 50°N (Austria, Czech
12 Republic and Germany) is described primarily by xanthophylls and carotenes. Locations which were
13 sampled both in 2012 and 2013 were always found to locate in the same cluster, indicating that
14 short term weather variations that may happen from one year to the next, did not substantially
15 affect the grouping in three clusters. Thus, across a 1500 km latitudinal gradient, plants show distinct
16 and repeatable patterns of metabolic composition in response to environmental, and especially UV-
17 exposure, conditions. By using genetically similar plants, this study has unmasked the physiological
18 component of plant responses to latitudinal gradients. It is concluded that plant physiological
19 plasticity, and especially metabolic plasticity, complement genetic adaptations along latitudinal
20 gradients.
21
22 **ABBREVIATIONS**
23 ABTS, 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic; avgGR, sumGR divided by the number of days
24 between bud break and leaf collection; avgUVR, sumUVR divided by the number of days between
25 bud break and leaf collection; BSTFA, N,O-Bis(trimethylsilyl)trifluoroacetamide; DD, Degree day
26 integral calculated over the period between bud break and veraison; DSSF, Downward surface

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1 shortwave flux; FRAP, ferric reducing antioxidant potential; PCA, principal component analysis; ROS,
2 reactive oxygen species; TEAC, Trolox equivalent antioxidant capacity; Trolox, 6-hydroxy-2,5,7,8-
3 tetramethylchroman-2-carboxylic acid; TMSC, trimethylchlorosilane; TPTZ, 2,4,6-tripyridin-2-yl-1,3,5-
4 triazine; sumGR, cumulated DSSF global radiation data calculated for the period from bud break to
5 leaf collection ; sumUVR, cumulated TEMIS-derived erythemal UV radiation data calculated for the
6 period from bud break to leaf collection; 10d-sumUVR, cumulated TEMIS-derived erythemal UV
7 radiation data calculated for the last 10 days before leaf collection; VAZ, the sum of the amounts of
8 three xanthophylls: violaxanthin, antheraxanthin, zeaxanthin.
9

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TABLES

Table 1
Pinot noir leaf sampling sites and collection years

*Map**	*Location*	*Latitude* (°N)	*Year(s)*
1	Jerez, Spain	36.69	2013
2	Vilajuïga, Spain	42.24	2012
3	La Rioja, Spain	42.29	2012, 2013
4	Sant Feliu de Buixalleu, Spain	42.47	2012, 2013
5	Villenave d'Ornon, France	43.36	2012, 2013
6	Florence, Italy	43.77	2012, 2013
7	Potoce, Slovenia	45.88	2013
8	Bilje, Slovenia	45.89	2013
9	Pécs, Hungary	46.07	2012, 2013
10	Retz, Austria	48.76	2012, 2013
11	Lednice, Czech Republic	48.80	2013
12	Geisenheim, Germany	49.99	2013

*Numbers refer to map in Fig.1.

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Table 2

Correlations between meteorological conditions at Pinot noir leaf sampling sites.

	Latitude	*sumGR* (MJ m^{-2})	*sumUVR* (kJ m^{-2})	*avgGR* (MJ m^{-2}d^{-1})	*avgUVR* (kJ m^{-2}d^{-1})	*DD* (∑ °C)	*Days*	*avgT* (°C)
Latitude (°N)		-0.4449 (0.0643)	**-0.5724 (0.0130)**	**-0.5881 (0.0102)**	**-0.6495 (0.0035)**	0.4154 (0.0864)	0.0435 (0.8640)	0.2617 (0.2942)
sumGR (MJ m^{-2})			0.4358 (0.0706)	**0.5960 (0.0090)**	0.0985 (0.6973)	0.2792 (0.2617)	**0.5960 (0.0090)**	0.0207 (0.9350)
sumUVR (kJ m^{-2})	– –			0.2296 (0.3593)	**0.5226 (0.0226)**	0.0503 (0.8462)	0.3102 (0.2103)	0.0258 (0.9188)
avgGR (MJ m^{-2}d^{-1})	– –	+ +			**0.5963 (0.0089)**	0.0807 (0.7503)	-0.2867 (0.2487)	0.2902 (0.2427)
avgUVR (kJ m^{-2}d^{-1})	– –		+	+ +		-0.1794 (0.4762)	**-0.4753 (0.0461)**	0.2101 (0.4025)
DD (∑ °C)							0.2417 (0.3340)	**0.8064 (5.3 10^{-5})**
Days (from bud break to veraison)		+ +			–			-0.3212 (0.1937)
avgT (°C)						+ +		

Above diagonal: Pearson's correlation coefficients and corresponding *P* values in parenthesis, n = 18. Significant correlations are highlighted in bold type.
Below diagonal: Statistically significant correlation marked as: $P < 0.01$ positive (+ +), $0.01 < P < 0.05$ positive (+), $P < 0.01$ negative (– –), or $0.01 < P < 0.05$ negative (–).
Meteorological parameters are: sumGR, cumulated DSSF global radiation data calculated for the period from bud break to leaf collection; sumUVR, cumulated TEMIS-derived erythemal UV radiation data calculated for the period from bud break to leaf collection; avgGR, sumGR divided by the number of days between bud break and leaf collection; avgUVR, sumUVR divided by the number of days between bud break and leaf collection; DD, degree day integral calculated over the period between bud break-veraison; Days, number of days between bud break and veraison; avgT, daily average temperature.

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Table 3

Correlations between meteorological parameters at sampling sites and morphological characteristics of Pinot noir leaves

	Fresh weight	Area	Circumference	Average lobe to indentation ratio
Latitude (°N)	+	+	+	
sumUVR (kJ m^{-2})	–	–		
avgUVR (kJ m^{-2} d^{-1})	–	–		
10d-sumUVR (kJ m^{-2})				
sumGR (MJ m^{-2})	– –	– –		
avgGR (MJ $m^{-2}d^{-1}$)	–	–		
DD (∑ °C)				
Days (from bud break to veraison)				
avgT (°C)				

Marked correlations are: $P < 0.01$ positive (+ +), $0.01 < P < 0.05$ positive (+), $P < 0.01$ negative (– –), or $0.01 < P < 0.05$ negative (–).

See Supporting Information Table S1 for Pearson's correlation coefficients (*R*) and corresponding *P* values.

Meteorological parameters are: sumUVR, cumulated TEMIS-derived erythemal UV radiation data calculated for the period from bud break to leaf collection; avgUVR, sumUVR divided by the number of days between bud break and leaf collection; 10d-sumUVR, cumulated TEMIS-derived erythemal UV radiation data calculated for the last 10 days before leaf collection; sumGR, cumulated DSSF global radiation data calculated for the period from bud break to leaf collection; avgGR, sumGR divided by the number of days between bud break and leaf collection; DD, degree day integral calculated over the period between bud break-veraison; avgT, daily average temperature.

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Table 4

Correlations between meteorological parameters at sampling sites and metabolic contents of Pinot noir leaves

	Latitude	*sumUVR*	*avgUVR*	*10d-sumUVR*	*DD*	*avgT*	*Days*
UV-B absorption		+				+	
UV-A absorption		+					
Total UV absorption		+					
Total antioxidant capacity (TEAC)							
Total antioxidant capacity (FRAP)		+					
α-tocopherol			−				+
Total phenolics	−	+					
Total phenolic acids		+		+			
Total flavonoids		+					
Quercetin-3-O-glucuronide		+					
Zeaxanthin	− −		+	+ +	− −		
Neoxanthin		−					
Violaxanthin	+	−		− −			
Antheraxanthin	− −						
Total xanthophylls		−					
VAZ	− −			+ +	− −	−	
β-carotene							
Lutein	+	− −		−			
Total carotenoids		−					
Total chlorophylls							

Marked correlations are: $P < 0.01$ positive (+ +), $0.01 < P < 0.05$ positive (+), $P < 0.01$ negative (− −), or $0.01 < P < 0.05$ negative (−).

See Supporting Information Table S2 for Pearson's correlation coefficients and corresponding P values, n = 18.

Meteorological parameters are: sumUVR, cumulated TEMIS-derived erythemal UV radiation data calculated for the period from bud break to leaf collection; avgUVR, sumUVR divided by the number of days between bud break and leaf collection; 10d-sumUVR, cumulated TEMIS-derived erythemal UV radiation data calculated for the last 10 days before leaf collection; DD, degree day integral calculated over the period between bud break-veraison; Days, number of days between bud break and veraison; avgT, daily average temperature.

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Table 5A

Correlations between metabolic characteristics of Pinot noir leaves

	UVBabs	*UVAabs*	*UVabs*	*TEAC*	*FRAP*	*α-Toc.*	*tot Phen*	*tot PhAc*	*tot Flav*	*Q-3-Glu*
UV-B absorption (UVBabs)		+ +	+ +	+	+ +		+ +	+	+ +	+ +
UV-A absorption (UVAabs)	+ +		+ +		+ +		+ +		+ +	+ +
Total UV absorption (UVabs)	+ +	+ +			+ +		+ +		+ +	+ +
Total antioxidant capacity (TEAC)	+				+ +	+	+	+ +		
Total antioxidant capacity (FRAP)	+ +	+ +	+ +	+ +		+	+ +	+ +	+ +	+ +
α-tocopherol (α-Toc)				+	+			+		
Total phenolics (tot Phen)	+ +	+ +	+ +	+	+ +			+ +	+ +	+ +
Total phenolic acids (tot PhAc)	+			+ +	+ +	+	+ +		+	+
Total flavonoids (tot Flav)	+ +	+ +	+ +		+ +		+ +	+		+ +
Quercetin-3-O-glucuronide (Q-3-Glu)	+ +	+ +	+ +		+ +		+	+	+ +	
Zeaxanthin (ZeaXa)										
Neoxanthin (NeoXa)	− −	− −	− −				− −	−	− −	− −
Violaxanthin (ViolXa)	−	−	−		−		− −	− −	−	−
Antheraxanthin (AntXa)										
Total xanthophylls (tot Xa)	− −	− −	− −		−		− −	−	− −	− −
VAZ										
β-carotene (β-Car)										
Lutein (Lut)	− −	−	− −		−		− −	− −	− −	−
Total carotenoids (tot Car)	− −	−	−				−		−	−
Total chlorophylls (tot Chl)	−	−	−		-		−	−	−	

Marked correlations are: $P < 0.01$ positive (+ +), $0.01 < P < 0.05$ positive (+), $P < 0.01$ negative (− −), or $0.01 < P < 0.05$ negative (−). For easier comparisons correlations are marked both above and below the diagonal (i.e. the cells in gray).

See Supporting Information S3A for values of Pearson's correlation coefficients and corresponding *P* values, n= 18.

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Table 5B

Correlations between metabolic characteristics of Pinot noir leaves

	ZeaXa	*NeoXa*	*ViolXa*	*AntXa*	*tot Xa*	*VAZ*	*β-Car*	*Lut*	*tot Car*
UV-B absorption (UVBabs)		– –	–		– –			– –	– –
UV-A absorption (UVAabs)		– –	–		– –			–	–
Total UV absorption (UVabs)		– –	–		– –			– –	–
Total antioxidant capacity (TEAC)									
Total antioxidant capacity (FRAP)			–		–			–	
α-tocopherol (α-Toc)									
Total phenolics (tot Phen)		– –	– –		– –			– –	-–
Total phenolic acids (tot PhAc)		–	– –		–			– –	
Total flavonoids (tot Flav)		– –	–		– –			– –	–
Quercetin-3-O-glucuronide (Q-3-Glu)		– –	–		– –				
Zeaxanthin (ZeaXa)						+ +			
Neoxanthin (NeoXa)			+ +		+ +		+	+ +	+ +
Violaxanthin (ViolXa)		+ +			+ +			+ +	+
Antheraxanthin (AntXa)									
Total xanthophylls (tot Xa)		+ +	+ +				+ +	+ +	+ +
VAZ	+ +								
β-carotene (β-Car)		+ +			+ +			+	+ +
Lutein (Lut)		+ +	+ +		+ +		+		+ +
Total carotenoids (tot Car)		+ +	+ +		+ +		+ +	+ +	
Total chlorophylls (tot Chl)		+ +	+ +		+ +		+ +	+ +	+ +

Marked correlations are: $P < 0.01$ positive (+ +), $0.01 < P < 0.05$ positive (+), $P < 0.01$ negative (– –), or $0.01 < P < 0.05$ negative (–). For easier comparisons correlations are marked both above and below the diagonal (i.e. the cells in gray).

See Supporting Information Table S3B for values of Pearson's correlation coefficients and corresponding P values, n = 18.

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1 **FIGURE LEGENDS**

2

3 **Figure 1**

4 Pinot noir leaf sampling sites. Numbers identify locations as listed in Table 1.

5

6 **Figure 2**

7 A strong linear correlation between latitude and average daily erythemal UV dose (avgUVR)

8 calculated for the period from bud break to leaf collection (see Materials and Methods for details).

9 The solid line indicates a linear regression between the data sets (R^2 = 0.422, P = 0.0035).

10

11 **Figure 3**

12 A strong linear correlation between leaf fresh weight and cumulated global radiation (sumGR)

13 calculated for the time between bud break and veraison (when leaves were collected) as downward

14 surface shortwave flux (DSSF). Data points and error bars represent averages and standard

15 deviations, respectively (n=5). The solid line indicates a linear regression between the data sets (R^2 =

16 0.6675, P = 0.0021).

17

18 **Figure 4**

19 Total phenolic contents of Pinot noir leaves. Numbers identify sampling sites according to Table 1

20 and sampling years. Q-3-Glu, quercetin-3-O-glucuronide; Q-3-Glc, quercetin-3-O-glucoside; Q-3-Rut,

21 quercetin-3-O-rutinoside; K-Gly, various glycosylated kaempferols including kaempferol-3-O-

22 glucuronide, kaempferol-7-O- glucoside and kaempferol-3-O- glucoside.

23

24 **Figure 5**

25 Carotenoid contents of Pinot noir leaves. Numbers identify sampling sites according to Table 1 and

26 sampling years.

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1

2 **Figure 6**

3 A strong linear correlation between total content of xanthophyll cycle pigments (VAZ) and UV

4 radiation during the last 10 d before sampling (10d-sumUVR). The solid line indicates a linear

5 regression between the data sets (R^2 = 0.463, P = 0.0019).

6

7 **Figure 7**

8 A strong linear correlation between total phenolic and total xanthophyll contents. The solid line

9 indicates a linear regression between the data sets (R^2 = 0.468, P = 0.0017).

10

11 **Figure 8**

12 Principal component analysis (PCA) of correlations.

13 (A) Original data points. The first number identifies the geographical location according to Table 1,

14 the second number (-2012 or -2013) is the year of sample collection. Circles correspond to sample

15 groups based on cluster analysis (Supporting Information Fig.S1.). Three groups were identified, and

16 these are indicated in the figure as -1, -2 and -3.

17 (B) Projections of the original variables. Solid lines show metabolic parameters (red, carotenoids; ligh

18 blue, phenolic compounds; black, α-tocopherol), antioxidant and UV-absorbing capacities (green).

19 Dashed lines correspond to latitude (black) and meteorological parameters (blue, UV radiation

20 parameters; orange, global radiation parameters; grey, other parameters).

21 α-Toc, α-tocopherol; β-Car, β-carotene; AntXa, antheraxanthin; avgGR, average daily global

22 radiation; avgT, average daily temperature; avgUVR, average daily UV radiation; days, number of

23 days between bud break and veraison; DD, degree day integral calculated over the period between

24 bud break-veraison; FRAP, total antioxidant capacity measured as ferric reducing potential; Lut,

25 lutein; NeoXa, neoxanthin; Q-3-Glu, quercetin-3-*O*-glucuronide; sumGR, cumulative global radiation

26 from bud break to veraison; sumUVR, cumulative UV radiation from bud break to veraison; 10d-

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1 sumUVR, cumulative UV radiation calculated for the last 10 days before veraison; TEAC, total
2 antioxidant capacity measured as Trolox equivalent antioxidant capacity; tot Car, total carotenoid
3 content; tot Chl, total chlorophyll content; tot Flav, total flavonoid content; tot PhAc, total phenolic
4 acid content; tot Phen, total phenolics content; tot Xa, total xanthophyll content; UVAabs, UV-B
5 absorbing pigments; UVBabs, UV-B absorbing pigments; VAZ, zeaxanthin+
6 antheraxanthin+violaxanthin; ViolXa, violaxanthin; ZeaX, zeaxanthin.

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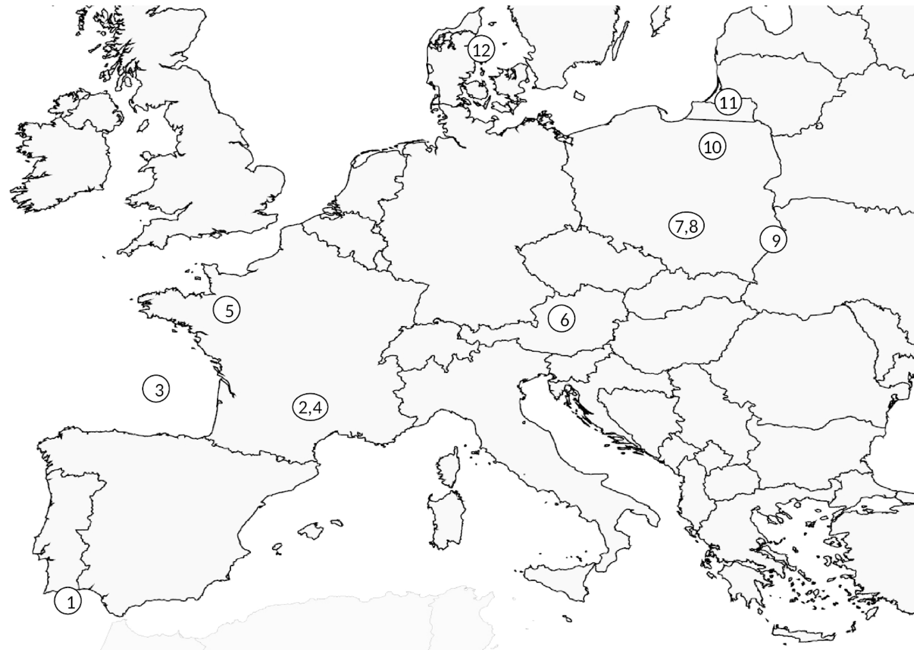

Figure 1

225x145mm (300 x 300 DPI)

ew Only

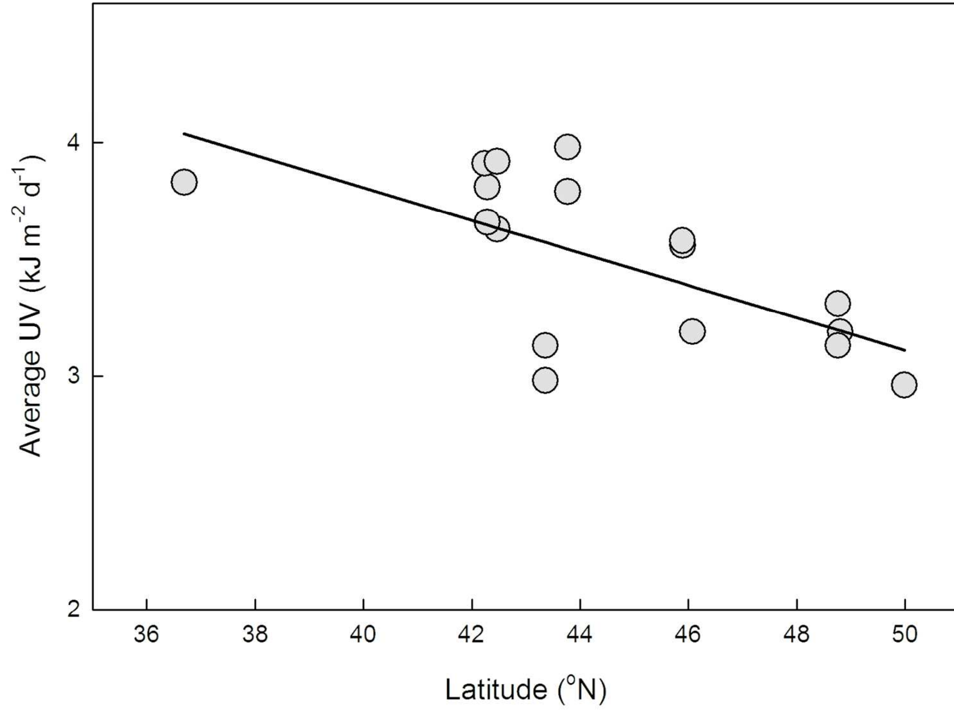

Figure 2

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Only

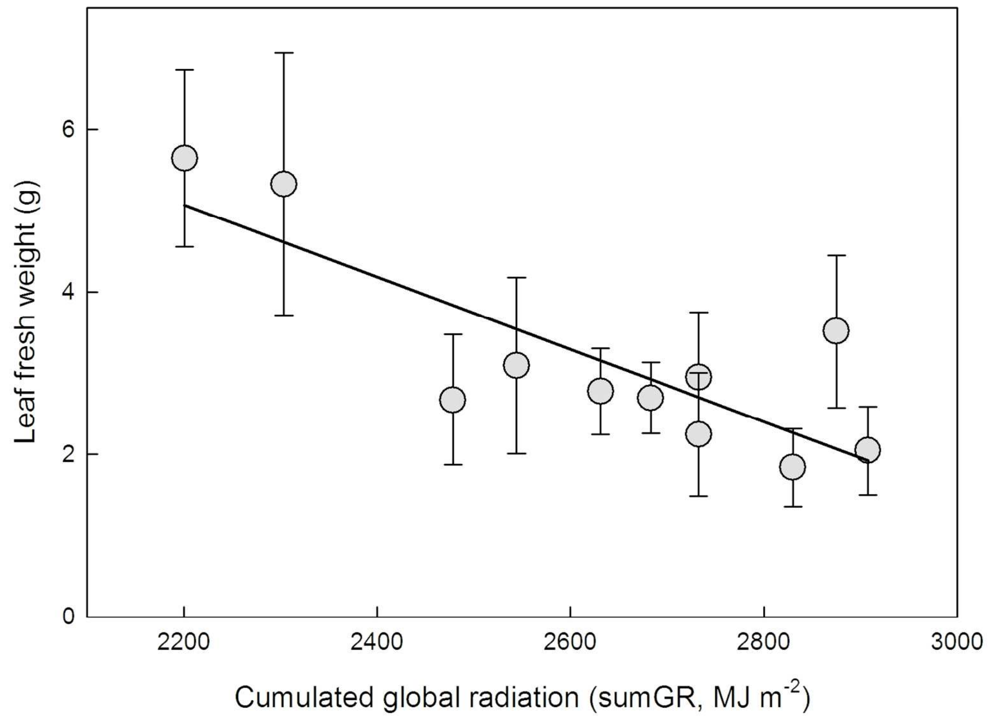

Figure 3

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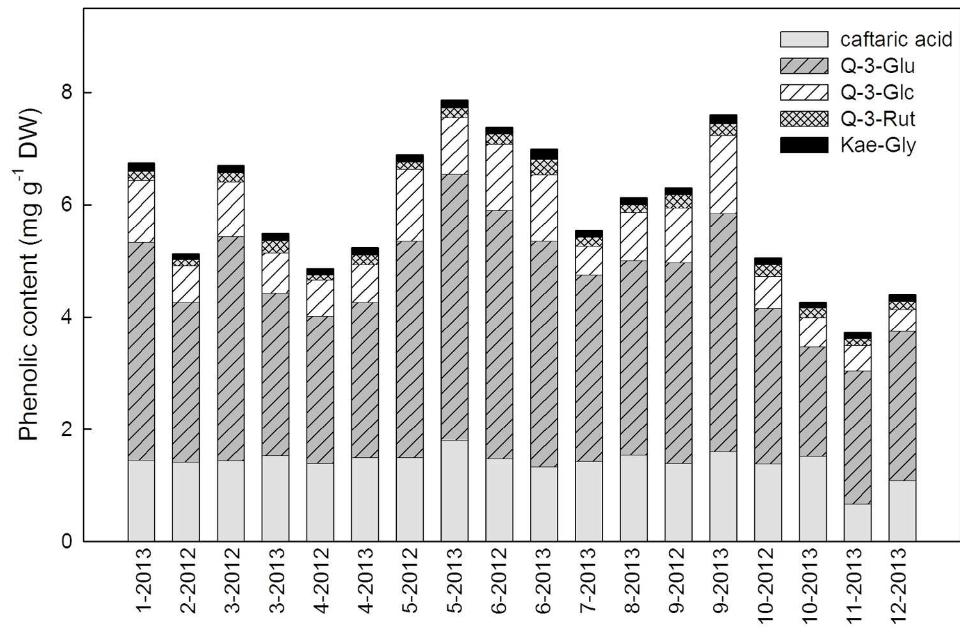

Figure 4

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w Only

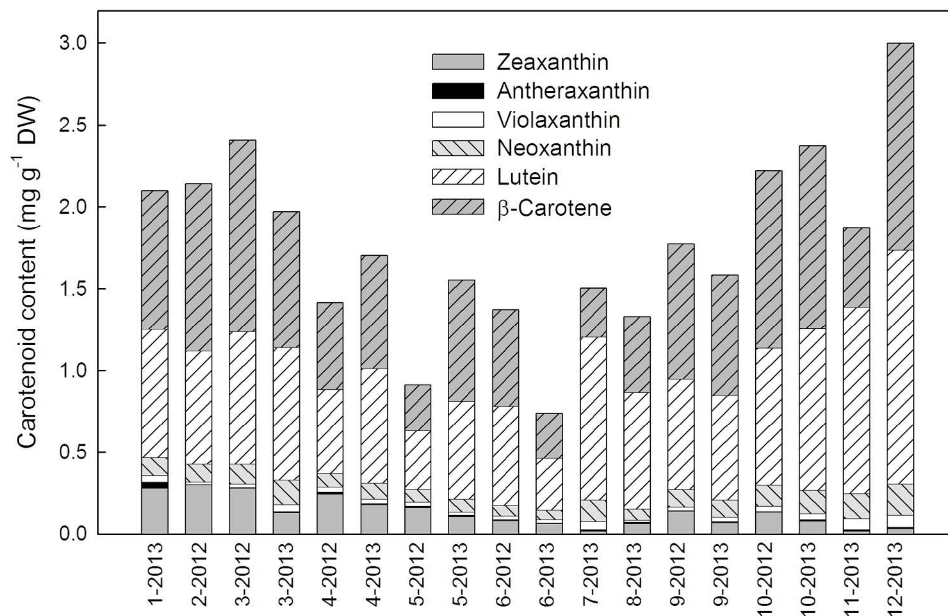

Figure 5

338x234mm (150 x 150 DPI)

w Only

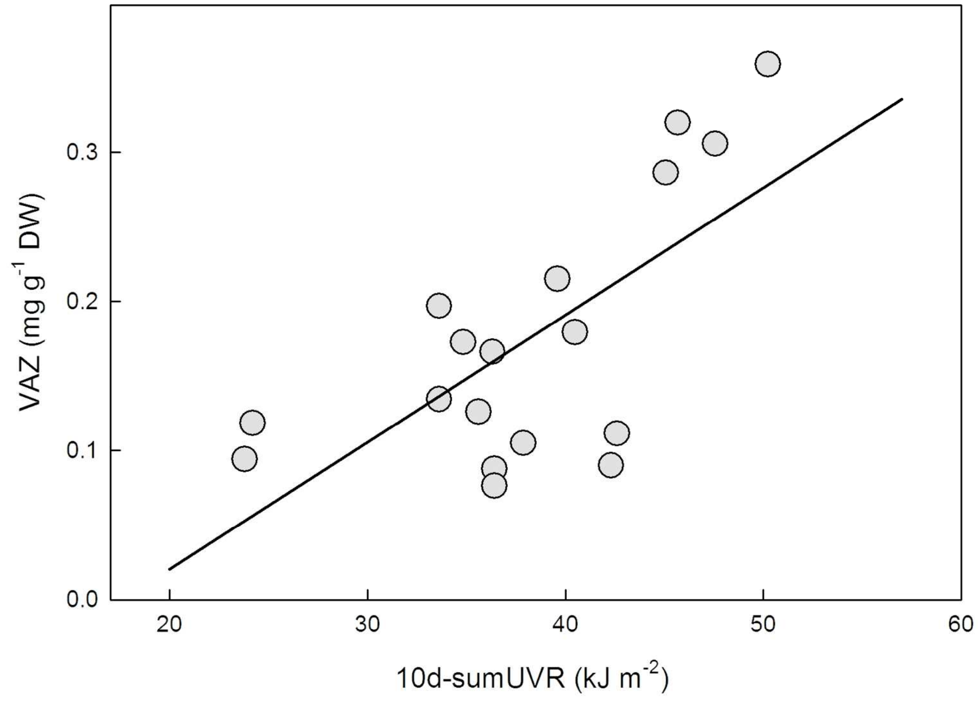

Figure 6

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Only

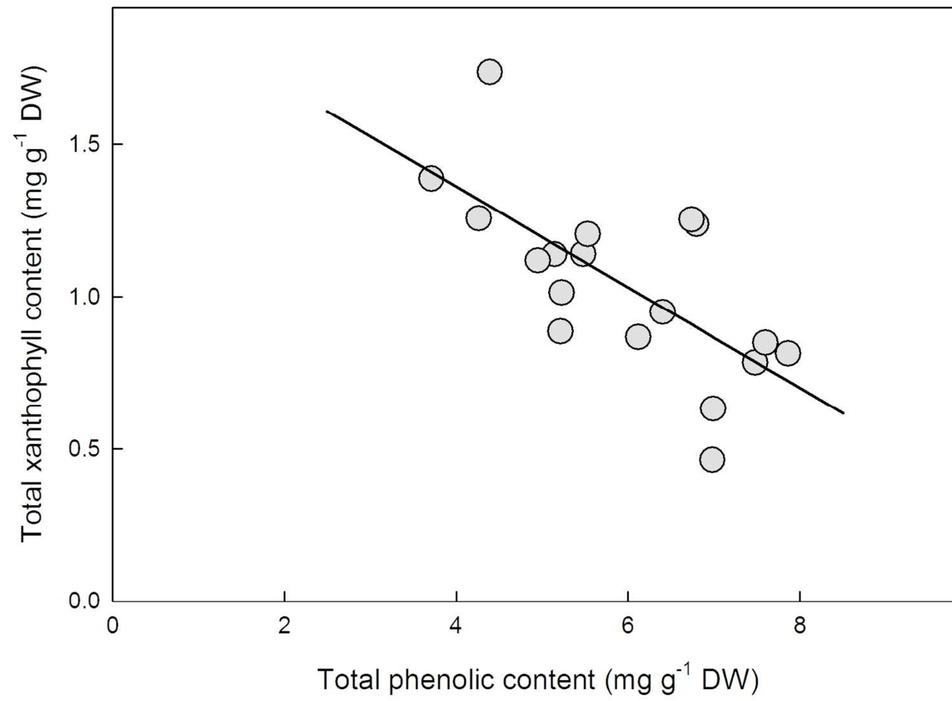

Figure 7

298x233mm (150 x 150 DPI)

Only

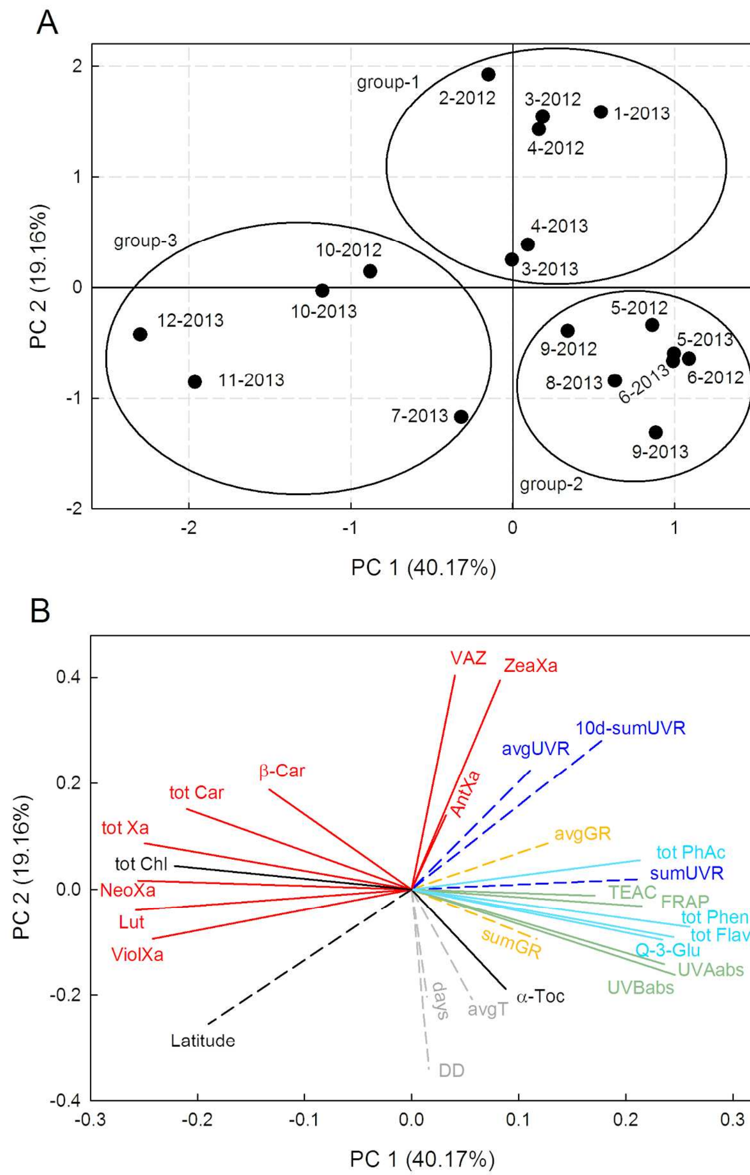

Figure 8

300x458mm (150 x 150 DPI)

SUPPORTING INFORMATION (on-line version only)

Table S1

Correlations between meteorological parameters at sampling sites and morphological characteristics of Pinot noir leaves

Pearson's correlation coefficients *R* and corresponding *P* values (in parenthesis, under *R* values), n=11. *P* < 0.05 correlations are highlighted in bold type.

	Fresh weight	Area	Circumference	Average lobe to indentation ratio
Latitude (°N)	**0.6888 (0.0191)**	**0.6686 (0.0245)**	**0.6232 (0.0405)**	0.2665 (0.4828)
sumUVR (kJ m^{-2})	**-0.7266 (0.0113)**	**-0.6893 (0.0189)**	-0.5988 (0.0516)	-0.1925 (0.5706)
avgUVR (kJ m^{-2} d^{-1})	**-0.6935 (0.0180)**	**-0.6456 (0.0319)**	-0.5892 (0.0565)	-0.1994 (0.5667)
10d-sumUVR (kJ m^{-2})	-0.5800 (0.0614)	-0.4942 (0.1223)	-0.3800 (0.2490)	-0.4609 (0.1536)
sumGR (MJ m^{-2})	**-0.8170 (0.0021)**	**-0.8133 (0.0023)**	**-0.7104 (0.0143)**	-0.3295 (0.32224)
avgGR (MJ $m^{-2}d^{-1}$)	**-0.7195 (0.0126)**	**-0.6439 (0.0325)**	-0.5413 (0.0855)	-0.5912 (0.0554)
DD (∑ °C)	-0.2344 (0.4879)	-0.3027 (0.3655)	-0.2928 (0.3822)	0.1926 (0.5703)
Days (from bud break to veraison)	-0.1914 (0.5728)	-0.1399 (0.6814)	-0.0179 (0.9582)	-0.4660 (0.1485)
avgT (°C)	-0.2565 (0.4469)	-0.1807 (0.5960)	-0.0538 (0.8752)	-0.5776 (0.0627)

Meteorological parameters are: sumUVR, cumulated TEMIS-derived erythemal UV radiation data calculated for the period from bud break to leaf collection; avgUVR, sumUVR divided by the number of days between bud break and leaf collection; 10d-sumUVR, cumulated TEMIS-derived erythemal UV radiation data calculated for the last 10 days before leaf collection; sumGR, cumulated DSSF global radiation data calculated for the period from bud break to leaf collection; avgGR, sumGR divided by the number of days between bud break and leaf collection; DD, degree day integral calculated over the period between bud break-veraison; Days, number of days between bud break and veraison; avgT, daily average temperature.

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Table S2

Correlations between meteorological parameters at sampling sites and metabolic characteristics of Pinot noir leaves.

Pearson's correlation coefficients *R* and corresponding *P* values (in parenthesis, under *R* values), n=18. *P* < 0.05 correlations are highlighted in bold type.

	Latitude	*sumUVR*	*avgUVR*	*10d-sumUVR*	*DD*	*avgT*	*Days*	*sumGR*	*avgGR*
UV-B absorption	-0.3120 (0.2075)	**0.5667 (0.0141)**	0.1518 (0.5476)	0.3293 (0.1508)	0.3293 (0.0672)	**0.4726 (0.0476)**	0.1389 (0.5823)	0.3983 (0.1017)	0.3669 (0.1342)
UV-A absorption	-0.3320 (0.1783)	**0.50637 (0.0320)**	0.1320 (0.6015)	0.3341 (0.1647)	0.3341 (0.1225)	0.4227 (0.0806)	0.1032 (0.6837)	0.3857 (0.1139)	0.3880 (0.1117)
Total UV (A+B) absorption	-0.3271 (0.1851)	**0.52677 (0.0247)**	0.1386 (0.5834)	0.3339 (0.1587)	0.3339 (0.1017)	0.4396 (0.0679)	0.1145 (0.6509)	0.3910 (0.1086)	0.3830 (0.1167)
Total antioxidant capacity (TEAC)	-0.4198 (0.0828)	0.42447 (0.0792)	0.0140 (0.9558)	0.2809 (0.1735)	0.2809 (0.5714)	-0.0155 (0.9513)	0.3533 (0.1504)	0.3678 (0.1332)	0.0914 (0.7183)
Total antioxidant capacity (FRAP)	-0.4382 (0.0688)	**0.51357 (0.0293)**	-0.0210 (0.9339)	0.3228 (0.1500)	0.3228 (0.6533)	-0.0064 (0.9800)	0.3302 (0.1808)	0.3434 (0.1630)	0.1033 (0.6835)
α-tocopherol content	0.0686 (0.7867)	0.09897 (0.6961)	**-0.5172 (0.0279)**	-0.1808 (0.6953)	-0.1808 (0.4225)	-0.0311 (0.9025)	**0.4978 (0.0355)**	0.3129 (0.2062)	-0.1232 (0.6262)
Total phenolics content	**-0.4793 (0.0441)**	**0.54157 (0.0203)**	0.1443 (0.5676)	0.4072 (0.0593)	0.4072 (0.4818)	0.2007 (0.4246)	0.1340 (0.5961)	0.3763 (0.1238)	0.3428 (0.1637)
Phenolic acids (total)	-0.4586 (0.0556)	**0.51037 (0.0305)**	0.1535 (0.5431)	**0.5054 (0.0127)**	0.5054 (0.7427)	-0.0423 (0.8676)	0.1077 (0.6705)	0.1848 (0.4629)	0.1219 (0.6298)
Flavonoids (total)	-0.4466 (0.0632)	**0.49557 (0.0365)**	0.1243 (0.6229)	0.3437 (0.1141)	0.3437 (0.3862)	0.2270 (0.)3651	0.1390 (0.5823)	0.4001 (0.0999)	0.3666 (0.1346)
Quercetin-3-O-glucuronide	-0.4315 (0.0738)	**0.49647 (0.0361)**	0.1340 (0.5959)	0.3088 (0.1389)	0.3088 (0.3487)	0.2234 (0.3726)	0.1506 (0.5509)	0.3951 (0.1047)	0.3506 (0.1538)
Zeaxanthin	**-0.7234 (0.0007)**	0.22077 (0.3787)	**0.5075 (0.0315)**	**0.7456 (0.0009)**	**0.7456 (0.0003)**	-0.4549 (0.0578)	-0.39922 (0.1008)	-0.1118 (0.6587)	0.2765 (0.2667)
Neoxanthin	0.4657 (0.0515)	**-0.54947 (0.0182)**	-0.3853 (0.1143)	**-0.4589 (0.0204)**	-0.4589 (0.7982)	-0.2857 (0.2505)	0.1147 (0.6506)	-0.1802 (0.4742)	-0.3483 (0.1566)
Violaxanthin	**0.4707 (0.0486)**	**-0.57977 (0.0117)**	-0.3916 (0.1079)	**-0.6218 (0.0044)**	-0.6218 (0.5356)	-0.1022 (0.6865)	0.1629 (0.5184)	-0.0715 (0.7781)	-0.2697 (0.2792)
Antheraxanthin	**-0.5914 (0.0097)**	-0.01347 (0.9578)	0.1947 (0.4387)	0.3346 (0.1124)	0.3346 (0.6476)	-0.1541 (0.5414)	0.0643 (0.7999)	0.3277 (0.1844)	0.3073 (0.2147)

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Table S2, continued

	Latitude	*sumUVR*	*avgUVR*	*10d-sumUVR*	*DD*	*avgT*	*Days*	*sumGR*	*avgGR*
Total xanthophyll content	0.3268 (0.1855)	**-0.56077 (0.0155)**	-0.2131 (0.3958)	-0.3307 (0.1159)	-0.3307 (0.8123)	-0.2205 (0.3792)	-0.0282 (0.9114)	-0.1574 (0.5327)	-0.1809 (0.4726)
VAZ	**-0.7104 (0.0009)**	0.11297 (0.6553)	0.4664 (0.0510)	**0.6804 (0.0037)**	**-0.7671 (0.0002)**	**-0.5060 (0.0322)**	-0.3761 (0.1240)	-0.1006 (0.6911)	0.2611 (0.2953)
β-Carotene	0.1636 (0.5167)	-0.41007 (0.0911)	-0.1332 (0.5980)	0.0276 (0.7064)	0.0276 (0.2567)	-0.2794 (0.2615)	-0.2061 (0.4119)	-0.3604 (0.1417)	-0.2291 (0.3606)
Lutein	**0.5388 (0.0211)**	**-0.59007 (0.0099)**	-0.3415 (0.1654)	**-0.5341 (0.0133)**	-0.5341 (0.4345)	-0.0397 (0.8757)	0.0783 (0.7576)	-0.1182 (0.6405)	-0.2422 (0.3329)
Total carotenoid content	0.2669 (0.2844)	**-0.53677 (0.0217)**	-0.1943 (0.2315)	-0.1671 (0.2936)	-0.1671 (0.4504)	-0.2808 (0.2590)	-0.1235 (0.6254)	-0.2770 (0.2658)	-0.2220 (0.3759)
Total chlorophyll content	0.2920 (0.2396)	-0.43777 (0.0692)	-0.2969 (0.4738)	-0.3640 (0.0553)	-0.3640 (0.9967)	-0.2615 (0.2946)	0.1612 (0.5227)	-0.1592 (0.8814)	-0.2655 (0.1300)

Meteorological parameters are: sumUVR, cumulated TEMIS-derived erythemal UV radiation data calculated for the period from bud break to leaf collection; avgUVR, sumUVR divided by the number of days between bud break and leaf collection; 10d-sumUVR, cumulated TEMIS-derived erythemal UV radiation data calculated for the last 10 days before leaf collection; DD, degree day integral calculated over the period between bud break-veraison; Days, number of days between bud break and veraison; avgT, daily average temperature.

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Table S3A

Correlations between metabolic characteristics of Pinot noir leaves

Pearson's correlation coefficients *R* and corresponding *P* values (in parenthesis, under R values), n=18. $P < 0.05$ correlations are highlighted in bold type. For easier comparisons, data are shown for both above and under diagonal (i.e. cells in gray).

	UVBabs	*UVAabs*	*UVabs*	*TEAC*	*FRAP*	*α-Toc.*	*tot Phen*	*tot PhAc*	*tot Flav*	*Q-3-Glu*
UV-B absorption (UVBabs)		**0.9822 (4.7 10^{-13})**	**0.9914 (1.5 10^{-15})**	**0.4701 (0.0490)**	**0.6983 (0.0013)**	0.3758 (0.1243)	**0.9025 (3.0 10^{-7})**	**0.5230 (0.0259)**	**0.8948 (5.4 10^{-7})**	**0.8597 (4.8 10^{-6})**
UV-A absorption (UVAabs)	**0.9822 (4.7 10^{-13})**		**0.9983 (2.8 10^{-21})**	0.3856 (0.1140)	**0.6482 (0.0036**	0.3222 (0.1923)	**0.9168 (8.9 10^{-8})**	0.4384 (0.0688)	**0.9277 (2.9 10^{-8})**	**0.8872 (9.2 10^{-7})**
Total UV absorption (UVabs)	**0.9914 (1.5 10^{-15})**	**0.9983 (2.8 10^{-21})**		0.4129 (0.0885)	**0.6660 (0.0025)**	0.3398 (0.1677)	**0.9159 (9.6 10^{-8})**	0.4660 (0.0513)	**0.9211 (5.9 10^{-8})**	**0.8821 (1.3 10^{-6})**
Total antioxidant capacity (TEAC)	**0.4701 (0.0490)**	0.3856 (0.1140)	0.4129 (0.0885)		**0.9141 (1.1 10^{-7})**	**0.4740 (0.0469)**	**0.5227 (0.0261)**	**0.7506 (3.3 10^{-4})**	0.4390 (0.0683)	0.4229 (0.0803)
Total antioxidant capacity (FRAP)	**0.6983 (0.0013)**	**0.6482 (0.0036)**	**0.6660 (0.0025)**	**0.9141 (1.1 10^{-7})**		**0.5086 (0.0312)**	**0.7387 (4.6 10^{-4})**	**0.7831 (1.2 10^{-4})**	**0.6650 (0.0026)**	**0.6299 (0.0051)**
α-tocopherol (α-Toc)	0.3758 (0.1243)	0.3222 (0.1923)	0.3398 (0.1677)	**0.4740 (0.0469)**	**0.5086 (0.0312)**		0.2938 (0.2367)	**0.5346 (0.0223)**	0.2281 (0.3627)	0.1712 (0.4970)
Total phenolics (tot Phen)	**0.9025 (3.0 10^{-7})**	**0.9168 (8.9 10^{-8})**	**0.9159 (9.6 10^{-8})**	**0.5227 (0.0261)**	**0.7387 (4.6 10^{-4})**	0.2938 (0.2367)		**0.6228 (0.0058)**	**0.9862 (6.5 10^{-14}**	**0.9707 (2.5 10^{-11})**
Total phenolic acids (tot PhAc)	**0.5230 (0.0259)**	0.4384 (0.0688)	0.4660 (0.0513)	**0.7506 (3.3 10^{-4})**	**0.7831 (1.2 10^{-4})**	**0.5346 (0.0223)**	**0.6228 (0.0058)**		**0.4881 (0.0399)**	**0.4723 (0.0478)**
Total flavonoids (tot Flav)	**0.8948 (5.4 10^{-7})**	**0.9277 (2.9 10^{-8})**	**0.9211 (5.9 10^{-8})**	0.4390 (0.0683)	**0.6650 (0.0026)**	0.2281 (0.3627)	**0.9862 (6.5 10^{-14})**	**0.4881 (0.0399)**		**0.9855 (9.2 10^{-14})**
Quercetin-3-O-glucuronide (Q-3-Glu)	**0.8597 (4.8 10^{-6})**	**0.8872 (9.2 10^{-7})**	**0.8821 (1.3 10^{-6})**	0.4229 (0.0803)	**0.6299 (0.0051)**	0.1712 (0.4970)	**0.9707 (2.5 10^{-11})**	**0.4723 (0.0478)**	**0.9855 (9.2 10^{-14})**	
Zeaxanthin (ZeaXa)	-0.1089 (0.6670)	-0.0610 (0.8100)	-0.0759 (0.7647)	0.1497 (0.5532)	0.1805 (0.4735)	-0.2470 (0.3231)	0.1203 (0.6345)	0.3397 (0.1678)	0.0557 (0.8261)	0.0373 (0.8833)
Neoxanthin (NeoXa)	**-0.6439 (0.0039)**	**-0.5917 (0.0097)**	**-0.6099 (0.0072)**	-0.3692 (0.1316)	-0.4610 (0.0541)	-0.1121 (0.6578)	**-0.6898 (0.0015)**	**-0.5405 (0.0206)**	**-0.6602 (0.0029)**	**-0.6270 (0.0054)**
Violaxanthin (ViolXa)	**-0.5633 (0.0149)**	**-0.5083 (0.0312)**	**-0.5271 (0.0246)**	-0.4439 (0.0650)	**-0.5487 (0.0184)**	-0.2117 (0.3991)	**-0.6300 (0.0051)**	**-0.7410 (4.2 10^{-4})**	**-0.5409 (0.0205)**	**-0.5015 (0.0340)**
Antheraxanthin (AntXa)	-0.0178 (0.9440)	0.0236 (0.9259)	0.0110 (0.9654)	0.2866 (0.2489)	0.1712 (0.4971)	-0.0896 (0.7236)	0.1080 (0.6696)	0.0345 (0.8920)	0.1272 (0.6151)	0.1329 (0.5990)
Total xanthophylls (tot Xa)	**-0.6876 (0.0016)**	**-0.6397 (0.0042)**	**-0.6568 (0.0031)**	-0.3518 (0.1522)	**-0.4993 (0.0349)**	-0.3131 (0.2059)	**-0.6843 (0.0017)**	**-0.5384 (0.0212)**	**-0.6558 (0.0031)**	**-0.5923 (0.0096)**
VAZ	-0.2261 (0.3670)	-0.1618 (0.5213)	-0.1821 (0.4696)	0.0932 (0.7129)	0.0940 (0.7105)	-0.3062 (0.2166)	0.0098 (0.9693)	0.2088 (0.4058)	-0.0378 (0.8815)	-0.0487 (0.8479)

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β-carotene (β-Car)	0.3407 (0.1666)	0.3197 (0.1960)	0.3273 (0.1849)	**0.4721** **(0.0479)**	**0.5763** **(0.0123)**	0.0670 (0.7916)	**0.4875** **(0.0402)**	**0.7034** **(0.0011)**	0.3868 (0.1128)	0.3309 (0.1799)
Lutein (Lut)	-0.3891 (0.1105)	-0.3431 (0.1634)	-0.3585 (0.1441)	-0.0533 (0.8337)	-0.0736 (0.7716)	-0.2031 (0.4190)	-0.2834 (0.2544)	-0.0258 (0.9191)	-0.3277 (0.1843)	-0.3158 (0.2018)
Total carotenoids (tot Car)	**-0.6062** **(0.0077)**	**-0.5811** **(0.0114)**	**-0.5910** **(0.0098)**	-0.3743 (0.1259)	**-0.5269** **(0.0247)**	-0.2325 (0.3531)	**-0.6748** **(0.0021)**	**-0.5981** **(0.0088)**	**-0.6312** **(0.0050)**	**-0.5611** **(0.0154)**
Total chlorophylls (tot Chl)	**-0.5944** **(0.0093)**	**-0.5430** **(0.0199)**	**-0.5608** **(0.0155)**	-0.2150 (0.3915)	-0.3103 (0.2101)	-0.2779 (0.2641)	**-0.5318** **(0.0231)**	-0.3053 (0.2179)	**-0.5410** **(0.0204)**	**-0.5001** **(0.0346)**
UV-B absorption (UVBabs)	**-0.5401** **(0.0207)**	**-0.4794** **(0.0441)**	**-0.4998** **(0.0347)**	-0.1779 (0.4801)	-0.2844 (0.2527)	-0.2772 (0.2654)	**-0.5283** **(0.0242)**	**-0.5179** **(0.0277)**	**-0.4850** **(0.0413)**	-0.4494 (0.0614)

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Table S3B (Table S3A continued)

Correlations between metabolic characteristics of Pinot noir leaves

Pearson's correlation coefficients *R* and corresponding *P* values (in parenthesis, under *R* values), n=18. *P* < 0.05 correlations are highlighted in bold type.

	ZeaXa	*NeoXa*	*ViolXa*	*AntXa*	*tot Xa*	*VAZ*	*β-Car*	*Lut*	*tot Car*	*ZeaXa*
UV-B absorption (UVBabs)	-0.1089 (0.6670)	**-0.6439 (0.0039)**	**-0.5633 (0.0149)**	-0.0178 (0.9440)	**-0.6876 (0.0016)**	-0.2261 (0.3670)	0.3407 (0.1666)	-0.3891 (0.1105)	**-0.6062 (0.0077)**	**0.5944 (0.0093)**
UV-A absorption (UVAabs)	-0.0610 (0.8100)	**-0.5917 (0.0097)**	**-0.5083 (0.0312)**	0.0236 (0.9259)	**-0.6397 (0.0042)**	-0.1618 (0.5213)	0.3197 (0.1960)	-0.3431 (0.1634)	**-0.5811 (0.0114)**	**-0.5430 (0.0199)**
Total UV absorption (UVabs)	-0.0759 (0.7647)	**-0.6099 (0.0072)**	**-0.5271 (0.0246)**	0.0110 (0.9654)	**-0.6568 (0.0031)**	-0.1821 (0.4696)	0.3273 (0.1849)	-0.3585 (0.1441)	**-0.5910 (0.0098)**	**-0.5608 (0.0155)**
Total antioxidant capacity (TEAC)	0.1497 (0.5532)	-0.3692 (0.1316)	-0.4439 (0.0650)	0.2866 (0.2489)	-0.3518 (0.1522)	0.0932 (0.7129)	**0.4721 (0.0479)**	-0.0533 (0.8337)	-0.3743 (0.1259)	-0.2150 (0.3915)
Total antioxidant capacity (FRAP)	0.1805 (0.4735)	-0.4610 (0.0541)	**-0.5487 (0.0184)**	0.1712 (0.4971)	**-0.4993 (0.0349)**	0.0940 (0.7105)	**0.5763 (0.0123)**	-0.0736 (0.7716)	**-0.5269 (0.0247)**	-0.3103 (0.2101)
α-tocopherol (α-Toc)	-0.2470 (0.3231)	-0.1121 (0.6578)	-0.2117 (0.3991)	-0.0896 (0.7236)	-0.3131 (0.2059)	-0.3062 (0.2166)	0.0670 (0.7916)	-0.2031 (0.4190)	-0.2325 (0.3531)	-0.2779 (0.2641)
Total phenolics (tot Phen)	0.1203 (0.6345)	**-0.6898 (0.0015)**	**-0.6300 (0.0051)**	0.1080 (0.6696)	**-0.6843 (0.0017)**	0.0098 (0.9693)	**0.4875 (0.0402)**	-0.2834 (0.2544)	**-0.6748 (0.0021)**	**-0.5318 (0.0231)**
Total phenolic acids (tot PhAc)	0.3397 (0.1678)	**-0.5405 (0.0206)**	**-0.7410 (4.2 10^{-4})**	0.0345 (0.8920)	**-0.5384 (0.0212)**	0.2088 (0.4058)	**0.7034 (0.0011)**	-0.0258 (0.9191)	**-0.5981 (0.0088)**	-0.3053 (0.2179)
Total flavonoids (tot Flav)	0.0557 (0.8261)	**-0.6602 (0.0029)**	**-0.5409 (0.0205)**	0.1272 (0.6151)	**-0.6558 (0.0031)**	-0.0378 (0.8815)	0.3868 (0.1128)	-0.3277 (0.1843)	**-0.6312 (0.0050)**	**-0.5410 (0.0204)**
Quercetin-3-O-glucuronide (Q-3-Glu)	0.0373 (0.8833)	**-0.6270 (0.0054)**	**-0.5015 (0.0340)**	0.1329 (0.5990)	**-0.5923 (0.0096)**	-0.0487 (0.8479)	0.3309 (0.1799)	-0.3158 (0.2018)	**-0.5611 (0.0154)**	**-0.5001 (0.0346)**
Zeaxanthin (ZeaXa)		-0.1901 (0.4500)	-0.4601 (0.0547)	0.3158 (0.2018)	-0.0319 (0.8999)	**0.9746 (8.0 10^{-12})**	**0.7444 (3.9 10^{-4})**	0.3229 (0.1913)	-0.3344 (0.1750)	-0.1023 (0.6862)
Neoxanthin (NeoXa)	-0.1901 (0.4500)		**0.8114 (4.4 10^{-5})**	-0.0023 (0.9928)	**0.9150 (1.0 10^{-7})**	-0.0367 (0.8849)	**-0.6029 (0.0081)**	**0.5956 (0.0091)**	**0.8998 (3.7 10^{-7})**	**0.8320 (1.9 10^{-5})**
Violaxanthin (ViolXa)	-0.4601 (0.0547)	**0.8114 (4.4 10^{-5})**		0.1899 (0.4505)	**0.7537 (3.0 10^{-4})**	-0.2622 (0.2932)	**-0.8661 (3.4 10^{-6})**	0.1649 (0.5132)	**0.8199 (3.1 10^{-5})**	**0.5065 (0.0320)**
Antheraxanthin (AntXa)	0.3158 (0.2018)	-0.0023 (0.9928)	0.1899 (0.4505)		0.1735 (0.4912)	0.4551 (0.0577)	0.0047 (0.9853)	-0.0765 (0.7627)	0.0425 (0.8670)	0.0579 (0.8196)
Total xanthophylls (tot Xa)	-0.0319 (0.8999)	**0.9150 (1.0 10^{-7})**	**0.7537 (3.0 10^{-4})**	0.1735 (0.4912)		0.1317 (0.6024)	**-0.5283 (0.0242)**	**0.6494 (0.0035)**	**0.9499 (1.7 10^{-9})**	**0.9075 (2.0 10^{-7}**

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VAZ	**0.9746 (8.0 10^{-12})**	-0.0367 (0.8849)	-0.2622 (0.2932)	0.4551 (0.0577)	0.1317 (0.6024)		**0.6011 (0.0083)**	0.3607 (0.1414)	**-0.7090 (0.0010)**	-0.2094 (0.4042)
β-carotene (β-Car)	**0.7444 (3.9 10^{-4})**	**-0.6029 (0.0081)**	**-0.8661 (3.4 10^{-6})**	0.0047 (0.9853)	**-0.5283 (0.0242)**	**0.6011 (0.0083)**		0.1467 (0.5614)	**-0.7090 (0.0010)**	-0.2094 (0.4042)
Lutein (Lut)	0.3229 (0.1913)	**0.5956 (0.0091)**	0.1649 (0.5132)	-0.0765 (0.7627)	**0.6494 (0.0035)**	0.3607 (0.1414)	0.1467 (0.5614)		**0.5252 (0.0252)**	**0.9085 (1.9 10^{-7})**
Total carotenoids (tot Car)	-0.3344 (0.1750)	**0.8998 (3.7 10^{-7})**	**0.8199 (3.1 10^{-5})**	0.0425 (0.8670)	**0.9499 (1.7 10^{-9})**	-0.1807 (0.4731)	**-0.7090 (0.0010)**	**0.5252 (0.0252)**		**0.8113 (4.4 10^{-5})**
Total chlorophylls (tot Chl)	0.1598 (0.5263)	**0.8320 (1.9 10^{-5})**	**0.5065 (0.0320)**	0.0579 (0.8196)	**0.9075 (2.0 10^{-7})**	0.2713 (0.2762)	-0.2094 (0.4042)	**0.9085 (1.9 10^{-7})**	**0.8113 (4.4 10^{-5})**	
UV-B absorption (UVBabs)	-0.1023 (0.6862)	**0.8656 (3.5 10^{-6})**	**0.7739 (1.6 10^{-4})**	0.2661 (0.2858)	**0.8838 (1.2 10^{-6})**	0.0710 (0.7794)	**-0.4829 (0.0424)**	**0.6182 (0.0063)**	**0.8471 (9.2 10^{-6})**	**0.8266 (2.4 10^{-5})**

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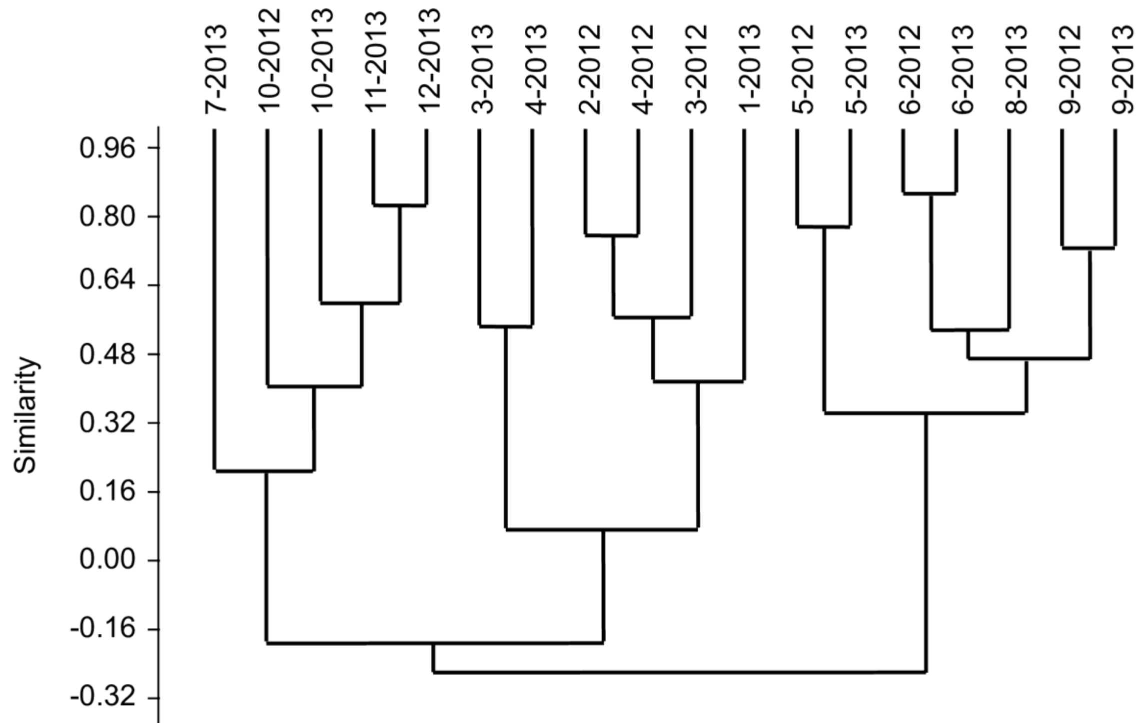

Figure S1

Cluster analysis according to similarities of samples. Numbers refer to sampling locations in Table 1 and sampling years.

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