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## Effects of defoliation on quality attributes of Nero di Troia (*Vitis vinifera* L.) grape and wine

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1 **Effects of defoliation on quality attributes of Nero di Troia (*Vitis vinifera* L.)**

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3 **grape and wine**  
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## Abstract

Field studies were conducted in Puglia (Italy) to evaluate the influence of defoliation around cluster zones on grape and wine quality. Nero di Troia grapes were subjected to four different treatments: N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and at pre-harvest also along the east side. Grapes of defoliated vines generally showed higher sugar content, lower titratable acidity, total flavonoids, flavonoids different from anthocyanins, and total phenolic content than grapes from non-defoliated vines while their total anthocyanin concentration was not affected by defoliation at a significant level. Concerning wines, alcohol content, residual soluble solids, different forms of anthocyanins but also volatile acidity were generally higher in samples from defoliated vines. Differences were also highlighted among the defoliation treatments: the best results in terms of dry matter, sugar and alcohol content were observed in the samples submitted to the more severe defoliation as a consequence of the higher light availability and berry temperature. Concerning the concentration of the individual phenolics, significant differences were highlighted for: caffeic and caftaric acids, peonidin- and malvidin- p-coumaroylglucoside, which were higher in the E wines; quercetin-3- glucoside, galactoside, and rhanoside, and procyanidins, which were higher in F wines.

## Chemical compounds studied in this article

Tartaric acid (PubChem CID: 8765); Malic acid (PubChem CID: 525); Citric acid (PubChem CID: 311); D-gluconic acid (PubChem CID: 10690); Malvidin-3-glucoside (PubChem CID: 443652); (+)-catechin (PubChem CID: 9064); Epicatechin (PubChem CID: 72276); Procyanidin B1 (PubChem CID: 11250133); Vitisin B (PubChem CID: 16138152).

**Keywords:** defoliation, oenological parameters, phenolics, photosynthetic photon flux, quality

## 1. Introduction

Canopy management embraces a range of viticulture practices aimed to obtain a desired shoot arrangement and avoid an excessive foliage density which would shade and make humid the fruit zone; these microclimatic conditions are known to reduce the vine fruitfulness, the expression of grape variety characters and the overall grape quality, beside to hamper the efforts at disease control. Leaf removal (defoliation) in the fruiting zone is a canopy management practice widely applied, at any time from fruit set to veraison, to enhance air circulation and light penetration in dense foliage (Smart and Robinson, 1991). Many studies showed that grapes well-exposed to sunlight have higher sugar, anthocyanin, and phenolic accumulation, and lower titratable acidity, pH and malic acid concentration than shaded grapes. As summarized by Dokoozlian & Kliewer (1996), the photoregulation of the invertase and phenylalanine-ammonia-lyase enzymes are thought to be primarily involved in these responses, together with the thermal regulation of the malic enzyme, considering that a rise in light availability normally induces also a rise of berry temperature.

Nevertheless, intense defoliations may expose the clusters to excess of light intensity and of temperature, especially in warm climates; it is proved that very high temperatures may reduce the skin colour (Price, Breen, Valladao & Watson, 1995) and lower too much the titratable acidity. Although the experimental results change with the grape variety and the growing environment, an average critical threshold for anthocyanin response might be individuated around 30°C, as suggested by Downey, Dokoozlian & Krstic (2006).

Beside the variety, the environment and the severity of leaf removal, the overall defoliation effect depends also on its timing. According to Diago, Villanova & Tardaguila (2010), “early” defoliation leads to musts richer in total soluble solids, especially when leaf removal is carried out at pre-bloom, and has little or no effect on acidity. In their study, Tempranillo wines from early defoliated vines exhibited higher alcohol content than the control wines, but, in general, neither pH nor titratable acidity were significantly altered. The increase in alcohol concentration might have helped in extracting larger amounts of anthocyanins. Early defoliation improved the phenolic composition of Tempranillo wines also by favouring the accumulation of hydroxycinnamics, flavonols and anthocyanins, thus enhancing wine quality in terms of colour and sensory properties (Diago, Ayestarán, Guadalupe, Garrido & Tardaguila, 2012). On the other hand, when Hunter, Ruffner, Volschenk & Le Roux (1995) analyzed the effects induced by two partial defoliation levels (33% and 66%), performed at different developmental stages, on grape skin colour and sugar content and on wine quality of Cabernet Sauvignon, They found that the anthocyanin content per berry was significantly higher in vines defoliated at veraison.

78 Since the concentration of phenolic compounds in the wine is intrinsically related to their  
79 concentration in the berries (Jensen, Demiray & Egebo MandMeyer, 2008), and considering that  
80 both anthocyanin and flavonol biosynthetic pathways are regulated by enzymes that are light- and  
81 temperature-sensitive (Downey, Harvey & Robinson, 2003; Hunter, Villiers & Watts, 1991), any  
82 changes in microclimatic conditions, such as those imparted by defoliation, might have a significant  
83 impact on the synthesis and accumulation of these compounds in the berries and their concentration  
84 in wine.

85 The general consensus is that, regardless of the defoliation timing, leaf removal is an effective  
86 technique for improving the quality of wines since noticeable increases in constituents  
87 (anthocyanins, phenolics), colour density, cultivar character intensity, and overall quality is  
88 generally found in wines from defoliated vines. Therefore, this work was aimed to establish how  
89 defoliation, performed at veraison according to the local custom, can influence the physico-  
90 chemical composition of Nero di Troia grapes grown in Southern Italy and of the corresponding  
91 wines. In particular, the effects of three leaf removal treatments, differing for vine defoliation side  
92 and amounts of removed leaves, were compared to each other and to the results coming from non-  
93 defoliated control vines, with a specific focus on their consequence on grape and wine phenolic  
94 composition and colour parameters.

## 95 96 **2. Materials and methods**

### 97 *2.1 Vineyard site and plant material*

98 The field trial was carried out, in 2012 summer, at a privately owned vineyard located in San  
99 Ferdinando (Foggia province, Apulia region, 41°19' N, 15°05', altitude 68 m a.s.l.).

100 The climate of this area is Mediterranean semi-arid according to the De Martonne (1926) scale  
101 (aridity index = 18 within the 15-20 range defined as semi-arid). The annual mean temperature is  
102 15.5 °C (maximum mean temperature 31.8 °C in July and August, minimum mean temperature 3.0  
103 °C in February); mean annual rainfall is 470 mm, 34% of which in the warmer period, that is May-  
104 September. (CliNO, 1971-2000). The area totalizes 2170 GDD (IV region of the Winkler scale).

105 The soil is deep, calcareous, medium textured, fertile, and retains moisture in the deep layers.

106 Nero di Troia is one of the main red wine grape variety grown in the Puglia and is the main  
107 component of many Controlled Designation of Origin wines. When grown in the Foggia province,  
108 this genotype shows a considerable vigour and produces lots of girth and large, rather compact,  
109 pyramidal clusters of violet coloured berries.

110 The vineyard was established in 2007 by planting vines of cv. Nero di Troia, grafted onto 140 Ru  
111 (*V. berlandieri* x *V. rupestris*) stock at 1.25 x 2.50 m apart, in N-S oriented rows. Vines were VSP

112 trained and spur-cordon pruned. The cordon was positioned 0.60 m above the ground while the  
113 highest trellis wire was at 1.80 m from the soil and the total canopy height reached about 2.20 m;  
114 the average main shoot length was 1.60 m.

115 In the year of the trial, the number of bunches per vine was  $32 \pm 1$ .

116 Fertilization was provided by means of soil applications, foliar nutrition and fertigation, with a total  
117 amount of about 45 kg N, 25 kg  $P_2O_5$ , 53 kg  $K_2O$ , 32 kg CaO, 20 kg MgO, 25 kg  $SO_3$  per hectare;  
118 moreover, foliar application provided also about 50 kg alginic acid and 125 kg organic matter (both  
119 strong water soluble) per hectare.

120 Irrigation supplied about  $1700 \text{ m}^2 \text{ ha}^{-1}$  of water, from July to early September, by a drip system.

121

## 122 *2.2 Leaf removal treatments and leaf area evaluation*

At complete veraison (mid August), the following four leaf removal treatments were manually  
24 applied:

- 25 • N: no leaf removal;
- 26 • E: 75% of fruit-zone leaves removed from the East canopy side;
- 27 • E/W: 75% removal of the fruit-zone leaves on the East and also on the West side of the  
28 canopy;
- 319 • F: Farm defoliation (2 steps), that is, almost 100% removal of fruit-zone leaves on the West  
320 side of the canopy at full veraison (1st step), plus almost 100% removal of fruit-zone leaves  
34 on the East side of the canopy about 15 days before grape harvest.

321 Defoliation percentage was visually estimated.

322 Treatments were replicated in three 4-row blocks; each replicate was assigned to one row and  
323 involved 16 vines.

324 In order to evaluate the amount of leaf area removed and retained on vine after the treatments were  
325 imposed, the leaves removed from each replicate were immediately enclosed in plastic bags and  
326 transported to the lab where, after weighing, the weight-to-area ratio was applied using 100 leaf  
327 dishes (28 mm diameter) per replicate.

328 Moreover, aiming to express the data in terms of percentage of the total vine leaf area, half canopy  
329 of 5 representative vines was entirely defoliated and was subjected to the same procedure already  
330 described.

331

## 332 *2.3 Field measurements*

333 Measurements were taken in cloudless days of late summer (August 30th and 31st).

145 Air temperature and relative humidity at 2.00 m above the soil were measured (thermo-hygrometer  
146 HD 8501 H, Delta Ohm, PD, Italy) under midday conditions; average values were  $33.37 \pm 0.12$  °C  
147 and  $34.90 \pm 0.31$ %.

148 When the East side of the canopy was fully lighted, the rate of photosynthetic active radiation  
149 (PAR) was measured as maximum photosynthetic photon flux (PPF) interceptable by orienting a  
150 solar bar (AccuPAR PAR/LAI LP-80, Decagon Dev. Inc. Pullman, WA, USA), and as PPF  
151 interceptable at the leaf surface of the East and of the West side of the vine canopy by positioning  
152 the solar bar along the canopy at 0.90 m above the cordon; 30 readings per type of measurements  
153 were recorded. The average values were the following: PPF max  $1994.70 \pm 2.63$   $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; PPF  
154 at East canopy side  $1238.50 \pm 6.71$   $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; PPF at West canopy side  $95.10 \pm 2.68$   $\mu\text{mol m}^{-2} \text{s}^{-1}$ .  
155 Immediately after, in order to assess the influence of the leaf removal treatments on the fruit-zone  
microclimate, PAR availability at East and at the West side of the vine canopy was measured by  
positioning the solar bar along the bunches and, moreover, the surface temperature of exposed  
bunches was measured using a non-contact infrared thermometer with laser pointer (TRI-88  
Lafayette Electronic Supply Inc., Indiana, USA); 10 readings per each replicate and each type of  
measurement were recorded. The same set of measurements was taken in the afternoon, when the  
West side of the canopy was fully lighted. The average values of these readings are shown in Table  
1. When the East canopy side was fully lighted, the photosynthetic photon flux intercepted at the  
east fruit-zone ranged from  $236.17$   $\mu\text{mol m}^{-2} \text{s}^{-1}$  in the non defoliated vines (control) to  $563.53$   $\mu\text{mol}$   
 $\text{m}^{-2} \text{s}^{-1}$  in the E treatment that improved bunch exposure by 139%. Nonetheless, according to  
Bergqvist, Dokoozlian & Ebisuda (2001), the bunch exposure of control vines was not limiting for  
phenol accumulation; similar PPF was found in F vines that, by the time of measurements, were had  
not been defoliated on the East face. This finding is at least partially due to the fact that, under the  
growing conditions of this trial, Nero di Troia produced big and prominent clusters. Compared to  
the control, the E/W defoliation enhanced sunlight penetration by 64%. When the east canopy face  
was fully lighted, the West face received diffused light between  $93$  and  $57$   $\mu\text{mol m}^{-2} \text{s}^{-1}$  of PAR; the  
PPF rates decreased as the defoliation intensity on the West side increased. The berry surface  
temperature measured at the east vine side in the morning reached  $37.9$  °C in the E treatment; the  
other treatments showed decreasing temperatures according their pattern of sun irradiance at the  
fruit-zone. Berry temperature of non-defoliated vines was  $33.87$  °C; this thermal level was quite  
close to that found in berries that were not exposed to direct sunlight, that is, in the morning those  
of bunches of the west side, and in the afternoon those of bunches of the east side. The highest  
absolute berry surface temperatures, about  $40$ °C, were recorded, in afternoon, in west defoliated  
vines, that is, E/W and F vines.

179

180 **I**n order to evaluate if leaf removal influenced the vine water status, stem water potential ( $\Psi_{\text{stem}}$ )  
181 was measured under midday conditions according to Turner (1981); 10 measurements per replicate  
182 were taken.

183 At farm harvest (October 4th), yield components were assessed on 10 vines per replicate, that is,  
184 vine total grape yield, number of bunches per vine, average bunch weight. The grape was  
185 immediately sent to the vinification.

186

#### 187 *2.4 Grapes and wine-making*

188 Grapes were picked early in the morning and immediately delivered to a pilot plant (Foggia, Italy)  
189 made of a crusher-destemmer, 20 stainless steel vats (100 L-capacity), a temperature management  
190 system, and 2 winepresses.

191 A traditional red wine-making was carried out with crusher-destemmer, addition of potassium  
192 metabisulphite (10 g/hL of must), fermentation-maceration performed at 25 °C for 7 days by *S.*  
193 *cerevisiae* (20 g/100 kg, AEB, Brescia, Italy), and two punching-down per day. Each vinification  
was replicated two times.

#### 96 *2.5 Conventional analyses of grape and wine*

97 The dry matter of the various parts of the berries were determined by separating skins from seeds  
98 and pulps and oven drying at 90°C until constant weight. Alcoholic strength at 20°C (expressed  
99 as% vol.), titratable acidity (expressed as g of tartaric acid/L), volatile acidity (g acetic acid/L),  
density (g/L), sugar content (g/L), dry extract (g/L), and free and total sulphur dioxide (mg/L) were  
determined according to the EEC Regulation 2676/90 (1990). The pH was also measured. The  
02 concentration of sugars (glucose, fructose, and their ratio), organic acids (g/L) and acetaldehyde  
03 (mg/L) were determined through a Hyperlab automatic multi-parametric analyzer (Steroglass, San  
04 Martino in Campo, Perugia, Italy) by means of enzymatic kits. Dissolved oxygen (mg/L) was  
05 measured by using an LDO-HQ10 portable oxygen meter (Hach, Düsseldorf, Germany).

206

50

#### 207 *2.6 Extraction of phenolic fractions from grapes*

208 Extraction of the phenolic fraction from skins, seeds and pulps was done according to Di Stefano &  
209 Cravero (1991).

10

#### 11 *2.7 Determination of phenolic compounds, colour measurements, and structure indices of wine*



212 The total phenolic content was measured at 765 nm through an UV–visible spectrophotometer  
213 (Cary 50 SCAN; Varian, Palo Alto, CA) according to the Folin–Ciocalteu method as reported by  
214 Singleton & Rossi (1965). Results were expressed as gallic acid equivalents (mg/L and mg/kg of  
215 grape). A calibration line was built on the basis of solutions of known and increasing concentrations  
216 of gallic acid (Extrasynthèse, Genay, France). The various phenolic classes (total anthocyanins,  
217 monomeric anthocyanins, total flavonoids, flavonoids different from anthocyanins,  
218 proanthocyanidins and flavans reactive with vanillin) were analysed according to the methods of Di  
219 Stefano, Cravero & Gentilizi (1989) and Di Stefano & Cravero (1991), while anthocyanins sensitive  
220 to SO<sub>2</sub> were determined according to Ribéreau-Gayon & Stonestreet (1965). When necessary,  
221 extracts were opportunely diluted with aliquots of the extraction solution. The results were  
222 expressed as mg per kg of skins, seeds, and pulps or per L of wine.

223 Colour was evaluated by the measurement of the Glories parameters (1984), i.e. colour intensity  
224 (CI), tonality (T), percentage of yellow, red and blue components (% yellow, % red and % blue,  
225 respectively), dA%, dAl%, dAT% and dTAT%, through an UV–visible spectrophotometer (Cary 50  
226 SCAN; Varian, Palo Alto, CA), using quartz cells of 0.1cm path length. HCl index, ethanol index,  
227 gelatin index, and PVPP index were determined according to Glories (1978), while polymeric  
pigments were performed as described in Habertson, Picciotto & Adams (2003).

228

229

### 230 231 *2.8 HPLC-DAD-ESI-MS/MS analysis of phenolics*

232 The chromatographic analyses were performed according to the method described in Crupi et al.  
233 (2012) with some modifications. A Capillary HPLC 1100 Series system, equipped with a degasser  
234 model G1379A, a binary pump model G1376A solvent delivery, an autosampler model G1377A, a  
235 thermostated column compartment model G1316A, a DAD model G1315C, and an XCT-trap Plus  
236 mass detector model G2447A (Agilent, Santa Clara, CA) coupled with an ESI interface was used.  
237 The reversed stationary phase employed was a Poroshell 120 SB-C18 2.7 µm (150 × 2.1 mm i.d.,  
238 Agilent Technologies) thermostated at 40 °C. The solvent A was water containing 1% formic acid  
239 while the solvent B was acetonitrile. The following gradient system was applied: 0 min, 0% B; 2  
240 min, 5% B; 10 min, 13% B; 25 min, 15% B; 30 min, 22% B; 50 min, 22% B; 55 min, 95% B; 65  
241 min, 95% B; 66 min, 5% B; stop time to 66 min followed by washing and re-equilibration of the  
242 column. The flow was maintained at 200 µL/min. The sample injection was 8 µL. Diode array  
243 detection was between 250 and 650 nm, and absorbance were recorded at 280, 313, 350 and 520  
244 nm. Both positive and negative electrospray mode were used for the molecule ionization with a  
245 capillary voltage of 3500 V and a skimmer voltage at 40 V. The nebulizer pressure was 40 psi and

246 the nitrogen flow rate was 8 L/min. The temperature of the drying gas was 350 °C. The monitored  
247 mass range was from m/z 50 to 1200. The wine samples were filtered through a 0.45 µm cellulose  
248 acetate filter prior to HPLC injection. Compounds identification was achieved by combining  
249 different information: elution pattern, UV-Vis and MS spectra, MS/MS fragmentation patterns and  
250 with the help of structural models already hypothesized in the literature. Quantitative  
251 determinations were made by using the external standard method with commercial standards. The  
252 calibration curves were obtained by injection of standard solutions under the same conditions of the  
253 samples analysed, over the range of concentrations observed. The compounds for which no  
254 standards were available were quantified on the curves of quercetin-3-rutinoside (flavonols and  
255 dihydroflavonols), trans-resveratrol (stilbenes), gallic acid (hydroxybenzoic acids), caffeic acid  
256 (hydroxycinnamic acids), (+)-catechin (flavan-3-ols) and malvidin-3-O-glucoside (anthocyanins).  
Therefore, flavonols, flavan-3-ols, hydroxybenzoic acids, hydroxycinnamic acids, stilbenes, and  
anthocyanins were respectively expressed in quercetin-3-rutinoside (QE, mg/L;  $R^2 = 0.9986$ ), (+)-  
catechin (CE, mg/L;  $R^2 = 0.9945$ ), gallic acid (GAE, mg/L;  $R^2 = 0.997$ ), caffeic acid (CAE, mg/L;  
 $R^2 = 0.9954$ ), trans-resveratrol (RE, mg/L;  $R^2 = 0.9894$ ) and malvidin-3-O-glucoside (ME, mg/L;  
 $R^2 = 0.9941$ ) equivalents.

## 63 2.9 Statistical Analysis

264 Each analysis was replicated at least three times. The averages and the standard deviations were  
265 calculated using Excel software V. 11.5.1 (Microsoft, Redmond, WA). The regression analysis was  
66 carried out at  $p < 0.05$ . The statistical data treatment of data will be performed using the package  
67 Statistica for Windows ver. 10 (Statsoft Inc., Tulsa, OK). The least significant difference (LSD) test  
68 ( $p < 0.05$ ) and the analysis of variance (ANOVA) were applied to determine the main effect of the  
69 defoliation on the chemical composition of grapes and wines. The Principal Component Analysis  
270 (PCA) were applied to the data sets aiming to discriminate grapes and wines on the base of the leaf  
271 removal treatments.

## 273 3. Results and discussion

### 274 3.1 Effects of defoliation treatments on fruit-zone microenvironment, vine water status and yield 275 components

276 Estimated total leaf area of Nero di Troia vines was approximately 8.7 m<sup>2</sup> (Table 2). Fruit-zone  
77 defoliation eliminated a small portion of the total leaf area, that is: 7.7% when about 70% of leaves  
78 were removed from the East canopy side, almost 11% when about 70% of leaves were removed  
79 from both sides, and almost 16% when about 100% of leaves were removed, on the two canopy

280 sides, from the shoot base up to the second node (second step of the F treatment). These data  
281 support two considerations: i) Nero di Troia leaves were not large in size, as confirmed by the  
282 general features of this variety; ii) the shoot growth, and thus the vine leaf surface, were not  
283 uniform at the two row sides: the East side was more vigorous and leafy.

284 Data showed that the increase of light availability at the fruit-zone did not correspond to the relative  
285 amount of removed leaves, either in terms of percentages or in terms of differences among  
286 treatments, and that the response differed between the two canopy sides. To explain these findings  
287 several factors should be considered, such as: i) vine organs different from leaves are involved in  
288 bunch shading, i.e. lateral shoot axes (their number, thickness and length); ii) vine tend to  
289 compensate leaf removal with lateral and sub-lateral shoot re-growth around the fruit-zone,  
290 especially in case of high vigour and irrigation water supply; iii) this compensatory response may  
291 be strongly stimulated in some cases, such as on the East side of vines defoliated on both canopy  
292 sides. In the afternoon, when the West vine face was fully lighted by direct solar radiation, the  
293 pattern of the sun irradiance available at fruit-zone varied again according to the defoliation  
294 intensity at that side (Table 1). Hence, vines of the two treatments that did not provide West  
295 defoliation had the lower bunch exposure ( $134\text{-}142 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), while bunches of farm-defoliated  
296 vines received the highest irradiance ( $586.43 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) which rate was similar to that found  
297 during the morning in the E vines; the E/W treatment performed intermediately. At the East canopy  
298 side, that in the afternoon received diffused light, the two treatments that did not provide east  
299 defoliation by the time of measurements (N and F) showed the lower bunch exposure ( $69\text{-}76 \mu\text{mol}$   
300  $\text{m}^{-2} \text{s}^{-1}$ ); compared with them, E improved bunch exposure by 46-61%, while E/W increased light  
301 penetration only by 18-30%. As for the relationship between defoliation intensity and sunlight  
302 interception, the considerations set out above should be repeated. On the whole, summing by thesis  
303 all PPF values as an index of bunch light exposure to light at the end of August, F treatment was the  
304 most lighted, followed by E, E/W, and finally N. Nonetheless, it can be thought that the differences  
305 between F and other treatments increased after the F second defoliation step (pre-harvest). The  
306 opinion that a high sun irradiance enhances grape phenol content (especially anthocyanins) at the  
307 opposite of a low light regime, is largely accepted (Price, Breen, Valladao & Watson, 1995),  
308 although other evidences show no limitation to total anthocyanin content in shaded grapes or  
309 decreasing total anthocyanin content with high sunlight cluster exposure (Hunter, Ruffner,  
310 Volschenk & Le Roux, 1995; Bergqvist, Dokoozlian & Ebisuda, 2001).

311 Concerning temperature of berry surface, it is known that the air heat accumulation increases after  
312 12 noon limiting the thermal exchange between from solid bodies that, as a consequence, show a  
313 rise in temperature. In this experiment, summing by thesis all the temperature values as an index of

314 bunch thermal exposure at the end of August, E/W treatment was the warmer (143 °C) as expected,  
315 E and F showed very similar temperatures (139-138 °C), N was 2-3 °C cooler. Nonetheless, it can  
316 be thought that the F increased temperature after its second defoliation step (pre-harvest).  
317 Temperature is widely recognized as a factor having a major influence on cell metabolism,  
318 including anthocyanin biosynthesis and accumulation; however, varieties may differ in their  
319 response. In facts, day temperature ranging between 30 and 35°C can inhibit anthocyanin  
320 biosynthesis in Cardinal berry skin (Kliewer & Torres, 1972), but do not affect Pinot noir grapes;  
321 the same thermal range has been supposed to be critical for anthocyanin accumulation in grapes of  
322 cv. Merlot (Spayd, Tarara, Mee & Ferguson, 2004).

323 Type and intensity of leaf removal may affect vine water status. As summarized by Downey,  
324 Dokoozlian & Krstic (2006), several studies pointed out that water deficit enhances berry phenol  
concentration mainly by limiting berry size (Kennedy, Matthews & Waterhouse, 2002) or by  
21 changing the skin structure (Roby & Matthews, 2004), while a direct effect on flavonoid  
22 biosynthesis is rarely admitted. In this trial, the vine midday stem water potential ranged between -  
23 0.95 ± 0,04 MPa in E/W (vines having the lowest total leaf area by the time of measurements) and -  
24 1.08 MPa in N (vines having the greatest transpiring leaf surface): according to Van Leeuwen,  
25 Trégoa, Choné, Bois, Pernet & Gaudillère (2009), all treatments had water status consistent with a  
26 moderate-to-weak water deficit.

31 As expected, vines gave a very high grape yield, which ranged from about 9.0 kg per vine (F and  
32 E/W treatments) to 10.6-10.7 kg (N and E treatments). Although these differences were not  
33 statistically significant, the higher grape yields were achieved with the two treatments that, at the  
34 harvest time, left the more spread leaf surfaces. These results were in agreement with previously  
35 scientific experiments that highlighted the depressing effects of defoliation grape yield (Hunter &  
36 Visser, 1990) as a consequence of the vine source-sink balance or to the cluster microclimate. When  
37 defoliation is performed at a quite advanced stage of ripening, i.e. at veraison, the effects of cluster  
38 microclimate are probably higher than those of the leaf-to-fruit ratio. The number of bunches per  
39 vine (32±1) was the same for not defoliated and defoliated vines.

### 341 342 *3.2 Effects of defoliation treatments on grape quality*

343 At harvest, the glucose-to-fructose ratio was 0.70±0.01 in all the samples. The highest sugar content  
344 (22.1±0.7 °Brix) was detected in the grapes of F vines, which were submitted to the removal of the  
345 highest leaf area most of which at pre-harvest on the West side, thus it is likely that berry juice was  
346 more concentrated. The sugar concentrations of the other grapes ranged from 20.4±0.3 to 21.0±0.7  
347 °Brix).

348 Non-defoliated and E/W defoliated grapes showed the highest titratable acidity ( $6.61\pm 0.05$  g tartaric  
349 acid/L), while the lowest values ( $6.00\pm 0.01$ ) were found in E grapes (which also showed the lowest  
350 malic acid concentration,  $1.16\pm 0.01$  g/L) and in F grapes (which also had the lowest tartaric acid  
351 concentration,  $2.07\pm 0.04$  g/L). This behaviour could be explained by the highest PPF densities  
352 received from the E and F samples (an average of 873.17 and 996,13  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively).  
353 According to Valdivia (2001), bunch exposure to higher radiation (as it is the case of defoliated  
354 plants) increases fruit cell respiration, with a greater consumption of organic acids. The highest  
355 concentrations of citric and D-gluconic acid ( $0.35$  and  $0.23\pm 0.03$  g/L) were found in E/W grapes.  
356 Low-light conditions are known to decrease the weight of grape skins and the skin to berry ratio. In  
357 fact, the highest and lowest skin dry matter percentages were found in F ( $43.7\pm 1.6$ ) and N  
358 ( $34.4\pm 1.1$ ) grapes, respectively, in agreement with the finding of Keller, Arnink & Hrazdina (1998).  
359 The dry matter percentages of pulps and seeds did not depend on defoliation treatments and were in  
360 the ranges 14.9-18.9 and 48.2-69.5, respectively.  
361 Leaf removal did not improve skin total anthocyanin concentration; differences among these were  
362 small (max. 16%) and not significant (Table 3). This result is consistent with the sufficient light  
363 intensity available at the fruit-zone for all treatments and/or with the late time of the defoliation  
364 treatments (complete veraison). The concentrations of flavans reactive with vanilline, and  
365 proanthocyanidins were not affected by the defoliation treatments, whereas total flavonoids,  
366 flavonoids different from anthocyanins, and the total phenolic content were higher in the skins of  
367 berries of non-defoliated vines (Table 3). Nevertheless, the phenolic profile of skins was modified  
368 by defoliation (Table 3). In particular, the flavonols myricetin--3-glucoside, quercetin-3-glucoside,  
369 quercetin-3-glucuronide, and laricitrin-3-rhamnose-7-tri-hydroxycinnamic acid decreased while  
370 laricitrin-3-glucoside increased as a consequence of all the defoliation treatments. The flavan-3-ols  
371 were not affected by leaf removal. Although the concentration of total anthocyanins remained  
372 unchanged in the defoliated grapes, the phenolic profile of skins was modified by the different leaf  
373 removal treatments. In fact, it has been demonstrated that the exposure of berries to changes of  
374 temperature and solar radiation alters the partitioning of anthocyanins between the various forms  
375 (Tarara, Lee, Spayd & Scagel, 2008). In the present trial, defoliation determined the increase of  
376 concentration of the highest number of anthocyanin species in grapes exposed to a PPF level equal  
377 to that of non-defoliated grapes and to temperatures only slightly higher than those of the not  
378 defoliated grapes. In fact, the F grapes showed the increase of the 3-glucoside forms of delphinidin,  
379 cyanidin, petunidin+peonidin, and of peonidin-3-p-coumaroylglucoside concentrations. Grapes  
380 submitted to the highest PPF and temperatures exhibited the increases of concentration of only 2  
381 compounds (delphinidin-3-glucosides and malvidin-3-p-coumaroylglucoside in E grapes; petunidin-

382 +peonidin-3-glucoside and peonidin-3-p-coumaroylglucoside in E/W grapes). Malvidin-3-glucoside  
383 decreased with all the defoliation treatments, while malvidin-3-acetylglucoside, malvidin-3-  
384 caffeoylglucoside, and petunidin-3- p-coumaroylglucoside remained unchanged.

385 Concerning the phenolic composition of seeds and skins (Table 3), the E samples showed the lowest  
386 concentrations of all the phenolic classes in the seeds and the highest total phenolic content in the  
387 skins. Defoliation significantly affected the composition of flavan-3-ols contained in grape seeds by  
388 increasing their concentrations especially as a consequence of the E and F treatments.

389 The E and F grapes showed the highest pulp total phenolic contents ( $2474\pm 6$  and  $2477\pm 172$  mg/kg  
390 of dry matter, followed by the not defoliated ( $2147\pm 73$  mg/kg) and E/W ( $1943\pm 108$  mg/kg) grapes.

### 392 *3.3 Effects of defoliation treatments on wine quality*

The oenological parameters of the wines produced from defoliated grapes are listed in Table 4. The  
94 leaf removal led to wines higher in alcohol content than those produced from not defoliated vines.  
95 Furthermore, the alcohol content increased with the increase of the removed leaf area (from E to F).  
96 These data were in agreement with those concerning the total soluble solids of the grapes and with  
the findings of Diago, Vilanova & Tardaguila (2010), who observed higher sugar and alcohol  
content in wines from the varieties Tempranillo, Graciano, and Mazuelo when plants were  
99 subjected to defoliation.

00 The highest values of dry matter were observed in wines from the vines submitted to the more  
01 severe defoliation. Since these data related well with the dry matter of the skins, this result could be  
02 due to the skin thickening (Pastore, Zenoni, Fasoli, Pezzotti, Tornielli & Filippetti, 2013).

The wines made from grapes of non-defoliated vines had the lowest volatile acidity, according to  
the findings of Ristic, Downey, Iland, Bindon, Francisi, Herderich & Robinson (2007) in Shiraz  
05 wines, while the highest values were observed in wines from grape subjected to leaf removal in the  
06 cluster area along the east side at complete veraison (E). The E wines also showed the lowest  
07 titratable acidity. The different behaviour showed by the east-side defoliation compared to other  
08 defoliation treatments, in terms of acidity, can be related to the different levels of irradiation and  
temperatures as already described in Table 1. In fact, a lower berry temperature leads to a higher  
10 preservation of the acidity level, as shown in previous studies (Morrison & Noble, 1990). Similar  
11 results were obtained by Ferrer, Pidocchi, Michelazzo, González G. & Carbonneau (2007), who  
12 explained that the high temperatures induced organic acids degradation.

13 The wine pH in the present study showed no significant differences between the various defoliation  
14 treatments ( $P<0.05$ ). Otero, Diago, Genisheva, Oliveira, Tubio, Álvarez & Vilanova (2010)  
reported that early defoliation had no influence on pH value in Albariño wines, and Diago,

416 Vilanova & Tardaguila (2010) affirmed that the pH in wines from Tempranillo, Graciano, and  
417 Mazuelo vines was not modified when plants underwent partial defoliation. Similar results were  
418 reported by Muñoz, Pérez, Pszczolkowski & Bordeu (2002) in experiments concerning Cabernet  
419 Sauvignon.

420 The amounts of the residual soluble solids were higher in the wines from the defoliated plants, in  
421 particular in E and F samples.

422 The titratable acidity in musts and wine is obviously mainly related to the accumulation of organic  
423 acids, especially tartaric and malic ones. Table 7 also shows the absence of significant differences  
424 among defoliation treatments for lactic, citric, pyruvic, and D-gluconic acids, while the lowest  
425 concentrations of tartaric and malic acids were found, respectively, in the F and E wines, in  
426 agreement with the results observed in the starting grapes.

427 Table 5 concerns the concentrations of specific phenolic classes, the distribution of anthocyanins  
428 among monomeric and polymeric forms, and the colour parameters. The E wines showed the  
429 highest concentrations of total anthocyanins, antocyanins sensitive to SO<sub>2</sub>, monomeric and small  
430 polymeric anthocyanins, while the highest concentrations of total flavonoids, flavonoids different  
431 from anthocyanins, and proanthocyanidins were detected in the F wines, and the highest total  
432 phenolic content was measured in the E/W samples. These results greatly differed from those  
433 detected on the grapes and already discussed. Nevertheless, it can be stated that partial defoliation  
434 had no marked effect on berry composition and volume but it generally improved wine quality  
435 (Hunter, De Villiers & Watts, 1991). Probably the different composition of the anthocyanin  
436 pigments in defoliated and non-defoliated grapes could have affected their extractability and  
437 stability during winemaking (Ristic, Downey, Iland, Bindon, Francisi, Herderich & Robinson.,  
438 2007). Furthermore, wine colour is the result of a complex series of reactions and is influenced by  
439 the amount and type of flavonoids in the fruit, the extent of extraction of these compounds during  
440 winemaking, and the stability of the pigments during fermentation and subsequent aging of the  
441 wine. While grape anthocyanins (especially monomeric) are initially the prominent contributor to  
442 wine colour, the levels and composition of other flavonoids such as tannins and flavonols in the  
443 fruit are also important as they influence anthocyanin stability both by acting as co-pigments and  
444 through the formation of stable adducts, such as the pigmented polymers. Many studies have shown  
445 that the level of polymerisation between anthocyanins and tannins and the stability of these  
446 pigments depends on their concentration and composition (Romero & Bakker, 2000). In the present  
447 study, the wine with the highest anthocyanin concentrations coincided with the experimental  
448 treatment in which the highest photosynthetic photon flux and the highest medium temperature at  
449 the bunch level were measured on the East side of the vines. The best regressions were just found

450 between the photosynthetic photon flux (sum of morning and afternoon measurements) on the east  
451 side of the vines and the total anthocyanins, according to the equation 1 ( $p < 0.05$ )

$$452 \quad 453 \quad [Totalanthocyanins] = 96.87 * \ln[Photosyntheticphotonflux] - 134.64 \quad R^2 = 0.950 \quad (1)$$

454  
455 and between the medium temperature on the east side of the vines and the total anthocyanins,  
456 according to the equation 2 ( $p < 0.05$ )

$$58 \quad [Totalanthocyanins] = 1645.4 * \ln[Mediumtemperature] - 535.3 \quad R^2 = 0.9722 \quad (2)$$

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18  
460 The total flavonoids showed an optimum regression with the photosynthetic photon flux (sum of  
0  
461 morning and afternoon measurements) on the west side of the vines, according to the equation 3 ( $p$   
62  $< 0.05$ )

$$463 \quad 464 \quad [Totalflavonoids] = 689.78 * \ln[Photosyntheticphotonflux] - 1707.5 \quad R^2 = 0.996 \quad (3)$$

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466 Table 5 also concerns some indexes showing different tannin attributes. The gelatin index measures  
467 the capacity of tannins to react with proteins, forming stable combinations and, since the maximum  
69 reactivity occurs with procyanidins that have a molecular weight around 2500 (eight flavanol units),  
70 it may be give an indication of astringency. The highest value of this index was shown by the F  
71 wines, in agreement with their highest proanthocyanidin contents and with the statement that tannin  
72 polymerization increase with aging. The ethanol index measures the condensed anthocyanin  
73 polysaccharides while the hydrochloric acid index measure the degree of polymerization of  
74 procyanidins. Both increase with aging. In the present study, there were no significant differences  
75 among wines for both the indices, and their intermediate values (the HCl index normally ranges  
76 from 5 to 40) are index of enough balanced wines. The PVPP index measure the amounts of  
77 anthocyanins bounded to tannins. According to the results of Table 5, they increased with  
78 defoliation, showing the highest concentrations in the E wines, which were also the wines with the  
79 highest concentration of total anthocyanins. Concerning the colour parameters, the only significant  
80 differences were found for the colour intensity, dAI% and dAT%. The first parameter exhibited the  
81 highest values in the E wines, in agreement with their higher anthocyanin (especially monomeric  
82 and small polymeric) contents. The F wines showed the lowest absorbance due to monomeric  
anthocyanins and the highest values absorbance due to polymeric pigments decolorized with SO<sub>2</sub>.



483 Also the specific phenolic profiles of wines strongly differed from those detected on the grapes  
484 (Table 6) and the effects of defoliation treatments was mitigated by wine-making. Among phenolic  
485 acids, differences among samples were exhibited only by the caftaric and caffeic, whose highest  
486 concentrations were shown by the E wines. Defoliation determined significant increases of  
487 quercetin-3-glucoside among flavonols, of (-)-epicatechin among flavan-3-ols, and of malvidin-3-p-  
488 coumaroylglucoside among anthocyanins.

489 The standardising effect of wine-making can be also inferred by the application of PCA to the all  
490 the data set of grapes and wines, respectively (Fig. 1a and b). Concerning grapes, the first two  
491 components explained about 65% of the total variability in the data and the samples were  
492 homogeneously grouped according to the defoliation practices. Taking into account a cut-off  
493 absolute value of 0.15, the variables associated with the first components (0.15 cut-off absolute  
494 value) were pH, titratable acidity, tartaric and malic acids, total pulp phenolics, catechin, picatechin,  
495 procyanidins B1, B2, B3, and B4 of seeds, procyanidin B3 of skins, malvidin-, myricetin-, and  
496 laricitrin-3-glucosides, petunidin-3-p-coumaroylglucoside. The variables referred to the second  
497 components included pulp and skin dry matter, citric and D-gluconic acids, petunidin- and  
498 peonidin-3-glucosides, malvidin-3-acetylglucoside and caffeoylglucoside, myricetin-3-glucosides,  
499 quercetin-3-glucoside, glucuronide, and galactoside.

500 Concerning wines, the variance explained by the first two components was about 69%, but the  
501 samples appeared not clearly distinguishable from each other due to the same values exhibited by  
502 E/W and F wines for the first component and the same values showed by E, E/W, and N wines for  
503 the second components. The first component included the effects of variables such as pH, volatile  
504 acidity, tartaric acid, anthocyanins sensitive to SO<sub>2</sub>, colour intensity, monomeric and small  
505 polymeric anthocyanins, large-to-small polymeric anthocyanin ratio, and the anthocyanins  
506 cyanidin-3-glucosides, peonidin- and malvidin-3-p-coumaroylglucosides. Alcohol content, malic,  
507 citric, and D-gluconic acids, total flavonoids, flavonoids different from anthocyanins,  
508 proanthocyanidins, tonality, percentage of red colour due to flavilium cation, % of yellow and red  
509 components, monomeric anthocyanins, polymeric pigments sensitive to SO<sub>2</sub>, gelatin index,  
510 quercetin-3-glucoside, galactoside, glucuronide, and rhamnoside, procyanidins B1, B2, and B4,  
511 epicatechin, vitisin B were associated to the second components.

## 513 **Conclusions**

514 Defoliation did not influence the total anthocyanin concentration of grapes but increased their sugar  
515 content and decreased the concentration of total flavonoids, flavonoids different from anthocyanins,  
516 and total phenolics. The effects of defoliation strongly depended on the side where defoliation was

517 applied and the amounts of removed and retained leaves, since these variables affected grape  
518 quality by influencing the light intensity available at cluster level and the berry temperature. As a  
519 result, grapes samples harvested from non-defoliated vines and those deriving from the three  
520 defoliation treatments were homogeneously grouped according to the defoliation practices. Apart  
521 from the higher alcohol content, the effects of defoliation were less evident in the corresponding  
522 wines due to the standardising action of vinification process on concentrations of most phenolic  
523 compounds (in particular anthocyanins), state of condensation between tannins and anthocyanins,  
524 level of pigment polymerization and, consequently, on colour parameters. The best results in terms  
525 of anthocyanin concentration were detected in wines deriving from grapes exposed to the highest  
526 light intensity and the highest medium temperature as measured, in late summer, on the east side of  
527 the vines.

## 529 **Conflict of Interest**

530 The author has no conflict of interest to declare.

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644 **Legends to Figures**

645  
646 **Figure 1:** PCA scatter plots for projection on the factor plane of: **a)** grapes; **b)** wines.

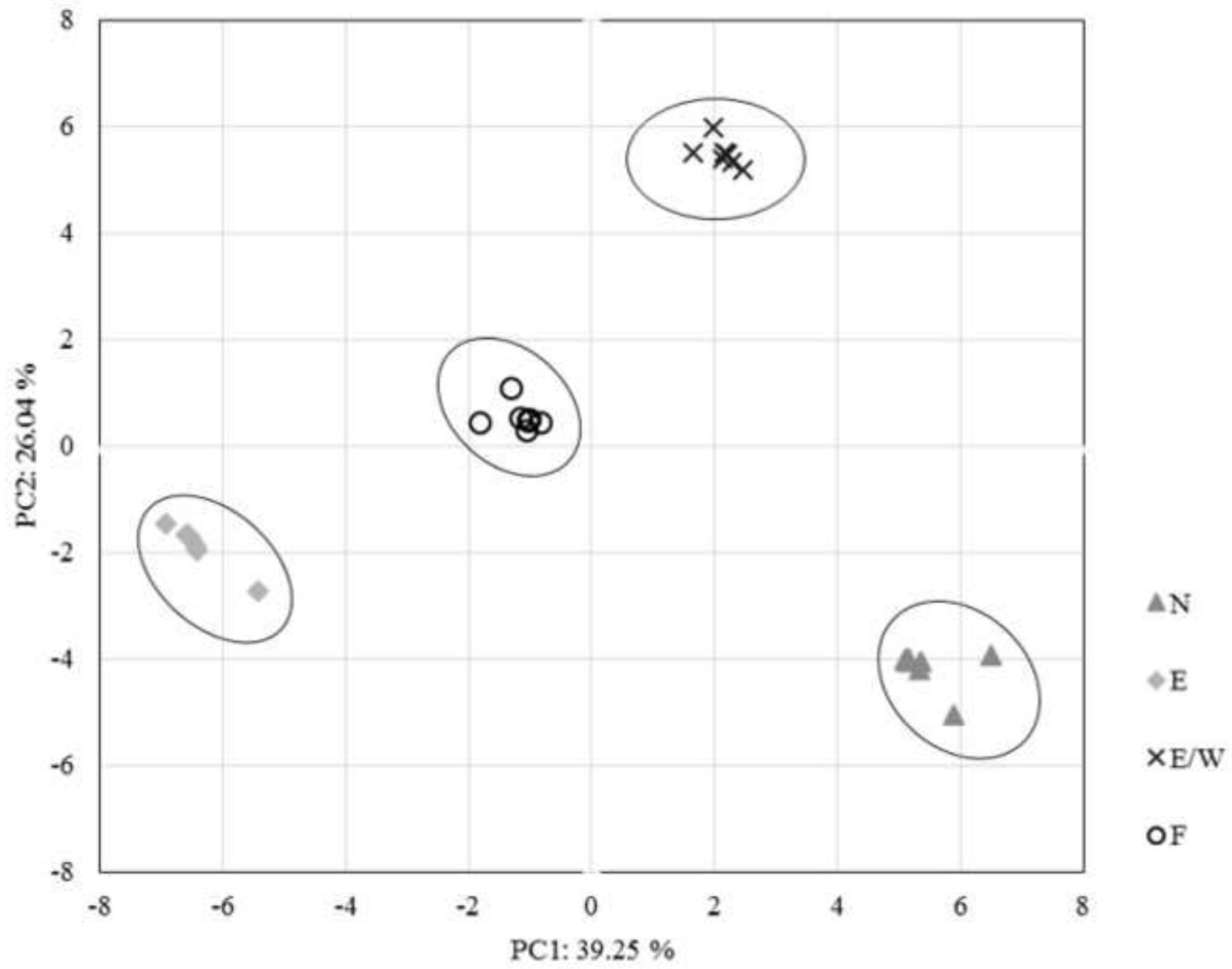
647 N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf  
648 removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal  
649 along the west side (at complete veraison) and in pre-harvest also along the east side.

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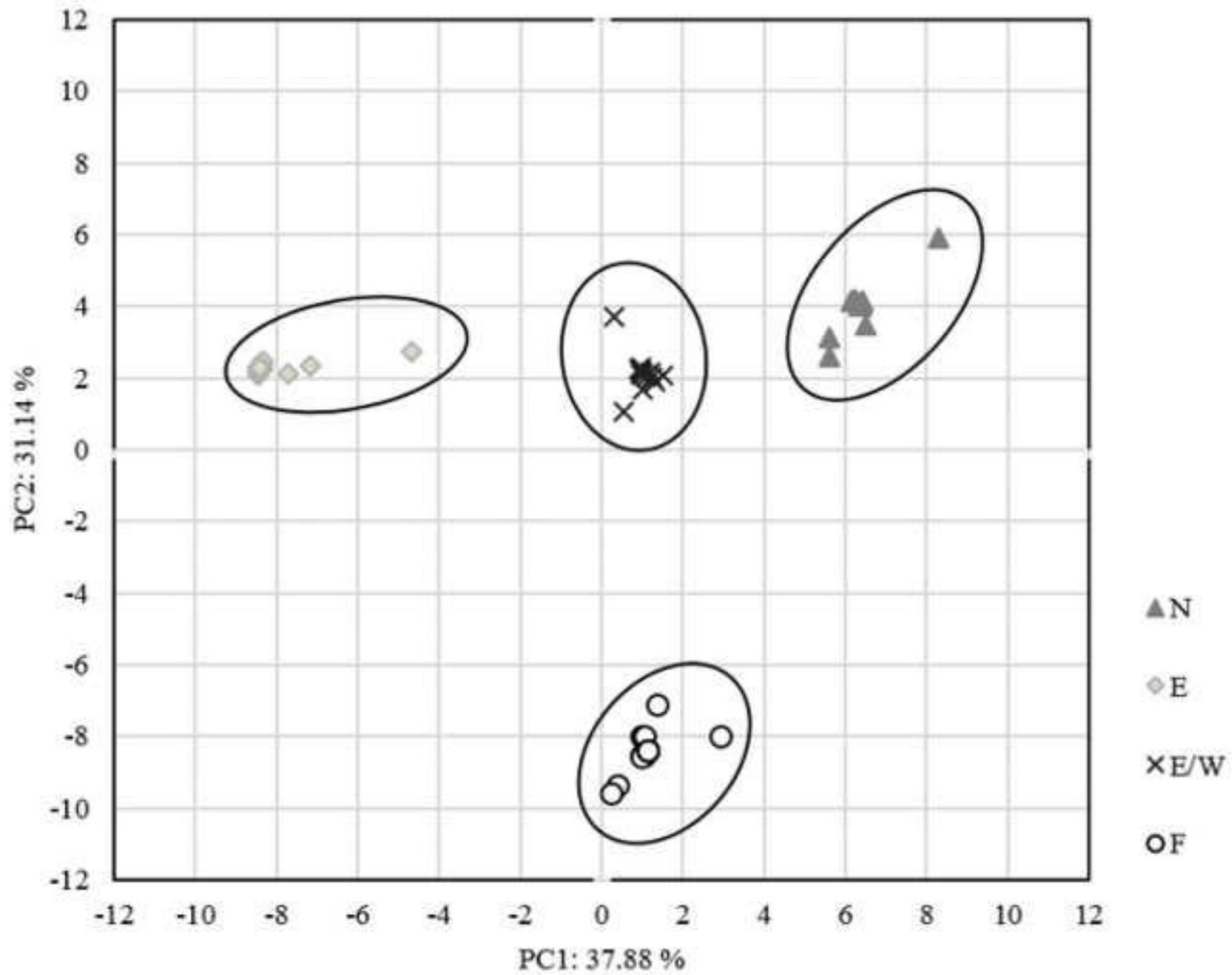
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Figure

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**Table 1** – Influence of fruit-zone leaf removal treatments on flux of photosynthetic active radiation available for bunches and on berry surface temperature of exposed bunches, in late summer (August 30<sup>th</sup> -31<sup>st</sup>, 15 after leaf removal).

Experimental Treatments	Photosynthetic Photon Flux (PPF, $\mu\text{mol m}^{-2} \text{s}^{-1}$ )				Temperature ( $^{\circ}\text{C}$ )			
	Morning		Afternoon		Morning		Afternoon	
	East	West	East	West	East	West	East	West
<b>N</b>	236.17 $\pm$ 28.03 <b>a</b>	57.60 $\pm$ 3.37 <b>a</b>	69.07 $\pm$ 3.84 <b>a</b>	133.70 $\pm$ 14.52 <b>a</b>	33.87 $\pm$ 0.17 <b>a</b>	32.67 $\pm$ 0.23 <b>b</b>	32.60 $\pm$ 0.12 <b>b</b>	37.07 $\pm$ 0.54 <b>a</b>
<b>E</b>	563.53 $\pm$ 34.16 <b>c</b>	57.20 $\pm$ 3.16 <b>a</b>	110.67 $\pm$ 3.65 <b>c</b>	141.77 $\pm$ 11.13 <b>a</b>	37.90 $\pm$ 0.41 <b>d</b>	33.17 $\pm$ 0.20 <b>b</b>	32.07 $\pm$ 0.15 <b>a</b>	36.07 $\pm$ 0.53 <b>a</b>
<b>E/W</b>	387.43 $\pm$ 27.34 <b>b</b>	74.13 $\pm$ 1.84 <b>b</b>	90.33 $\pm$ 2.89 <b>b</b>	243.43 $\pm$ 23.21 <b>b</b>	36.20 $\pm$ 0.42 <b>c</b>	32.43 $\pm$ 0.54 <b>b</b>	34.20 $\pm$ 0.19 <b>c</b>	40.53 $\pm$ 0.1 <b>b</b>
<b>F</b>	240.44 $\pm$ 31.02 <b>a</b>	93.03 $\pm$ 3.37 <b>c</b>	76.23 $\pm$ 3.63 <b>a</b>	586.43 $\pm$ 14.52 <b>c</b>	35.00 $\pm$ 0.32 <b>b</b>	31.27 $\pm$ 0.17 <b>a</b>	32.37 $\pm$ 0.15 <b>bc</b>	39.30 $\pm$ 0.65 <b>b</b>

N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and at pre-harvest also along the east side.

In column, different letters indicate significant differences at  $p < 0.05$  by LSD multiple range test.

**Table 2** – Leaf area removed and retained on vine after the fruit-zone leaf removal treatments.

Experimental treatments	Leaf area per vine			
	Removed		Retained	
	m <sup>2</sup>	% <sup>x</sup>	m <sup>2</sup>	% <sup>x</sup>
<b>N</b>	- <b>a</b>	- <b>a</b>	8.71 ± 0.88 <b>b</b>	100.00 <b>d</b>
<b>E</b>	0.67 ± 0.10 <b>b</b>	7.73 ± 1.18 <b>b</b>	8.03 ± 0.10 <b>a</b>	92.27 ± 1.18 <b>c</b>
<b>E/W</b>	0.95 ± 0.13 <b>c</b>	10.90 ± 1.44 <b>c</b>	7.75 ± 0.13 <b>a</b>	89.10 ± 1.44 <b>b</b>
<b>F</b>	0.40 <sup>y</sup> ± 0.14 <b>b</b>	4.69 <sup>y</sup> ± 0.16 <b>b</b>	8.31 <sup>y</sup> ± 0.15 <b>a</b>	95.41 <sup>y</sup> ± 2.50 <b>c</b>
	1.35 <sup>z</sup> ± 0.18 <b>d</b>	15.57 <sup>z</sup> ± 2.06 <b>d</b>	7.35 <sup>z</sup> ± 0.18 <b>a</b>	84.43 <sup>z</sup> ± 2.06 <b>a</b>

<sup>x</sup> percentage on total leaf area per vine

<sup>y</sup> first defoliation step

<sup>z</sup> second defoliation step

N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and at pre-harvest also along the east side.

In column, different letters indicate significant differences at  $p < 0.05$  by LSD multiple range test.

**Table 3** – Effect of fruit-zone leaf removal treatments on phenolic composition and profile of skins and seeds of *Nero di Troia* grape.

	Experimental Treatments			
	N	E	E/W	F
<b>SKINS</b>				
Phenolic classes				
<i>Total Anthocyanins (mg malvidin-3-glucoside/kg dry skins)</i>	31296 ± 1629	26192 ± 5499	27837 ± 4390	29713 ± 3168
<i>Total Flavonoids (mg (+)-catechin/kg dry skins)</i>	90574 ± 5930 <b>b</b>	70939 ± 7592 <b>a</b>	74880 ± 14891 <b>a</b>	83666 ± 7337 <b>ab</b>
<i>Flavonoids different from anthocyanins (mg (+)-catechin/kg dry skins)</i>	45009 ± 7069 <b>b</b>	32805 ± 797 <b>a</b>	34351 ± 9407 <b>a</b>	40406 ± 3148 <b>ab</b>
<i>Flavans reactive with vanillin (mg (+)-catechin/kg dry skins)</i>	31531 ± 2788	30488 ± 12147	27422 ± 5705	28804 ± 3843
<i>Proanthocyanidins (mg cyanidin chloride/kg dry skins)</i>	48242 ± 8557	51304 ± 14363	52180 ± 7895	44568 ± 9755
<i>Total Phenolic Compounds (mg gallic acid/kg dry skins)</i>	77478 ± 3229 <b>b</b>	65395 ± 16294 <b>a</b>	69215 ± 11474 <b>ab</b>	74781 ± 6212 <b>ab</b>
Phenolic profiles				
<i>Flavonols (mg QE/kg dry skins)</i>				
Myricetin-3-glc	166.16 ± 1.22 <b>b</b>	106.62 ± 16.31 <b>a</b>	104.07 ± 16.24 <b>a</b>	122.60 ± 5.86 <b>a</b>
Quercetin-3-glc	52.49 ± 2.77 <b>c</b>	35.12 ± 5.94 <b>b</b>	23.72 ± 4.45 <b>a</b>	26.56 ± 1.84 <b>ab</b>
Quercetin-3-gler	164.23 ± 12.38 <b>b</b>	144.00 ± 27.97 <b>b</b>	74.62 ± 6.44 <b>a</b>	123.82 ± 7.63 <b>b</b>
Quercetin-3-galac	174.15 ± 31.99	163.30 ± 32.72	154.43 ± 27.32	153.30 ± 19.00
Laricitrin-3-glc	38.14 ± 2.57 <b>a</b>	62.15 ± 0.33 <b>c</b>	47.93 ± 3.13 <b>b</b>	53.41 ± 2.77 <b>b</b>
Syringetin-3-galac	35.73 ± 0.84	31.65 ± 1.49	39.82 ± 7.89	31.12 ± 0.30
Laricitrin-3-rhamnose-7-tri-hydroxycinnamic acid	1124.22 ± 70.99 <b>b</b>	919.50 ± 110.89 <b>b</b>	558.02 ± 85.67 <b>a</b>	639.24 ± 92.27 <b>a</b>
<i>Flavan-3-ols (mg CE/kg dry skins)</i>				
Procyanidin B3	77.08 ± 6.90	57.26 ± 10.64	64.89 ± 4.03	58.01 ± 7.48
(+)-Catechin	61.01 ± 6.74	69.88 ± 13.39	62.80 ± 4.67	60.61 ± 4.49
<i>Anthocyanins (mg ME/kg dry skins)</i>				
Dp-3-glc	112.48 ± 20.81 <b>a</b>	173.09 ± 4.50 <b>b</b>	118.77 ± 0.63 <b>a</b>	186.18 ± 31.72 <b>b</b>
Cy-3-glc	285.76 ± 2.38 <b>c</b>	126.30 ± 0.26 <b>a</b>	211.80 ± 21.91 <b>b</b>	327.51 ± 15.70 <b>d</b>
Pt-3-glc + Pn-3-glc	1512.85 ± 115.96 <b>b</b>	1070.52 ± 185.70 <b>a</b>	2698.75 ± 140.48 <b>c</b>	2390.83 ± 25.36 <b>c</b>
Mv-3-glc	5887.29 ± 317.52 <b>b</b>	4641.30 ± 552.06 <b>a</b>	5298.63 ± 92.80 <b>ab</b>	5279.52 ± 439.17 <b>ab</b>
Mv-3-acetylglc	3659.60 ± 651.52	3003.86 ± 339.13	2635.92 ± 204.64	3035.35 ± 455.89

Mv-3-caffeoylglc	322.16 ± 37.06	320.53 ± 43.63	270.06 ± 20.79	263.42 ± 12.83
Pt-3- <i>p</i> -coumglc	286.24 ± 22.80	342.23 ± 18.87	306.66 ± 60.71	364.64 ± 69.68
Pn-3- <i>p</i> -coumglc	118.45 ± 1.57 <b>a</b>	153.70 ± 2.17 <b>a</b>	454.30 ± 44.96 <b>b</b>	493.41 ± 0.62 <b>b</b>
Mv-3- <i>p</i> -coumglc	6647.31 ± 324.88	7540.75 ± 622.15 <b>c</b>	4782.58 ± 550.49 <b>a</b>	5616.91 ± 622.64
	<b>bc</b>		<b>ab</b>	<b>ab</b>
<b>SEEDS</b>				
Phenolic classes				
<i>Total Flavonoids (mg (+)-catechin/kg dry matter)</i>	132416 ± 34576 <b>b</b>	92938 ± 9630 <b>a</b>	104934 ± 11981 <b>ab</b>	126495 ± 16322 <b>b</b>
<i>Flavans reactive with vanillin (mg (+)-catechin/kg dry matter)</i>	78750 ± 21412 <b>c</b>	56509 ± 10159 <b>a</b>	62645 ± 3294 <b>ab</b>	72672 ± 5475 <b>bc</b>
<i>Proanthocyanidins (mg cyanidin chloride/kg dry matter)</i>	104934 ± 11981 <b>ab</b>	131117 ± 13586 <b>a</b>	132941 ± 1533 <b>a</b>	223416 ± 41269 <b>b</b>
<i>Total Phenolic Compounds (mg gallic acid/kg dry matter)</i>	164974 ± 34821 <b>b</b>	126447 ± 3218 <b>a</b>	141368 ± 7023 <b>a</b>	167942 ± 11672 <b>b</b>
Phenolic profiles				
<i>Flavan-3-ols (mg CE/kg dry seeds)</i>				
Procyanidin B3	851.98 ± 1.04 <b>a</b>	4867.19 ± 993.34 <b>c</b>	421.83 ± 36.18 <b>a</b>	2720.23 ± 335.71 <b>b</b>
(+)-Catechin	1525.93 ± 26.85 <b>b</b>	6375.43 ± 522.94 <b>d</b>	586.66 ± 71.20 <b>a</b>	3705.21 ± 218.27 <b>c</b>
Procyanidin	319.69 ± 14.99 <b>a</b>	2248.72 ± 70.48 <b>c</b>	631.98 ± 14.63 <b>b</b>	2651.20 ± 106.13 <b>d</b>
Procyanidin B4	585.61 ± 59.47 <b>a</b>	3951.59 ± 688.09 <b>c</b>	1254.64 ± 31.61 <b>ab</b>	1570.89 ± 143.77 <b>b</b>
(-)-Epicatechin	1290.23 ± 52.77 <b>a</b>	6519.57 ± 820.57 <b>c</b>	744.77 ± 144.78 <b>a</b>	2891.24 ± 320.88 <b>b</b>
Procyanidin B2	311.96 ± 22.56 <b>a</b>	1926.20 ± 298.10 <b>c</b>	596.29 ± 13.91 <b>ab</b>	864.78 ± 22.66 <b>b</b>
(-)-Epicatechin-3- <i>O</i> -gallate	421.01 ± 59.30 <b>a</b>	1765.37 ± 196.70 <b>c</b>	699.25 ± 79.30 <b>a</b>	1089.44 ± 138.60 <b>b</b>

N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and at pre-harvest also along the east side.

glc: glucoside, glcr: glucuronide, galac: galactoside, Dp: delphinidin, Cy: cyanidin, Pt: petunidin, Pn: peonidin, Mv: malvidin, acetylglc: acetylglucoside, *p*-coumglc: *p*-coumaroylglucoside, caffeoylglc: caffeoylglucoside.

In row, different letters indicate significant differences at  $p < 0.05$  by LSD multiple range test.

**Table 4** – Effect of fruit-zone leaf removal treatments on quali-quantitative characteristics and organic acid content of Nero di Troia wines at racking.

	Experimental Treatments			
	N	E	E/W	F
<b>OENOLOGICAL PARAMETERS</b>				
Alcohol (% vol)	11.44 ± 0.11 a	11.85 ± 0.11 b	11.73 ± 0.07 b	12.25 ± 0.26 c
Dry Matter (g/L)	33.7 ± 2.2 ab	32.8 ± 0.2 ab	30.3 ± 3.7 a	35.3 ± 1.5 b
Volatile Acidity (g acetic acid/L)	0.12 ± 0.01 a	0.25 ± 0.03 c	0.16 ± 0.01 b	0.17 ± 0.01 b
Titrateable Acidity (g tartaric acid/L)	6.46 ± 0.10 b	6.14 ± 0.02 a	6.47 ± 0.08 b	6.59 ± 0.19 b
pH	3.70 ± 0.05	3.77 ± 0.01	3.71 ± 0.02	3.72 ± 0.08
Total Soluble Solids (°Brix)	7.0 ± 0.3 a	7.6 ± 0.2 b	7.1 ± 0.3 ab	7.5 ± 0.3 b
Acetaldehyde (mg/L)	52 ± 3	61 ± 4	57 ± 8	57 ± 9
<b>ORGANIC ACIDS (expresses per L of wine)</b>				
Tartaric acid	2.40 ± 0.03 b	2.43 ± 0.10 b	2.40 ± 0.04 b	2.27 ± 0.05 a
L-Malic acid	2.66 ± 0.27 ab	2.48 ± 0.03 a	2.66 ± 0.04 ab	2.86 ± 0.12 b
L-Lactic acid	0.05 ± 0.00	0.05 ± 0.01	0.07 ± 0.01	0.06 ± 0.01
Acetic acid	0.11 ± 0.00 b	0.12 ± 0.01 b	0.13 ± 0.01 c	0.09 ± 0.01 a
Citric acid	0.58 ± 0.05	0.58 ± 0.06	0.57 ± 0.01	0.66 ± 0.06
Pyruvic acid	0.06 ± 0.01	0.06 ± 0.00	0.06 ± 0.01	0.06 ± 0.00
D-Gluconic acid	0.70 ± 0.16	0.65 ± 0.07	0.58 ± 0.08	1.03 ± 0.28

N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and at pre-harvest also along the east side.

In row, different letters indicate significant differences at  $p < 0.05$  by LSD multiple range test.

**Table 5** – Effect of fruit-zone leaf removal treatments on phenolic composition, monomeric and polymeric pigments, structure indices and colour parameters of Nero di Troia wines at racking.

	Experimental Treatments			
	N	E	E/W	F
<b>PHENOLIC COMPOSITION (expresses per L of wine)</b>				
Total Anthocyanins (mg malvidin-3-glucoside/L)	411 ± 56 <b>a</b>	491 ± 50 <b>b</b>	472 ± 9 <b>ab</b>	428 ± 24 <b>a</b>
Anthocyanins sensitive to SO <sub>2</sub> (mg malvidin-3-glucoside/L)	393 ± 24 <b>a</b>	486 ± 42 <b>b</b>	393 ± 19 <b>a</b>	384 ± 26 <b>a</b>
Monomeric anthocyanins (mg malvidin-3-glucoside/L)	255 ± 11 <b>b</b>	286 ± 18 <b>c</b>	243 ± 13 <b>ab</b>	236 ± 16 <b>a</b>
Total Flavonoids (mg (+)-catechin/L)	1899 ± 265 <b>a</b>	1931 ± 379 <b>a</b>	2301 ± 280 <b>ab</b>	2776 ± 319 <b>b</b>
Flavonoids different from anthocyanins (mg (+)-catechin/L)	1300 ± 184 <b>a</b>	1096 ± 320 <b>a</b>	1615 ± 285 <b>a</b>	2153 ± 353 <b>b</b>
Flavans reactive with vanillin (mg (+)-catechin/L)	1537 ± 92 <b>b</b>	1256 ± 144 <b>a</b>	1466 ± 176 <b>b</b>	1189 ± 185 <b>a</b>
Proanthocyanidins (mg cyanidin chloride/L)	2604 ± 148 <b>a</b>	2513 ± 179 <b>a</b>	2422 ± 338 <b>a</b>	5601 ± 791 <b>b</b>
Total Phenolic Compounds (mg gallic acid/L)	2379 ± 72 <b>a</b>	2420 ± 105 <b>ab</b>	2520 ± 251 <b>b</b>	2438 ± 202 <b>ab</b>
<b>PIGMENTS AND STRUCTURE INDICES</b>				
MP	0.54 ± 0.06 <b>a</b>	0.72 ± 0.12 <b>b</b>	0.63 ± 0.01 <b>a</b>	0.64 ± 0.03 <b>a</b>
SPP	0.16 ± 0.02 <b>a</b>	0.24 ± 0.03 <b>c</b>	0.19 ± 0.00 <b>ab</b>	0.21 ± 0.01 <b>b</b>
LPP	0.12 ± 0.02	0.13 ± 0.01	0.13 ± 0.02	0.13 ± 0.03
I <sub>gelatin</sub>	62.8 ± 4.1 <b>a</b>	58.9 ± 6.6 <b>a</b>	60.3 ± 5.0 <b>a</b>	87.4 ± 1.7 <b>b</b>
I <sub>EtOH</sub>	20.7 ± 3.7	24.2 ± 3.2	20.6 ± 0.6	21.0 ± 2.3
I <sub>HCl</sub>	28.3 ± 4.5	25.4 ± 4.7	29.5 ± 4.6	31.1 ± 5.3
I <sub>PVPP</sub>	35.0 ± 2.0 <b>a</b>	40.9 ± 3.0 <b>c</b>	38.2 ± 0.6 <b>b</b>	38.6 ± 2.6 <b>b</b>
<b>COLOUR PARAMETERS</b>				
CI	5.670 ± 0.585 <b>a</b>	7.340 ± 1.024 <b>b</b>	6.431 ± 0.042 <b>ab</b>	6.298 ± 0.092 <b>a</b>
T	0.565 ± 0.002	0.566 ± 0.013	0.565 ± 0.004	0.593 ± 0.044
dA(%)	63.1 ± 0.7	62.6 ± 0.7	62.6 ± 0.3	61.1 ± 3.1

<b>% yellow</b>	32.5 ± 0.1	32.4 ± 0.5	32.3 ± 0.2	33.3 ± 1.3
<b>% red</b>	57.5 ± 0.4	57.2 ± 0.4	57.2 ± 0.2	56.3 ± 2.0
<b>% blue</b>	10.0 ± 0.6	10.4 ± 0.2	10.5 ± 0.2	10.3 ± 0.7
<b>dAI%</b>	10.6 ± 0.2 <b>b</b>	10.5 ± 0.3 <b>b</b>	10.9 ± 0.0 <b>b</b>	7.8 ± 1.6 <b>a</b>
<b>dAT%</b>	89.4 ± 0.2 <b>a</b>	89.5 ± 0.2 <b>a</b>	89.2 ± 0.1 <b>a</b>	92.2 ± 1.6 <b>b</b>
<b>dTAT%</b>	0.0	0.0	0.0	0.0

MP: absorbance at 520 nm due to monomeric pigments; SPP: absorbance 520 nm due to small polymeric pigments; LPP: absorbance 520 nm due to large polymeric pigments.

I<sub>gelatin</sub>: gelatin index; I<sub>EtOH</sub>: ethanol index; I<sub>HCl</sub>: hydrochloric acid index; I<sub>PVPP</sub>: PVPP index.

CI: color intensity; T: tonality; dA(%): percentage of red color due to flavilium cation of free and combined anthocyanins; % yellow: percentage of yellow component; % red: percentage of red component; % blue: percentage of blue component; dAI: absorbance at 520 nm due to monomeric anthocyanins; dAT: absorbance at 520 nm due to polymeric pigments decolorized with SO<sub>2</sub>; dTAT: absorbance at 520 nm due to polymeric pigments not decolorized.

N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and at pre-harvest also along the east side.

In row, different letters indicate significant differences at  $p < 0.05$  by LSD multiple range test.

**Table 6** – Effect of fruit-zone leaf removal treatments on the phenolic profile of Nero di Troia wines at racking.

Phenolic compounds	t <sub>r</sub> (min)	MS (m/z)	MS–MS fragments (m/z)	N	E	E/W	F
<b>Phenolic acids (mg GAE/L; mg CAE/L)</b>		[M-H] <sup>-</sup>					
Gallic acid	5.6	169	125	18.8 ± 1.5	18.6 ± 1.1	19.0 ± 1.8	19.4 ± 0.9
Caftaric acid	12.5	311	179, 149	9.7 ± 0.5 <b>a</b>	17.8 ± 3.2 <b>b</b>	11.1 ± 0.4 <b>a</b>	11.2 ± 1.7 <b>a</b>
Caffeic acid	12.8	179	135	13.5 ± 0.7 <b>a</b>	17.7 <b>c</b>	14.9 ± 0.2 <b>b</b>	15.6 ± 0.6 <b>b</b>
<i>p</i> -Coumaric acid	15.3	163	119	6.7 ± 0.4	7.3 ± 1.9	7.3 ± 0.1	7.0 ± 0.1
Ferulic acid	17.2	193	178, 149, 134	6.8 ± 0.3	7.7 ± 1.3	7.6 ± 0.3	7.0 ± 0.3
<b>Stilbens (mg RE/L)</b>		[M-H] <sup>-</sup>					
<i>cis</i> -Piceid	24.7	389	227	0.6 ± 0.1	0.8 ± 0.1	0.7	0.8 ± 0.1
<i>trans</i> -Piceid	35.3	389	227	0.2	0.4	0.3	0.3
<b>Flavonols (mg QE/L)</b>		[M-H] <sup>-</sup>					
Myricetin-3-glc	22.3	479	316/317	6.6 ± 1.7	9.7 ± 2.4	10.1 ± 1.8	10.8 ± 1.2
Myricetin-3-rha	26.2	463	317	0.5 ± 0.1	0.6 ± 0.1	0.4	0.4
Quercetin-3-glc	27.4	463	301	0.6 ± 0.1 <b>a</b>	1.1 <b>b</b>	1.2 ± 0.2 <b>bc</b>	1.5 <b>c</b>
Quercetin-3-glcr	28.6	477	301	3.9 ± 0.1	3.6 ± 0.5	3.4 ± 0.6	4.3 ± 0.2
Quercetin-3-galac	28.9	463	301	3.3 <b>a</b>	3.6 ± 0.6 <b>a</b>	4.5 ± 0.8 <b>a</b>	7.4 ± 0.6 <b>b</b>
Laricitrin-3-glc	30.7	493	331	2.7 ± 0.2	3.3 ± 0.4	3.9 ± 0.5	3.4 ± 0.6
Quercetin-3-rha	35.1	447	301	0.8 ± 0.2 <b>a</b>	1.1 ± 0.2 <b>a</b>	0.9 <b>a</b>	1.5 <b>b</b>
Syringetin-3-galac	36.5	507	344/345	2.8 ± 0.1	3.4 ± 0.6	3.6 ± 0.4	3.0 ± 0.4
Laricitrin-3-rhamnose-7-tri-hydroxycinnamic acid	37.5	655	509, 501, 475, 347, 329, 314, 303	1.6	2.0 ± 0.4	1.9 ± 0.2	1.9 ± 0.1
<b>Flavan-3-ols (mg CE/L)</b>		[M-H] <sup>-</sup>					
Procyanidin B3	13.5	577	451, 425, 407, 289	108.4 ± 0.9	106.1 ± 8.2	117.7 ± 7.2	103.9 ± 10.9
(+)-Catechin	14.5	289	245, 205, 179	13.0 ± 3.4	12.2 ± 1.4	15.0 ± 4.8	13.6 ± 0.3
Procyanidin B1	15.7	577	451, 425, 407, 289	5.0 ± 1.2 <b>a</b>	8.3 ± 1.1 <b>b</b>	4.9 ± 0.6 <b>a</b>	10.2 ± 0.1 <b>b</b>
Procyanidin B4	16.4	577	451, 425, 407, 289	11.3 ± 0.8 <b>a</b>	11.0 ± 0.4 <b>a</b>	11.3 ± 0.3 <b>a</b>	15.0 ± 0.2 <b>b</b>
(-)-Epicatechin	17.5	289	245, 205, 179	12.4 ± 3.0 <b>a</b>	14.2 ± 3.0 <b>ab</b>	18.4 ± 1.2 <b>ab</b>	21.9 ± 3.8 <b>b</b>



Procyanidin B2	20.0	577	451, 425, 407, 289	8.8 ± 1.3	8.3 ± 0.7	8.3 ± 0.1	7.6 ± 1.2
<i>Anthocyanins (mg ME/L)</i>		[M-2H] <sup>-</sup>					
Dp-3-glc	15.3	463	301	9.7 ± 1.6	13.8 ± 4.4	8.6 ± 0.1	10.6 ± 1.7
Cy-3-glc	17.2	447	285	0.4 ± 0.1 <b>ab</b>	0.7 ± 0.2 <b>b</b>	0.3 <b>a</b>	0.6 ± 0.1 <b>ab</b>
Pt-3-glc	18.0	477	315	17.1 ± 3.2	24.4 ± 7.7	15.5 ± 1.5	18.1 ± 4.7
Pn-3-glc	20.4	461	299	6.8 ± 1.2	11.3 ± 2.4	7.5 ± 0.4	9.1 ± 2.1
Mv-3-glc	21.5	491	329	141.2 ± 6.5	171.8 ± 32.6	135.9 ± 11.8	138.8 ± 9.2
Dp-3-acetylglc	25.1	505	463, 301	1.8 ± 0.4	2.4 ± 0.5	2.0	2.1 ± 0.1
Pyrano-Mv-3-glc (Vitisin B)	27.6	515	353	0.3 ± 0.1 <b>c</b>	0.3 <b>c</b>	0.2 <b>b</b>	nd <b>a</b>
Pt-3-acetylglc	32.8	519	477, 315	4.6 ± 0.4	6.7 ± 2.2	4.0 ± 0.5	4.6 ± 0.4
Pn-3-acetylglc	35.8	503	299	5.8	6.5 ± 0.4	7.1 ± 0.9	6.0 ± 1.5
Mv-3-acetylglc	36.1	533	329	65.6 ± 2.1	70.2 ± 12.3	62.3 ± 1.4	58.7 ± 5.9
Pyrano-Mv-3- <i>p</i> -coumglc ( <i>p</i> -coum-Vitisin B)	37.1	661	353	0.6	0.8 ± 0.2	0.7 ± 0.1	0.7 ± 0.1
Mv-3-caffeoylglc	38.1	653	491, 329	1.7 ± 0.1	2.1 ± 0.3	1.8 ± 0.1	2.0 ± 0.1
Pt-3- <i>p</i> -coumglc	38.8	623	477, 315	2.3	3.0 ± 0.6	2.6	2.5
Mv-coumglc-8-ethyl-(epi)cat	41.9	953	801, 663, 645, 355	4.5	5.4 ± 1.4	4.8 ± 0.9	5.2 ± 0.5
Pn-3- <i>p</i> -coumglc	42.4	607	299	18.4 ± 0.9 <b>a</b>	22.6 ± 2.7 <b>b</b>	19.8 ± 0.4 <b>ab</b>	20.5 ± 0.3 <b>ab</b>
Mv-3- <i>p</i> -coumglc	42.4	637	491, 329				

nd: not detected.

N: no leaf removal; E: leaf removal in the area of the clusters along the east side (at complete veraison); E/W: leaf removal in the area of the clusters along the east and west side (at complete veraison); F: almost complete leaf removal along the west side (at complete veraison) and at pre-harvest also along the east side.

glc: glucoside, glcr: glucuronide, gall: gallate, rha: rhamnoside, galac: galactoside, Dp: delphinidin, Cy: cyanidin, Pt: petunidin, Pn: peonidin, Mv: malvidin, acetylglc: acetylglucoside, *p*-coumglc: *p*-coumaroylglucoside, caffeoylglc: caffeoylglucoside, cat: catechin.

In row, different letters indicate significant differences at  $p < 0.05$  by LSD multiple range test.