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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 reduces 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 changes 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.

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

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

2. Materials and methods

2.1 Vineyard site and plant material

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

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

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

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

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

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

In the year of the trial, the number of bunches per vine was 32±1.

Fertilization was provided by means of soil applications, foliar nutrition and fertigation, with a total 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; moreover, foliar application provided also about 50 kg alginic acid and 125 kg organic matter (both strong water soluble) per hectare.

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

2.2 Leaf removal treatments and leaf area evaluation

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

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

Defoliation percentage was visually estimated.

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

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

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

2.3 Field measurements

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

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

When the East side of the canopy was fully lighted, the rate of photosynthetic active radiation (PAR) was measured as maximum photosynthetic photon flux (PPF) interceptable by orienting a solar bar (AccuPAR PAR/LAI LP-80, Decagon Dev. Inc. Pullman, WA, USA), and as PPF interceptable at the leaf surface of the East and of the West side of the vine canopy by positioning the solar bar along the canopy at 0.90 m above the cordon; 30 readings per type of measurements were recorded. The average values were the following: PPF max 1994.70 ± 2.63 μmol m^{-2} s^{-1}; PPF at East canopy side 1238.50 ± 6.71 μmol m^{-2} s^{-1}; PPF at West canopy side 95.10 ± 2.68μmol m^{-2} s^{-1}. 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 μmol m^{-2} s^{-1} in the non defoliated vines (control) to 563.53 μmol m^{-2} 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 μmol m^{-2} 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.

In order to evaluate if leaf removal influenced the vine water status, stem water potential (Ψstem) was measured under midday conditions according to Turner (1981); 10 measurements per replicate were taken.

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

2.4 Grapes and wine-making

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

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

2.5 Conventional analyses of grape and wine

The dry matter of the various parts of the berries were determined by separating skins from seeds and pulps and oven drying at 90°C until constant weight. Alcoholic strength at 20°C (expressed 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 concentration of sugars (glucose, fructose, and their ratio), organic acids (g/L) and acetaldehyde (mg/L) were determined through a Hyperlab automatic multi-parametric analyzer (Steroglass, San Martino in Campo, Perugia, Italy) by means of enzymatic kits. Dissolved oxygen (mg/L) was measured by using an LDO-HQ10 portable oxygen meter (Hach, Düsseldorf, Germany).

2.6 Extraction of phenolic fractions from grapes

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

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

The total phenolic content was measured at 765 nm through an UV–visible spectrophotometer (Cary 50 SCAN; Varian, Palo Alto, CA) according to the Folin–Ciocalteu method as reported by Singleton & Rossi (1965). Results were expressed as gallic acid equivalents (mg/L and mg/kg of grape). A calibration line was built on the basis of solutions of known and increasing concentrations of gallic acid (Extrasynthèse, Genay, France). The various phenolic classes (total anthocyanins, monomeric anthocyanins, total flavonoids, flavonoids different from anthocyanins, proanthocyanidins and flavans reactive with vanillin) were analysed according to the methods of Di Stefano, Cravero & Gentilizi (1989) and Di Stefano & Cravero (1991), while anthocyanins sensitive to SO_2 were determined according to Ribéreau-Gayon & Stonestreet (1965). When necessary, extracts were opportunely diluted with aliquots of the extraction solution. The results were expressed as mg per kg of skins, seeds, and pulps or per L of wine.

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

2.8 HPLC-DAD-ESI-MS/MS analysis of phenolics

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

the nitrogen flow rate was 8 L/min. The temperature of the drying gas was 350 °C. The monitored mass range was from m/z 50 to 1200. The wine samples were filtered through a 0.45 µm cellulose acetate filter prior to HPLC injection. Compounds identification was achieved by combining different information: elution pattern, UV-Vis and MS spectra, MS/MS fragmentation patterns and with the help of structural models already hypothesized in the literature. Quantitative determinations were made by using the external standard method with commercial standards. The calibration curves were obtained by injection of standard solutions under the same conditions of the samples analysed, over the range of concentrations observed. The compounds for which no standards were available were quantified on the curves of quercetin-3-rutinoside (flavonols and dihydroflavonols), trans-resveratrol (stilbenes), gallic acid (hydroxybenzoic acids), caffeic acid (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.

2.9 Statistical Analysis

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

3. Results and discussion

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

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

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

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

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

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

Type and intensity of leaf removal may affect vine water status. As summarized by Downey, 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 changing the skin structure (Roby & Matthews, 2004), while a direct effect on flavonoid biosynthesis is rarely admitted. In this trial, the vine midday stem water potential ranged between -0.95 ± 0,04 MPa in E/W (vines having the lowest total leaf area by the time of measurements) and -1.08 MPa in N (vines having the greatest transpiring leaf surface): according to Van Leeuwen, Trégoa, Choné, Bois, Pernet & Gaudillère (2009), all treatments had water status consistent with a moderate-to-weak water deficit.

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

3.2 Effects of defoliation treatments on grape quality

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

Non-defoliated and E/W defoliated grapes showed the highest titratable acidity (6.61±0.05 g tartaric acid/L), while the lowest values (6.00±0.01) were found in E grapes (which also showed the lowest malic acid concentration, 1.16±0.01 g/L) and in F grapes (which also had the lowest tartaric acid concentration, 2.07±0.04 g/L). This behaviour could be explained by the highest PPF densities received from the E and F samples (an average of 873.17 and 996,13 μmol m^{-2} s^{-1}, respectively). According to Valdivia (2001), bunch exposure to higher radiation (as it is the case of defoliated plants) increases fruit cell respiration, with a greater consumption of organic acids. The highest concentrations of citric and D-gluconic acid (0.35 and 0.23±0.03 g/L) were found in E/W grapes.

Low-light conditions are known to decrease the weight of grape skins and the skin to berry ratio. In fact, he highest and lowest skin dry matter percentages were founds in F (43.7±1.6) and N (34.4±1.1) grapes, respectively, in agreement with the finding of Keller, Arnink & Hrazdina (1998). The dry matter percentages of pulps and seeds did not depend on defoliation treatments and were in the ranges 14.9-18.9 and 48.2-69.5, respectively.

Leaf removal did not improve skin total anthocyanin concentration; differences among theses were small (max. 16%) and not significant (Table 3). This result is consistent with the sufficient light intensity available at the fruit-zone for all treatments and/or with the late time of the defoliation treatments (complete veraison). The concentrations of flavans reactive with vanilline, and proanthocyanidins were not affected by the defoliation treatments, whereas total flavonoids, flavonoids different from anthocyanins, and the total phenolic content were higher in the skins of berries of non-defoliated vines (Table 3). Nevertheless, the phenolic profile of skins was modified by defoliation (Table 3). In particular, the flavonols myricetin--3-glucoside, quercetin-3-glucoside, quercetin-3-glucuronide, and laricitrin-3-rhamnose-7-tri-hydroxycinnamic acid decreased while laricitrin-3-glucoside increased as a consequence of all the defoliation treatments. The flavan-3-ols were not affected by leaf removal. Although the concentration of total anthocyanins remained unchanged in the defoliated grapes, the phenolic profile of skins was modified by the different leaf removal treatments. In fact, it has been demonstrated that the exposure of berries to changes of temperature and solar radiation alters the partitioning of anthocyanins between the various forms (Tarara, Lee, Spayd & Scagel, 2008). In the present trial, defoliation determined the increase of concentration of the highest number of anthocyanin species in grapes exposed to a PPF level equal to that of non-defoliated grapes and to temperatures only slightly higher than those of the not defoliated grapes. In fact, the F grapes showed the increase of the 3-glucoside forms of delphinidin, cyanidin, petunidin+peonidin, and of peonidin-3-p-coumaroylglucoside concentrations. Grapes submitted to the highest PPF and temperatures exhibited the increases of concentration of only 2 compounds (delphinidin-3-glucosides and malvidin-3-p-coumaroylglucoside in E grapes; petunidin-

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

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

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

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 leaf removal led to wines higher in alcohol content than those produced from not defoliated vines. Furthermore, the alcohol content increased with the increase of the removed leaf area (from E to F). 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 subjected to defoliation.

The highest values of dry matter were observed in wines from the vines submitted to the more severe defoliation. Since these data related well with the dry matter of the skins, this result could be 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 wines, while the highest values were observed in wines from grape subjected to leaf removal in the cluster area along the east side at complete veraison (E). The E wines also showed the lowest titratable acidity. The different behaviour showed by the east-side defoliation compared to other 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 preservation of the acidity level, as shown in previous studies (Morrison & Noble, 1990). Similar results were obtained by Ferrer, Pidocchi, Michelazzo, González G. & Carbonneau (2007), who explained that the high temperatures induced organic acids degradation.

The wine pH in the present study showed no significant differences between the various defoliation 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,

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

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

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

Table 5 concerns the concentrations of specific phenolic classes, the distribution of anthocyanins among monomeric and polymeric forms, and the colour parameters. The E wines showed the highest concentrations of total anthocyanins, antocyanins sensitive to SO_2, monomeric and small polymeric anthocyanins, while the highest concentrations of total flavonoids, flavonoids different from anthocyanins, and proanthocyanidins were detected in the F wines, and the highest total phenolic content was measured in the E/W samples. These results greatly differed from those detected on the grapes and already discussed. Nevertheless, it can be stated that partial defoliation had no marked effect on berry composition and volume but it generally improved wine quality (Hunter, De Villiers & Watts, 1991). Probably the different composition of the anthocyanin pigments in defoliated and non-defoliated grapes could have affected their extractability and stability during winemaking (Ristic, Downey, Iland, Bindon, Francisi, Herderich & Robinson,, 2007). Furthermore, wine colour is the result of a complex series of reactions and is influenced by the amount and type of flavonoids in the fruit, the extent of extraction of these compounds during winemaking, and the stability of the pigments during fermentation and subsequent aging of the wine. While grape anthocyanins (especially monomeric) are initially the prominent contributor to wine colour, the levels and composition of other flavonoids such as tannins and flavonols in the fruit are also important as they influence anthocyanin stability both by acting as co-pigments and through the formation of stable adducts, such as the pigmented polymers. Many studies have shown that the level of polymerisation between anthocyanins and tannins and the stability of these pigments depends on their concentration and composition (Romero & Bakker, 2000). In the present study, the wine with the highest anthocyanin concentrations coincided with the experimental treatment in which the highest photosynthetic photon flux and the highest medium temperature at the bunch level were measured on the East side of the vines. The best regressions were just found

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

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

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

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

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

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

Table 5 also concerns some indexes showing different tannin attributes. The gelatin index measures the capacity of tannins to react with proteins, forming stable combinations and, since the maximum reactivity occurs with procyanidins that have a molecular weight around 2500 (eight flavanol units), it may be give an indication of astringency. The highest value of this index was shown by the F wines, in agreement with their highest proanthocyanidin contents and with the statement that tannin polymerization increase with aging. The ethanol index measures the condensed anthocyanin polysaccharides while the hydrochloric acid index measure the degree of polymerization of procyanidins. Both increase with aging. In the present study, there were no significant differences among wines for both the indices, and their intermediate values (the HCl index normally ranges from 5 to 40) are index of enough balanced wines. The PVPP index measure the amounts of anthocyanins bounded to tannins. According to the results of Table 5, they increased with defoliation, showing the highest concentrations in the E wines, which were also the wines with the highest concentration of total anthocyanins. Concerning the colour parameters, the only significant differences were found for the colour intensity, dAl% and dAT%. The first parameter exhibited the highest values in the E wines, in agreement with their higher anthocyanin (especially monomeric 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_2.

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

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

Concerning wines, the variance explained by the first two components was about 69%, but the samples appeared not clearly distinguishable from each other due to the same values exhibited by E/W and F wines for the first component and the same values showed by E, E/W, and N wines for the second components. The first component included the effects of variables such as pH, volatile acidity, tartaric acid, anthocyanins sensitive to SO_2, colour intensity, monomeric and small polymeric anthocyanins, large-to-small polymeric anthocyanin ratio, and the anthocyanins cyanidin-3-glucosides, peonidin- and malvidin-3-p-coumaroylglucosides. Alcohol content, malic, citric, and D-gluconic acids, total flavonoids, flavonoids different from anthocyanins, proanthocyanidins, tonality, percentage of red colour due to flavilium cation, % of yellow and red components, monomeric anthocyanins, polymeric pigments sensitive to SO_2, gelatin index, quercetin-3-glucoside, galactoside, glucuronide, and rhamnoside, procyanidins B1, B2, and B4, epicatechin, visitin B were associated to the second components.

Conclusions

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

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

Conflict of Interest

The author has no conflict of interest to declare.

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Legends to Figures

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

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 in pre-harvest also along the east side.

Figure

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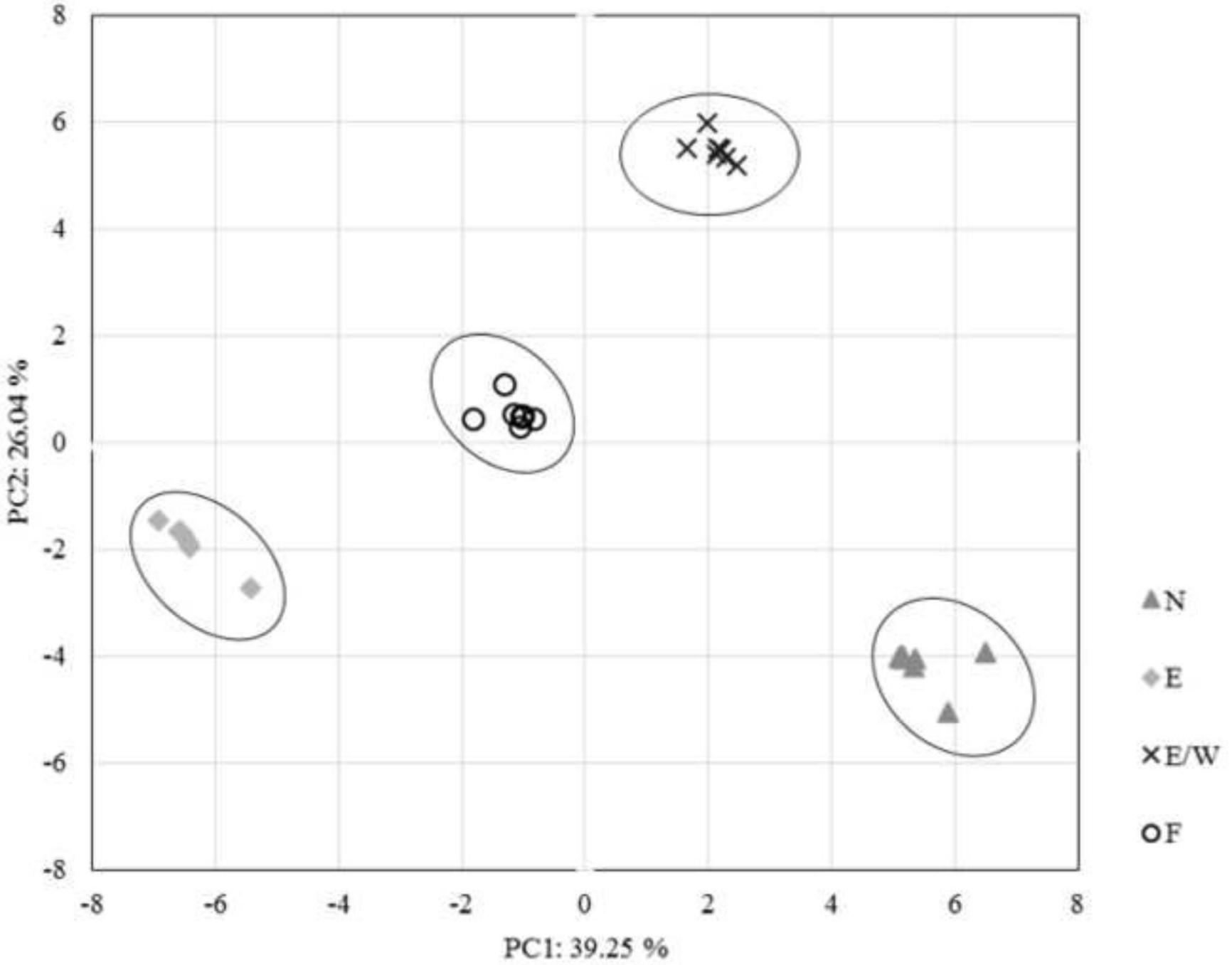

Figure

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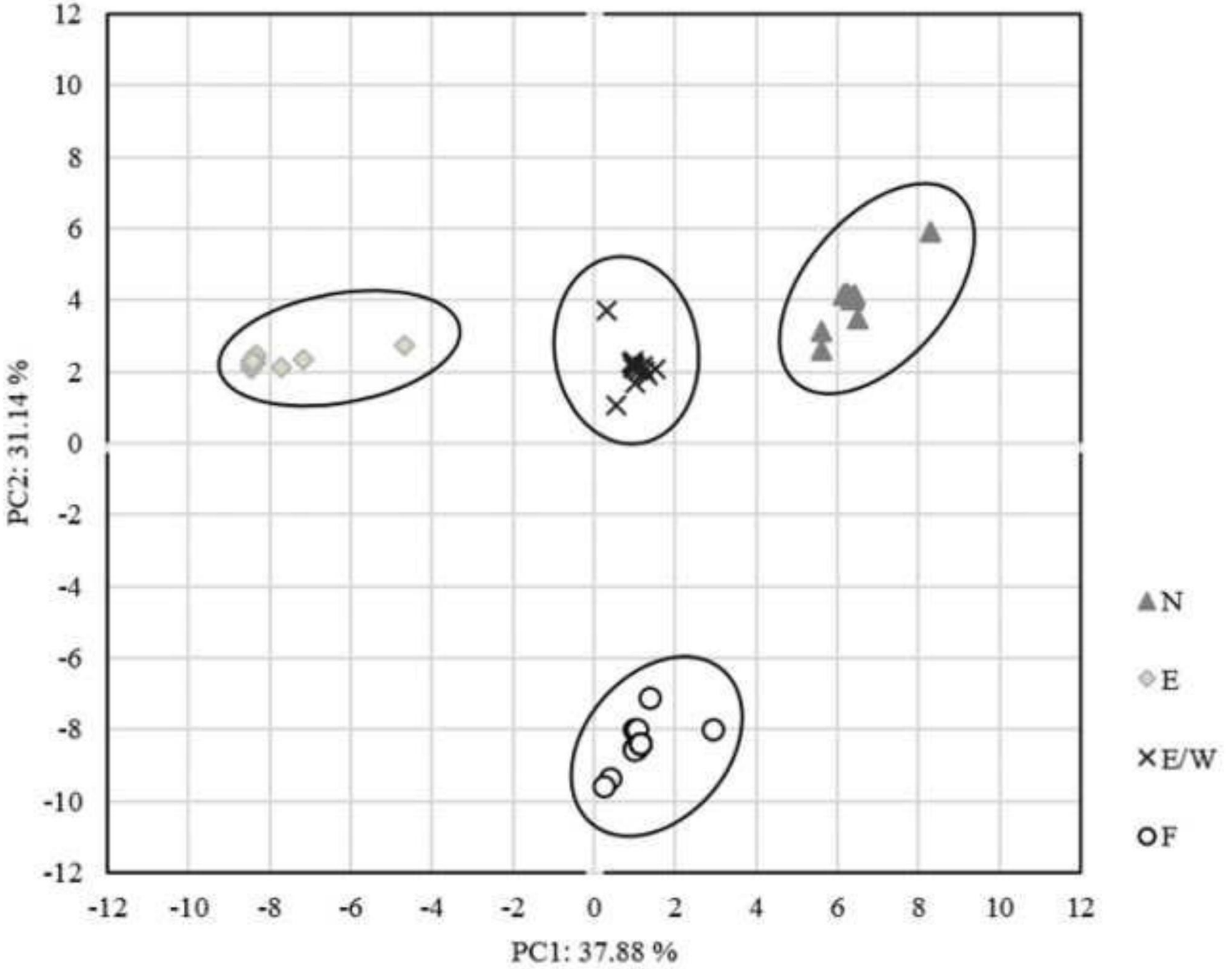

Table

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 30th -31st, 15 after leaf removal).

Experimental Treatments	Photosynthetic Photon Flux (PPF, µmol m^{-2} s^{-1})				Temperature (°C)			
	Morning		Afternoon		Morning		Afternoon	
	East	West	East	West	East	West	East	West
N	236.17 ± 28.03 **a**	57.60 ± 3.37 **a**	69.07 ± 3.84 **a**	133.70 ± 14.52 **a**	33.87 ± 0.17 **a**	32.67 ± 0.23 **b**	32.60 ± 0.12 **b**	37.07 ± 0.54 **a**
E	563.53 ± 34.16 **c**	57.20 ± 3.16 **a**	110.67 ± 3.65 **c**	141.77 ± 11.13 **a**	37.90 ± 0.41 **d**	33.17 ± 0.20 **b**	32.07 ± 0.15 **a**	36.07 ± 0.53 **a**
E/W	387.43 ± 27.34 **b**	74.13 ± 1.84 **b**	90.33 ± 2.89 **b**	243.43 ± 23.21 **b**	36.20 ± 0.42 **c**	32.43 ± 0.54 **b**	34.20 ± 0.19 **c**	40.53 ± 0.1 **b**
F	240.44 ± 31.02 **a**	93.03 ± 3.37 **c**	76.23 ± 3.63 **a**	586.43 ± 14.52 **c**	35.00 ± 0.32 **b**	31.27 ± 0.17 **a**	32.37 ± 0.15 **bc**	39.30 ± 0.65 **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^2	% [x]	m^2	% [x]
N	**- a**	**- a**	8.71 ± 0.88 **b**	100.00 **d**
E	0.67 ± 0.10 **b**	7.73 ± 1.18 **b**	8.03 ± 0.10 **a**	92.27 ± 1.18 **c**
E/W	0.95 ± 0.13 **c**	10.90 ± 1.44 **c**	7.75 ± 0.13 **a**	89.10 ± 1.44 **b**
F	0.40 [y] ± 0.14 **b**	4.69 [y] ± 0.16 **b**	8.31 [y] ± 0.15 **a**	95.41 [y] ± 2.50 **c**
	1.35 [z] ± 0.18 **d**	15.57 [z] ± 2.06 **d**	7.35 [z] ± 0.18 **a**	84.43 [z] ± 2.06 **a**

[x] percentage on total leaf area per vine
[y] first defoliation step
[z] 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**
	SKINS			
Phenolic classes				
Total Anthocyanins (mg malvidin-3-glucoside/kg dry skins)	31296 ± 1629	26192 ± 5499	27837 ± 4390	29713 ± 3168
Total Flavonoids (mg (+)-catechin/kg dry skins)	90574 ± 5930 **b**	70939 ± 7592 **a**	74880 ± 14891 **a**	83666 ± 7337 **ab**
Flavonoids different from anthocyanins (mg (+)-catechin/kg dry skins)	45009 ± 7069 **b**	32805 ± 797 **a**	34351 ± 9407 **a**	40406 ± 3148 **ab**
Flavans reactive with vanillin (mg (+)-catechin/kg dry skins)	31531 ± 2788	30488 ± 12147	27422 ± 5705	28804 ± 3843
Proanthocyanidins (mg cyanidin chloride/kg dry skins)	48242 ± 8557	51304 ± 14363	52180 ± 7895	44568 ± 9755
Total Phenolic Compounds (mg gallic acid/kg dry skins)	77478 ± 3229 **b**	65395 ± 16294 **a**	69215 ± 11474 **ab**	74781 ± 6212 **ab**
Phenolic profiles				
Flavonols (mg QE/kg dry skins)				
Myricetin-3-glc	166.16 ± 1.22 **b**	106.62 ± 16.31 **a**	104.07 ± 16.24 **a**	122.60 ± 5.86 **a**
Quercetin-3-glc	52.49 ± 2.77 **c**	35.12 ± 5.94 **b**	23.72 ± 4.45 **a**	26.56 ± 1.84 **ab**
Quercetin-3-glcr	164.23 ± 12.38 **b**	144.00 ± 27.97 **b**	74.62 ± 6.44 **a**	123.82 ± 7.63 **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 **a**	62.15 ± 0.33 **c**	47.93 ± 3.13 **b**	53.41 ± 2.77 **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**	919.50 ± 110.89 **b**	558.02 ± 85.67 **a**	639.24 ± 92.27 **a**
Flavan-3-ols (mg CE/kg dry skins)				
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
Anthocyanins (mg ME/kg dry skins)				
Dp-3-glc	112.48 ± 20.81 **a**	173.09 ± 4.50 **b**	118.77 ± 0.63 **a**	186.18 ± 31.72 **b**
Cy-3-glc	285.76 ± 2.38 **c**	126.30 ± 0.26 **a**	211.80 ± 21.91 **b**	327.51 ± 15.70 **d**
Pt-3-glc + Pn-3-glc	1512.85 ± 115.96 **b**	1070.52 ± 185.70 **a**	2698.75 ± 140.48 **c**	2390.83 ± 25.36 **c**
Mv-3-glc	5887.29 ± 317.52 **b**	4641.30 ± 552.06 **a**	5298.63 ± 92.80 **ab**	5279.52 ± 439.17 **ab**
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-*p*-coumglc	286.24 ± 22.80	342.23 ± 18.87	306.66 ± 60.71	364.64 ± 69.68
Pn-3-*p*-coumglc	118.45 ± 1.57 **a**	153.70 ± 2.17 **a**	454.30 ± 44.96 **b**	493.41 ± 0.62 **b**
Mv-3-*p*-coumglc	6647.31 ± 324.88 **bc**	7540.75 ± 622.15 **c**	4782.58 ± 550.49 **a**	5616.91 ± 622.64 **ab**
SEEDS				
Phenolic classes				
Total Flavonoids ***(mg (+)-catechin/kg dry matter)***	132416 ± 34576 **b**[b]	92938 ± 9630 **a**	104934 ± 11981 **ab**	126495 ± 16322 **b**
Flavans reactive with vanillin ***(mg (+)-catechin/kg dry matter)***	78750 ± 21412 **c**	56509 ± 10159 **a**	62645 ± 3294 **ab**	72672 ± 5475 **bc**
Proanthocyanidins ***(mg cyanidin chloride/kg dry matter)***	104934 ± 11981 **ab**	131117 ± 13586 **a**	132941 ± 1533 **a**	223416 ± 41269 **b**
Total Phenolic Compounds ***(mg gallic acid/kg dry matter)***	164974 ± 34821 **b**	126447 ± 3218 **a**	141368 ± 7023 **a**	167942 ± 11672 **b**
Phenolic profiles				
Flavan-3-ols (mg CE/kg dry seeds)				
Procyanidin B3	851.98 ± 1.04 **a**	4867.19 ± 993.34 **c**	421.83 ± 36.18 **a**	2720.23 ± 335.71 **b**
(+)-Catechin	1525.93 ± 26.85 **b**	6375.43 ± 522.94 **d**	586.66 ± 71.20 **a**	3705.21 ± 218.27 **c**
Procyanidin	319.69 ± 14.99 **a**	2248.72 ± 70.48 **c**	631.98 ± 14.63 **b**	2651.20 ± 106.13 **d**
Procyanidin B4	585.61 ± 59.47 **a**	3951.59 ± 688.09 **c**	1254.64 ± 31.61 **ab**	1570.89 ± 143.77 **b**
(-)-Epicatechin	1290.23 ± 52.77 **a**	6519.57 ± 820.57 **c**	744.77 ± 144.78 **a**	2891.24 ± 320.88 **b**
Procyanidin B2	311.96 ± 22.56 **a**	1926.20 ± 298.10 **c**	596.29 ± 13.91 **ab**	864.78 ± 22.66 **b**
(-)-Epicathechin-3-*O*-gallate	421.01 ± 59.30 **a**	1765.37 ± 196.70 **c**	699.25 ± 79.30 **a**	1089.44 ± 138.60 **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**
OENOLOGICAL PARAMETERS				
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**
Titratable 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
ORGANIC ACIDS (expresses per L of wine)				
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**
PHENOLIC COMPOSITION (expresses per L of wine)				
Total Anthocyanins (mg malvidin-3-glucoside/L)	411 ± 56 **a**	491 ± 50 **b**	472 ± 9 **ab**	428 ± 24 **a**
Anthocyanins sensitive to SO2 (mg malvidin-3-glucoside/L)	393 ± 24 **a**	486 ± 42 **b**	393 ± 19 **a**	384 ± 26 **a**
Monomeric anthocyanins (mg malvidin-3-glucoside/L)	255 ± 11 **b**	286 ± 18 **c**	243 ± 13 **ab**	236 ± 16 **a**
Total Flavonoids (mg (+)-catechin/L)	1899 ± 265 **a**	1931 ± 379 **a**	2301 ± 280 **ab**	2776 ± 319 **b**
Flavonoids different from anthocyanins (mg (+)-catechin/L)	1300 ± 184 **a**	1096 ± 320 **a**	1615 ± 285 **a**	2153 ± 353 **b**
Flavans reactive with vanillin (mg (+)-catechin/L)	1537 ± 92 **b**	1256 ± 144 **a**	1466 ± 176 **b**	1189 ± 185 **a**
Proanthocyanidins (mg cyanidin chloride/L)	2604 ± 148 **a**	2513 ± 179 **a**	2422 ± 338 **a**	5601 ± 791 **b**
Total Phenolic Compounds (mg gallic acid/L)	2379 ± 72 **a**	2420 ± 105 **ab**	2520 ± 251 **b**	2438 ± 202 **ab**
PIGMENTS AND STRUCTURE INDICES				
MP	0.54 ± 0.06 **a**	0.72 ± 0.12 **b**	0.63 ± 0.01 **a**	0.64 ± 0.03 **a**
SPP	0.16 ± 0.02 **a**	0.24 ± 0.03 **c**	0.19 ± 0.00 **ab**	0.21 ± 0.01 **b**
LPP	0.12 ± 0.02	0.13 ± 0.01	0.13 ± 0.02	0.13 ± 0.03
$I_{gelatin}$	62.8 ± 4.1 **a**	58.9 ± 6.6 **a**	60.3 ± 5.0 **a**	87.4 ± 1.7 **b**
I_{EtOH}	20.7 ± 3.7	24.2 ± 3.2	20.6 ± 0.6	21.0 ± 2.3
I_{HCl}	28.3 ± 4.5	25.4 ± 4.7	29.5 ± 4.6	31.1 ± 5.3
I_{PVPP}	35.0 ± 2.0 **a**	40.9 ± 3.0 **c**	38.2 ± 0.6 **b**	38.6 ± 2.6 **b**
COLOUR PARAMETERS				
CI	5.670 ± 0.585 **a**	7.340 ± 1.024 **b**	6.431 ± 0.042 **ab**	6.298 ± 0.092 **a**
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

% yellow	32.5 ± 0.1	32.4 ± 0.5	32.3 ± 0.2	33.3 ± 1.3
% red	57.5 ± 0.4	57.2 ± 0.4	57.2 ± 0.2	56.3 ± 2.0
% blue	10.0 ± 0.6	10.4 ± 0.2	10.5 ± 0.2	10.3 ± 0.7
dAl%	10.6 ± 0.2 **b**	10.5 ± 0.3 **b**	10.9 ± 0.0 **b**	7.8 ± 1.6 **a**
dAT%	89.4 ± 0.2 **a**	89.5 ± 0.2 **a**	89.2 ± 0.1 **a**	92.2 ± 1.6 **b**
dTAT%	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.
$I_{gelatin}$: gelatin index; I_{EtOH}: ethanol index; I_{HCl}: hydrochloric acid index; I_{PVPP}: 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; dAl: absorbance at 520 nm due to monomeric anthocyanins; dAT: absorbance at 520 nm due to polymeric pigments decolorized with SO_2; 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_r (min)	MS (*m/z*)	MS–MS fragments (*m/z*)	N	E	E/W	F
Phenolic acids (mg GAE/L; mg CAE/L)		$[M-H]^-$					
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 **a**	17.8 ± 3.2 **b**	11.1 ± 0.4 **a**	11.2 ± 1.7 **a**
Caffeic acid	12.8	179	135	13.5 ± 0.7 **a**	17.7 **c**	14.9 ± 0.2 **b**	15.6 ± 0.6 **b**
p-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
Stilbens (mg RE/L)		$[M-H]^-$					
cis-Piceid	24.7	389	227	0.6 ± 0.1	0.8 ± 0.1	0.7	0.8 ± 0.1
trans-Piceid	35.3	389	227	0.2	0.4	0.3	0.3
Flavonols (mg QE/L)		$[M-H]^-$					
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 **a**	1.1 **b**	1.2 ± 0.2 **bc**	1.5 **c**
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 **a**	3.6 ± 0.6 **a**	4.5 ± 0.8 **a**	7.4 ± 0.6 **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 **a**	1.1 ± 0.2 **a**	0.9 **a**	1.5 **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
Flavan-3-ols (mg CE/L)		$[M-H]^-$					
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 **a**	8.3 ± 1.1 **b**	4.9 ± 0.6 **a**	10.2 ± 0.1 **b**
Procyanidin B4	16.4	577	451, 425, 407, 289	11.3 ± 0.8 **a**	11.0 ± 0.4 **a**	11.3 ± 0.3 **a**	15.0 ± 0.2 **b**
(-)-Epicatechin	17.5	289	245, 205, 179	12.4 ± 3.0 **a**	14.2 ± 3.0 **ab**	18.4 ± 1.2 **ab**	21.9 ± 3.8 **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
Anthocyanins (mg ME/L)		$[M-2H]^-$					
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 **ab**	0.7 ± 0.2 **b**	0.3 **a**	0.6 ± 0.1 **ab**
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 **c**	0.3 **c**	0.2 **b**	nd **a**
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-*p*-coumglc (*p*-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-*p*-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-*p*-coumglc	42.4	607	299	18.4 ± 0.9 **a**	22.6 ± 2.7 **b**	19.8 ± 0.4 **ab**	20.5 ± 0.3 **ab**
Mv-3-*p*-coumglc	42.4	637	491, 329				

nd: not detected.

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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.