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LEAF REMOVAL, VINE PHYSIOLOGY AND WINE QUALITY IN CV. NEGROAMARO (*VITIS VINIFERA* L.)

Laura de PALMA [1]*, Luigi TARRICONE [2], Gaia MUCI [3], Patrizio LIMOSANI [1], Michele SAVINO [2], Vittorino NOVELLO [4]

[1] Dipartimento di Scienze Agroambientali, Chimica e Difesa Vegetale, Università degli Studi di Foggia, Via Napoli, 25, 71100 Foggia
[2] CRA, Unità di Ricerca per l'Uva da Tavola e la Vitivinicoltura in Ambiente Mediterraneo, Via Casamasima 148, 70010 Turi (BA)
[3] Corso di Laurea Specialistica Interateneo in Scienze Viticole ed Enologiche
[4] Dipartimento di Colture Arboree, Università degli Studi di Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO)
*Corresp. author: Laura de Palma, +390881-589221, +390881-589342, Email: l.depalma@unifg.it

Summary

Negroamaro has a very dense canopy. Farmers improve the bunch microclimate by removing leaves and laterals in that zone. As a consequence, clusters are exposed to a very high summer irradiance and are susceptible to sunburn. In order to individuate a best defoliation practice for Negroamaro, the effects on canopy microclimate, leaf functioning, berry and wine composition of three treatments were compared: i) cluster zone farm hand defoliation (FD), ii) cluster zone low intensity mechanical defoliation (MD_l), iii) hand canopy defoliation (CD) removing the main leaf and the lateral shoot, at alternate nodes, from the canopy base to the top. Treatments were imposed at berry set. FD removed about 63 % of the total vine leaf area, MD_l about 32 %, CD about 48 %. In late June, the shade at the cordon level was almost the double in MD_l compared to both FD and CD. At veraison, the photosynthetic rate was higher in FD and CD than in MD_l, while stomatal conductance and leaf transpiration did not change significantly. At veraison, the berry internal temperature, measured when the air temperature reached the maximum value (40 °C), was very proximal to this value, independently from the skin color (still green or blue) or the defoliation treatment. Also cluster sunburn damages did not differ among treatments. Compared to FD, the grape yield increased by 16 % in CD and 35 % in MD_l. Wine alcohol and phenol content, as well as the anthocyanin content, were higher in FD and CD. The tasting panel preferred FD wines for color and CD wine for flavor.

INTRODUCTION

Negroamaro, the main cultivar for red wine production in the most Southern part of the Apulia region (Salento, Italy), has very vigorous shoots, many laterals and big leaves that limit the air circulation and light penetration into the canopy. Leaf shading is known to penalize photosynthesis and berry sugar accumulation; high fruit shading is known to lower berry quality, especially as concerns the synthesis of phenols, although cultivars may differ in their sensitivity. This aspect has been poorly investigated in cv. Negroamaro. Farmers improve cluster microclimate by removing leaves and laterals from the bunch zone. As a consequence, clusters are exposed to high summer irradiance and temperatures which, in turn, may cause berry sunburns, loss of acidity, increase of Potassium content and pH, decrease of anthocyanins (Bergqvist *et al*., 2001; Haselgrove *et al*., 2000; Spayd *et al*., 2002).

In this trial, different modalities of leaf removal were tested to assess their effects on canopy microclimate, vine ecophysiology, berry and wine composition. The ultimate goal is to individuate a best practice for Negroamaro.

MATERIAL AND METHODS

The study was carried out in 2009 at a private farm (Conti Zecca, Leverano, Italy) on VSP trained and spur pruned vines (2.5 x 1.0 m apart), which shoots were topped after overcoming the last trellis wire. Soil was deep; rainfalls were abundant during winter, thus the vineyard did non receive irrigation water supply.

At the developmental stage of "berry pea size" (early June) the following treatments were applied, each on 3 adjacent vine rows: i) farm defoliation (FD), that is, hand removal of all leaves and laterals from shoot base until the first node over the last cluster; ii) mechanical defoliation at low intensity (MD_l) obtained by appropriate machine regulation; iii) canopy defoliation (CD), that is, hand removal of the main leaf and the lateral shoot, at alternate nodes, from the base to the top of the canopy. The removed leaf area was estimated using the weight-to-area ratio (250 leaf dishes per treatment, of known area, were cut and immediately weighted). Measurements were taken on 5 single-vine replications per treatment. Observations on vegetative growth were also done at different developmental stages.

Microclimatic and ecophysiological parameters were assessed, in two typical cloudless summer days, at the phenological stage of "majority of berries touching", that is, in late June, when summer climatic conditions were still mild, and at the stage of "veraison", in late July, when climate became hot and dry. Air temperature and relative humidity were measured, at different hours, with a thermo hygrometer. Photosynthetic photon flux (PPF) at the leaf surface was measured, 1 m above the cordon, when the East side of the canopy was fully lighted (solar bar, 10 measurements per treatment). Red to Far red ratio (R:Fr) of light available at the cordon level was measured when sun was over the vine row, as an indicator of canopy shading (660/730 nm light sensor, 25 data per treatment). Predawn leaf water potential was evaluated by means of a pressure bomb (10 data per treatment). At veraison, other parameters were also assessed. Midday temperature was measured, with an infra-red thermometer, at the surface of exterior leaves (30 data per treatment) and of clusters (30 data per treatment), as well as at the interior surface of sun exposed and shaded berries, either still green or blue, just detached and cut (75 data per treatment). At this stage, shoots were about 1.30 m long. Gas exchange per area unit of main and lateral leaves was measured at about 0.5-0.6 and 0.9-1.0 m above the cordon, that is, in the middle-low and in the middle-high shoot portions; measurements were done between 9:30 and 12:30 solar time, orienting the leaf chamber to intercept maximum PPF intensity (portable infra red gas analyzer, 20 leaves per treatment). At the same canopy height, PPF reaching the interior leaf layer was measured with a solar bar, positioning the probe parallel to the vine row (10 measurements per treatment).

At farm harvest (late September), yield components were evaluated on 10 vines per treatment. On five 100-berry sample per treatment, total soluble solids, pH and titratable acidity (expressed as tartaric acid) were assessed. About 100 kg of grapes per treatment were wine-processed according to a protocol already described (Suriano and Tarricone, 2006). At wine bottling, chemical and physical parameters were assessed according to the EC 2676/90 regulation. Moreover, concentrations of total polyphenols and flavonoids (both expressed as (+)catechin), anthocyanins (expressed as malvidin monoglucoside), proanthocyanidins (expressed as cyanidin chloride), and flavans reacting with vanillin (expressed as (+)catechin) were analyzed according to Di Stefano and coll. (1989). Wine sensory evaluation was done by a panel of 12 researchers.

Data were statistically processed by means of ANOVA and Duncan test ($p \leq 0.05$). Per each parameter and stage of measurement, average values are reported.

RESULTS AND DISCUSSION

Removed leaf area. Assuming that CD treatment removed about 50 % of the foliage, Negroamaro total leaf area per vine at the stage of "berry pea-size" was estimated about 2 m^2. Since at that developmental stage shoots were still young, most of the leaf area was concentrated in the proximal third of the canopy, that is, at the bunch zone. Assuming that all vines had same total leaf area when the three treatments were applied, FD removed about 63 % of the vine leaf area, resulting the most severe treatment, while MD_l removed about 32 %, that is, almost one half of the leaf area removed by FD. CD resulted in a quite intermediate defoliation intensity (fig. 1).

Microclimatic and physiological parameters at the developmental stage of "majority of berries touching". At the end of June, midday air temperature and relative humidity were about 28 °C and 48 %. PPF available at the East canopy surface, when this was fully lighted, was 1200-1300 umol m^{-2} s^{-1}.

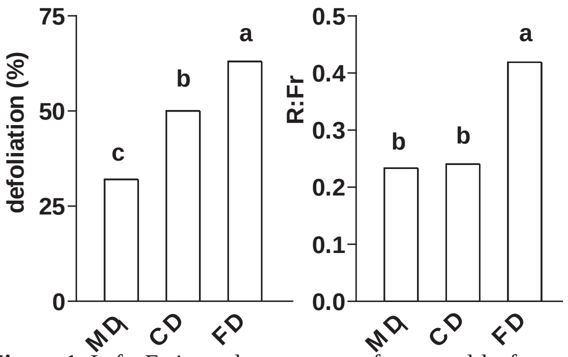

Figure 1. Left: Estimated percentage of removed leaf area at the stage of "berry pea size". Right: R:FR of sunlight filtering at the cordon level, at the stage of "majority of berries touching" *(different letters indicate statistically different values, p≤0.05).*

The R:Fr of sunlight measured at the cordon level was 0.42 in FD and almost half in MD_l (fig. 1): the "shading difference" between these theses was coherent with their "defoliation difference". On the other hand, light at the bottom of MD_l and CD canopies had same R:Fr: in CD, the leaf removal along all the foliage modified the relationship between defoliation intensity and canopy shading.

Predawn leaf water potential was -0.47 MPa in FD and almost the same in MD_l, while it was about 40 % more negative in CD (fig. 2). Vine water stress in late June ranged from light (MD_l and FD) to medium (CD), according to Carbonneau (1998); however it did not seem related to the defoliation percentage since vines treated with either minimum or maximum defoliation intensity showed a very similar water status.

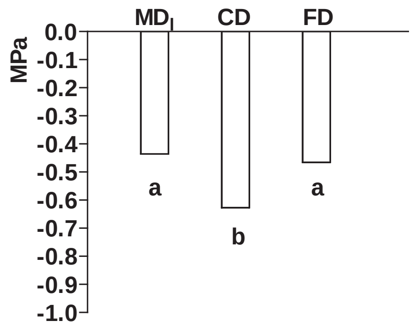

Figure 2. Predawn leaf water potential at the stage of "majority of berries touching" (different letters indicate statistically different values, p≤0.05).

Since MD_l and FD defoliation were concentrated around the bunch zone, while CD defoliation was distributed along the canopy, a role of the modality of leaf removal could be again hypothesized. The transpiration rate has been found to decline in the deeper leaves of the canopy (Hunter and Visser, 1988); in the present trial, CD, due to the removal of laterals along the main shoot, had less "deep leaves", thus, its canopy could have transpired to a grater extent, lowering the vine water status.

Microclimatic and physiological parameters at "veraison". At the end of July, midday air temperature and relative humidity were about 35 °C and 37 %, with extreem values of 40 °C and 21 %. PPF available at the East canopy surface did not change from late June. R:Fr of light at the cordon level decreased because of the shoot growth, however, this decrement was significant only in FD where it reached about -30 %. Nevertheless, FD R:Fr was still about 50 % higher than that found in the other treatments.

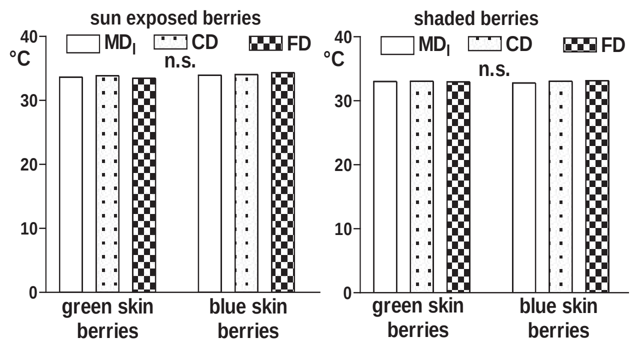

Figure 3. Temperature of internal surface of sun exposed and shaded berries having either green or blue skin at veraison *(n.s. = non significant difference).*

Temperature of exterior leaves ranged from 37 to 39 °C, that of clusters between 35 and 37 °C; the lowest values were found in CD, the highest in FD. Nevertheless, the berry interior temperature measured in the second of two consecutive days with max. air temperature of 40 °C, was always about 33-34 °C in both sun exposed and shaded berries, irrespectively of the skin color (still green or blue) or the treatment (fig. 3). A similar result was reported by Dokoozlian and Kliewer (1996).

The percentage of bunches with sunburn damages was further observed and it was found very similar among treatments (15-18 %). However, we ascertain that the severity of bunch damages increased progressively from MD_l to CD to FD; the presence of some desiccated bunches was noticed, especially in FD.

At this developmental stage, that is, when berries are known to become a very important sink for photosynthates, net CO_2 uptake per leaf area unit was about 20 % higher in FD and CD than in MD_l (fig. 4). The enhancement of photosynthesis per leaf area unit is a possible vine response to leaf removal, apt to face the carbohydrate requirement; thus, it may increase with the defoliation intensity (Hunter and Visser, 1988). In the present trial, stomatal conductance and leaf transpiration did not change significantly, supporting the evidence that the above mentioned photosynthetic rise had a non stomatal origin. Our results matched quite well those obtained by other studies on partial vine defoliation (Hunter, 1992).

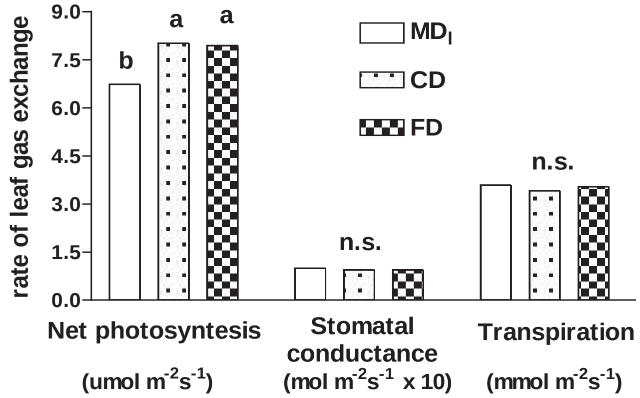

Figure 4. Rate of leaf gas exchange of mature leaves, at veraison (different letters indicate statistically different values, p≤0.05; n.s. = non significant difference).

Comparing CO_2 assimilation of leaves located in the middle-low shoot portions and that of leaves of the middle-high portion, a higher rate was reached in the latter (+80 %), which were "mature" but not as aged as the former. Similarly, leaves of lateral shoots, naturally younger than those of the main shoot, assimilated more CO_2 per leaf area unit, although the difference was "only" +34 %. Photosynthetic response to leaf age is well-known in grapevine starting from the studies of Kriedman and coll. (1970). In the present trial no significant interaction was found between type of leaf and treatment. In the middle-high canopy portion, PPF filtering through the exterior leaf layers was about 390 umol m^{-2} s^{-1} in CD (32 % of exterior PPF) and decreased progressively in FD (24 %) and MD_l (16 %). The best CD light microclimate seemed related to the regular thinning of main leaf and lateral shoots along the entire canopy; the worst MD_l light microclimate was related to the sprouting of long laterals from the nodes above the last bunch, that shaded the upper foliage; this behavior was specific of the vines belonging to this thesis.

Predawn leaf water potentials were more negative than those recorded in late June, as expected as a consequence of soil water exploitation. The pattern of difference among treatments did not change, but the differences were attenuated; the values ranged between -0.58 MPa in MD_l to -0.68 MPa in CD (15 % of decrement) and indicated a medium water stress level in all the treatments.

Yield components, berry and wine composition, wine sensory properties. The grape yield increased with the leaf surface left at the stage of "berry pea size"; compared to FD, which produced about 2.6 kg per vine, the increase was 16 % in CD and 35 % in MD_l (fig. 5). This response seemed more related to the number of bunches achieving ripeness than to the berry weight: in fact, compared to FD, where berry mass was 2.4 g, CD berry was 13 % lighter and MD_l berry was 33 % heavier.

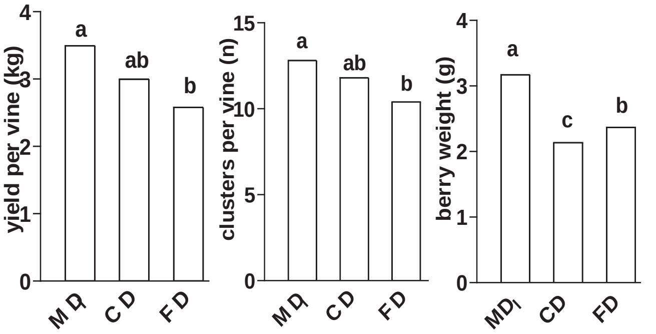

Figure 5. Yield components at harvest (different letters indicate statistically different values, p≤0.05).

Berry juice reached about 22 °Brix in CD, 21 °Brix in FD (-5 %), but only 19 °Brix in MD_l (-12 %) which did not achieve an optimal ripeness (fig. 6). Hence, more sugar was found in the grapes of vines that joined a higher photosynthetic rate, a better canopy light penetration at the canopy height where a highly photosynthetic active leaves were located, and a lower grape yield, as it was likely to expect. The amount of titratable acidity into the Negroamaro berry juice was penalized by the high summer temperature, especially in FD and CD treatments where clusters were less leaf-protected than in MD_l; the pattern of differences of acidity among theses was opposite to that of sugar. The pH did not differed significantly.

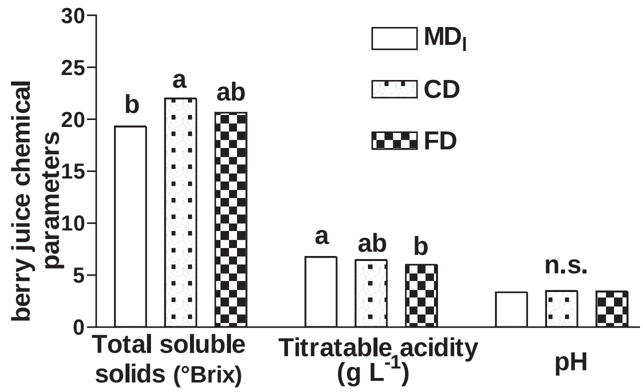

Figure 6. Berry juice composition at harvest (different letters indicate statistically different values, p≤0.05; n.s. = non significant difference).

In the wines, the differences of alcohol content reflected those of juice sugar (fig. 7). Other chemical and physical parameters showed very small or non significant differences among theses.

The Negroamaro wine obtained from "farm defoliated" vines was the richest in total polyphenols (+17 % respect to

CD, +37% respect to MD_l) and total anthocyanins (+14 % respect to CD, 100 % compared to MD_l), while the wine obtained from "canopy defoliated" vines was the richest in total flavonoids and in proanthocyanidins (fig. 8, fig. 9).

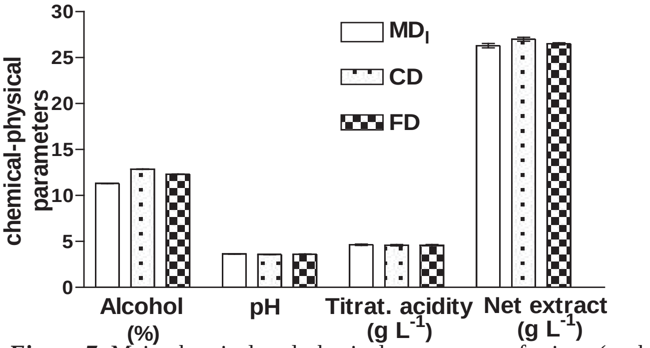

Figure 7. Main chemical and physical parameters of wines (each parameter was tested three times. The bar indicates the standard error of the readings).

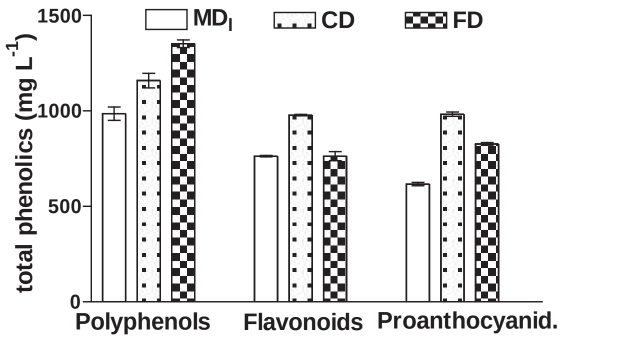

Figure 8. Wine phenol content (each analysis was repeated three times. The bar indicates the standard error of the three readings).

The anthocyanin richness of FD wine resulted in the highest wine color intensity and was coherent with the highest R:Fr of the light filtering at the cordon level during the grape development, that is, with a less shaded grape. The same micro-environmental conditions could have also induced the greatest total polyphenol accumulation in these grapes. The importance of a good light regime on anthocyanin and total phenolics accumulation has been deeply investigated (Dokoozlian and Kliewer, 1996)

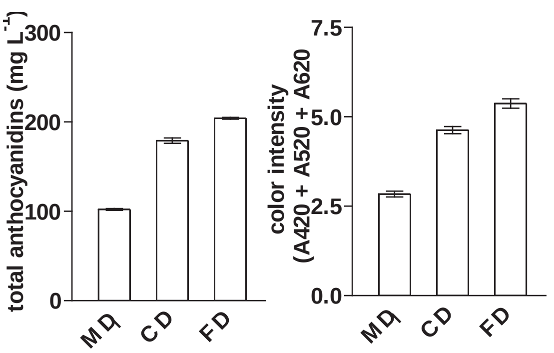

Figure 9. Wine anthocyanin content and color intesity (each parameter was tested three times. The bar indicates the standard error of the readings).

On the other hand, a high grape sunlight regime stimulates skin tannin accumulation until veraison, but penalize the final extractability of these compounds, as reminded by Keller (2007). In the present trial, the greatest amount of total proanthocyanidins, and also of total flavonoids, was found in CD wine, that is the treatment that joined a moderate grape sunlight regime to the best illumination of leaves of middle-high shoot portion, which might be useful to enhance the synthesis of carbon skeleton directed also to phenol production.

The wine sensory evaluation panel preferred the wine obtained from "canopy defoliated" vines; the wine obtained from "farm defoliated" vines had a 6 % lower score, that from vines which were mechanically defoliated at low intensity had a 9 % lower score. CD wine was particularly appreciated for the absence of faults, the intense herbaceous olfactory sensations such as mint and laurel, the spicy sensation of vanilla, the best taste balance. However, FD wine received the best score for the color intensity; moreover, it was appreciated for the intense fruity olfactory sensations such as strawberry, cherry, and peach.

In conclusion, the "strategy" of leaf removal at the developmental stage of "berry pea size" showed a significant influence on Negroamaro vine physiology and wine quality. Defoliation at 30 % level, concentrated around the fruit zone, did not assure a sufficient grape ripeness, penalizing the wine phenolic content and its sensory attributes. Doubling the defoliation at the fruit zone, CO_2 uptake rate increased, grape ripened quite well and the wine was enriched in phenolics and sensory attributes, especially anthocyanins and color intensity; thus it is useful to improve particularly the wine visual aspect. Defoliation at about 50 % along the foliage enhanced even more grape ripeness, and, moreover, the wine tannin content, aroma and flavor development; hence, it may be chosen to improve especially the wine taste.

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