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ORIGINAL PAPER

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A COMPARATIVE STUDY ON EFFECTS OF REFLECTIVE MULCH AS AN ALTERNATIVE TO SOME OTHER PREHARVEST APPLICATIONS TO IMPROVE PHENOLIC COMPOUNDS PROFILE AND ANTHOCYANIN ACCUMULATION OF CV. SYRAH WINE GRAPE (*V. vinifera* L.)

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ABSTRACT

In today's modern viticulture, reflective mulches and summer pruning practices are remarkable tools that may facilitate grape growers to increase yield and improve the grape quality. This study aimed to assess how reflective mulch application (RM) affects components of phenolic compounds and anthocyanin of cv. Syrah the compared to other preharvest applications such as basal leaf removal application (BLR), foliar proline application (PRO) and their various combinations, including applications of basal leaf removal + proline (BLR+PRO), basal leaf removal + reflective mulch (BLR+RM), proline + reflective mulch (PRO+RM) and basal leaf removal + proline + reflective mulch (BLR+PRO+RM). The study findings demonstrated that reflective mulch application (RM) may considerably enhance light distribution in the canopy of grapevine by increasing reflected light from the ground. In the current study, it was observed that combined applications had significant roles on improving yield and quality characteristics. In terms of total phenolic compounds content, the highest values were obtained from applications of BLR+RM and PRO+RM. Moreover, all applications had a rise to crucial increases in total anthocyanin content of cv. Syrah wine grape when the compared with C application.

Key words: wine grape, reflective mulch application, basal leaf removal application, foliar proline application, phenolic compounds, anthocyanins

INTRODUCTION

Sunlight is one of the main climatic factors, determining fruit yield and quality although other factors have considerable effects on yield and quality in fruit crops [Jifon and Syvertsen 2001].

Sufficient interception and distribution of light within canopy has important roles on grapevine productivity, photosynthetic activity, grape yield and quality [Smart and Robinson 1991].

Sunlight intensity within a grapevine canopy exhibits a considerable variation and outer parts of grapevine generally receive adequate irradiation than inner parts of grapevine. In this context, values of sunlight intensity in outer parts of grapevine canopy are over 2000 μmol $m^{-2}s^{-1}$ in spite of the fact that values in inner parts of dense grape canopy can be less than 10 μmol $m^{-2}s^{-1}$ [Smart and Robinson 1991].

In wine grape growing, frequently there have been utilized different strategies to increase grape quality such as canopy management practices such as shoot thinning [Reynolds et al. 2005] and leaf removal

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[Kok 2016, Kok and Bal 2018b] and different foliar applications [Kok and Bal 2016, Kok and Bal 2018a, Kok 2018].

Grape quality depends on various factors, including grape cultivars [He and Giusti 2010], various agronomical practices [Fenoll et al. 2009, Lutz et al. 2011, Kok 2011, Kok 2016, Kok 2018, Kok and Bal 2016, Kok and Bal 2018a, Kok and Bal 2018b] and climatic factors [Koundouras et al. 2006].

Traditional leaf removal is a common viticultural practice, leading to improvements in microclimate of the bunch zone through reduced leaf density and increased sunlight penetration to the bunch [Mosetti et al. 2016].

Foliar applications of different biostimulants in wine grape growing have also crucial roles on grape quality, including stimulating the synthesis of aroma [Martinez-Gil et al. 2012] and phenolic compounds [Pardo-Garcia et al. 2014, Kok 2018].

Proline is an abundant amino acid in grapes and foliar applications of proline have favorable effects on wine grape quality [Garde-Cerdan et al. 2014].

In recent years, there has been a growing interest in using reflective mulches with the purpose of directing more sunlight into grapevine canopy [Garde-Cerdan et al. 2014] where it will be intercepted by leaves and bunches, especially in viticulture regions with cool or cloudy weather conditions [Hostetler et al. 2007].

Reflective ground film is a shiny bright white or silver plastic cover that might increase sunlight in bunch zone and consequently enhance skin coloration, soluble solids content and flavor development. The advantage of mulch application compared to leaf removal and shoot thinning is that they would not decrease the total photosynthetic capacity of grapevines and could alter the canopy microclimate to improve ripening and disease suppression [Hostetler et al. 2007].

Reflective mulching materials can enhance light distribution in the canopy by rising reflected light from the floor, contributing to enhancements in photosynthetic effectiveness and regulation sugar metabolism as well as the activity of anthocyanin synthesis-related enzymes, thereby affecting fruit sugar accumulation and coloration [Jiang et al. 2014].

The aim of the present study was to compare the effects of the reflective mulch application with the preharvest applications of basal leaf removal, foliar proline and their different combinations in terms of contents of total phenolic compounds and total anthocyanin in winegrape cv. Syrah.

MATERIALS AND METHODS

Research site. This study was undertaken in the course of the 2017 growing season in a commercial vineyard (41°01'07"N; 27°40'25"E; 60 m above the sea level) in Tekirdağ, Turkey.

The vineyard was planted with cv. Syrah grapevines grafted onto Kober 5BB (Berlandieri × Riparia Teleki 8B, selection Kober 5BB) grapevine rootstock with a distance of 2.5 m between rows and 1.2 m between plants within row. Grapevines were trained on a vertical shoot positioned trellis with bilateral cordon with three wires.

In the research, the vineyard was managed according to local standard viticulture practices and a standard disease and pest control programs were employed.

Climatic characteristics of research region are Mediterranean-like with a warm-temperate climate. Moreover, soils in study region are mostly clay-loam and rich in the amount of potassium.

Measurements and analysis of yield and quality. In current study, yield characteristics, including berry length (mm), berry width (mm), berry weight (g), bunch length (cm), bunch width (cm) and bunch weight were measured. As well as total soluble solids content (%), p-value (μW), titratable acidity (g L^{-1}), must pH, total phenolic compounds content (mg GAE kg^{-1} fw) and total anthocyanin content (mg GAE kg^{-1} fw) were determined as quality characteristics.

Preharvest applications of basal leaf removal, foliar proline and reflective mulch and application times. In this study, basal leaf removal application, foliar proline application and reflective mulch application were utilized and these preharvest applications were respectively performed following sequence:

Basal leaf removal application (BLR). Basal leaf removal applications were conducted by hand at 10 days before vérasion period on two to five basal leaves around the bunch zones on each shoot.

Foliar proline application (PRO). L-proline (≥ 99%, Sigma-Aldrich) was used as foliar application. For this purpose, aqueous solution was made ready at dose of 6.5 mM added with tween 80 as wetting agent (0.1%) and foliar applications were carried out twice in the vineyard. First application was carried out at 10 days before vérasion period and second was repeated 10 days after vérasion. During the foliar proline applications, prepared solutions were sprayed on the grapevine canopy by using a back-pump sprayer until leaf surfaces were wetted.

Reflective mulch application (RM). Tyvek® (DuPont™) high density non-woven polyethylene material with high diffusive reflectivity, waterproof and breathable, soft and tough, safe and environmentally friendly was utilized as reflective mulch (Tab. 1). Reflective mulch was laid on the soil under the grapevine canopy at vérasion period.

Determination of grape harvest time and preparation of berry samplings. When the total soluble solids contents of berries from Control grapevines attained to 24%, bunches were manually harvested in cv. Syrah wine grape.

In the study, 250-berries were stored for analyzes of total soluble solids content, titratable acidity, pH and p-value of must. Besides, 300-berries samples were also used for determining total phenolic compounds content and total anthocyanin content. All berry samples were stored at –25°C temperature until the analyzes of total phenolic compounds content and total anthocyanin content. Prior to the analyzes of phenolic compounds and anthocyanins, berry samples were withdrawn from –25°C temperature, allowed to thaw overnight at 4°C and then homogenized in a commercial laboratory blender for 20 s.

Estimation of p-value of must. P-value, comprising redox potential (mV), must pH, P-value (µW) and resistivity (Ω) were formulated with an equation clarified by Hoffmann [1991]. In the present study, p-val-

Table 1. Miscellaneous properties of reflective mulch Tyvek® (DuPont™)

Basis weight ($g\ m^{-2}$)	Delamination ($N\ 2.54^{-1}$)	Thickness (µm)	Opacity (%)	Tensile (MD) ($N\ 2.54^{-1}$)	Elongation (%)	Mullenburst (kPa)
75.0	1.75	210	96.5	200	18.5	1200

Table 2. Influences of different preharvest applications on yield characteristics of cv. Syrah

Applications	Berry length (mm)	Berry width (mm)	Berry weight (g)	Bunch length (cm)	Bunch width (cm)	Bunch weight (g)
C	14.45	13.44	1.74^{d}	14.80^{c}	8.10	164.78^{b}
BLR	14.69	13.57	1.89^{cd}	15.20^{bc}	9.05	197.28^{ab}
PRO	14.79	13.71	1.92^{c}	15.60^{bc}	9.30	200.19^{ab}
RM	14.88	13.76	1.96^{bc}	15.90^{b}	9.55	216.36^{a}
BLR+PRO	14.96	13.89	2.08^{ab}	18.07^{a}	9.82	222.62^{a}
BLR+RM	14.99	14.00	2.11^{ab}	18.17^{a}	10.00	233.31^{a}
PRO+RM	15.08	14.24	2.16^{a}	18.22^{a}	10.25	233.82^{a}
BLR+PRO+RM	14.91	13.84	2.03^{abc}	16.00^{b}	9.67	209.89^{a}
$LSD_{5\%}$	NS	NS	0.16	1.08	NS	39.99

Different letters in column indicate the significant differences in the mean at 5% level by LSD multiple comparison test C: Control; BLR: Basal Leaf Removal; PRO: Proline; RM: Reflective Mulch; BLR+PRO: Basal Leaf Removal + Proline BLR+RM: Basal Leaf Removal + Proline + Reflective Mulch; PRO+RM: Proline + Reflective Mulch; BLR+PRO+RM: Basal Leaf Removal + Proline + Reflective Mulch; NS: Not significant ues in grape must samples from various preharvest applications were calculated in accordance with the equation mentioned above.

Spectrophotometric measurements. Determination of total phenolic compounds content was performed according to Folin-Ciocalteu method declared by Singleton et al. [1978]. However, total anthocyanin content was assessed according to a spectrophotometric method informed by Di Stefano and Cravero [1991]. Results of both analyzes were expressed as mg gallic acid equivalent per kg of fresh weight (mg GAE kg^{-1} fw). All spectrophotometric measurements were conducted with a UV-VIS spectrophotometer.

Statistical analysis. The research was performed in a completely randomized block design with four replicates. Results from preharvest applications were subjected to analysis of variance (ANOVA) by using TARIST statistical software. Differences among the applications were compared by using Fisher's Least Significant Difference (LSD) test at 5% level.

RESULTS AND DISCUSSION

Effects of preharvest applications on yield parameters. Yield characteristics of cv. Syrah depicted in Table 2 were unaffected by different preharvest applications except for berry weight, bunch length and bunch weight ($P \leq 0.05$).

Berry and bunch sizes in grapes may change in accordance with grape cultivar, ecological conditions and viticultural practices [Gougoulias and Masheva 2010, Kok 2017]. Table 2 summarizes the influences of preharvest applications on berry length and the highest berry length was recorded for PRO+RM application (15.08 mm) while the lowest mean was 14.45 mm for Control application (C), but no statistical differences were detected among the applications.

In the matter of berry width indicated in Table 2, non-significant differences among the preharvest applications were observed but PRO+RM application led to a highest berry width (14.24 mm) and C application led to the lowest (13.44 mm).

As elucidated in Table 2, berry weight was significantly influenced by preharvest applications ($P \leq 0.05$) and the highest berry weight was observed for PRO+RM application (2.16 g) and C application to the lowest (1.74 g).

Preharvest applications in Table 2 showed great differences in bunch length ($P \leq 0.05$). The applications of BLR+PRO, BLR+RM and PRO+RM resulted in the highest bunch length (respectively, 18.07, 18.17 and 18.22 cm) than C application (14.80 cm).

As represented in Table 2, bunch width was not affected significantly by preharvest applications ($P \leq 0.05$) but PRO+RM application exhibited the highest bunch width (10.25 cm) while the lowest mean was 8.10 cm for C application.

In this study, preharvest applications contributed to the significant increases in bunch weights ($P \leq 0.05$) and the highest bunch weights were respectively obtained from applications of BLR+PRO+RM (209.89 g), RM (216.36 g), BLR+PRO (222.62 g), BLR+RM (233.31 g) and PRO+RM (233.82 g) when the compared to C application (164.78 g) (Tab. 2).

Effects of preharvest applications on quality parameters. Figure 1 pointed out that different preharvest applications except for titratable acidity significantly affected quality characteristics of cv. Syrah ($P \leq 0.05$).

Soluble solids content in wine grapes has a key role in wine quality because they determine the alcohol rate of the wines. Total soluble solids contents in red wine grapes varies between 20.5 and 23.5% [Boulton et al. 1996]. In current study, preharvest applications significantly affected total soluble solids content ($P \leq 0.05$). As illustrated in Figure 1A, BLR+RM application caused the highest total soluble solids content (26.00%) than C application (24.05%).

P-value is notable tool that determine the quality characteristics of foods for degrading products [Kok 2017, Kok and Bal 2017b] and low p-value means better product quality [Wolf and Rey 1997]. Results of p-value denoted in Figure 1B informed that different preharvest applications had significant effects on P-value ($P \leq 0.05$). The lowest mean was recorded for BLR+RM (102.16 μW) while the highest p-value of must was 117.60 μW for C application (Fig. 1B).

Organic acids have pivotal effects on quality criteria of fruits such as stability, color and flavor [Romero and Munoz 1993] and grapes also contain significant amounts of organic acids. In this study, significant changes in titratable acidity were not observed among the preharvest applications ($P \leq 0.05$) and titratable acidy means ranged from 8.10 (BLR+RM) to 9.37 g L^{-1} (C) (Fig. 1C).

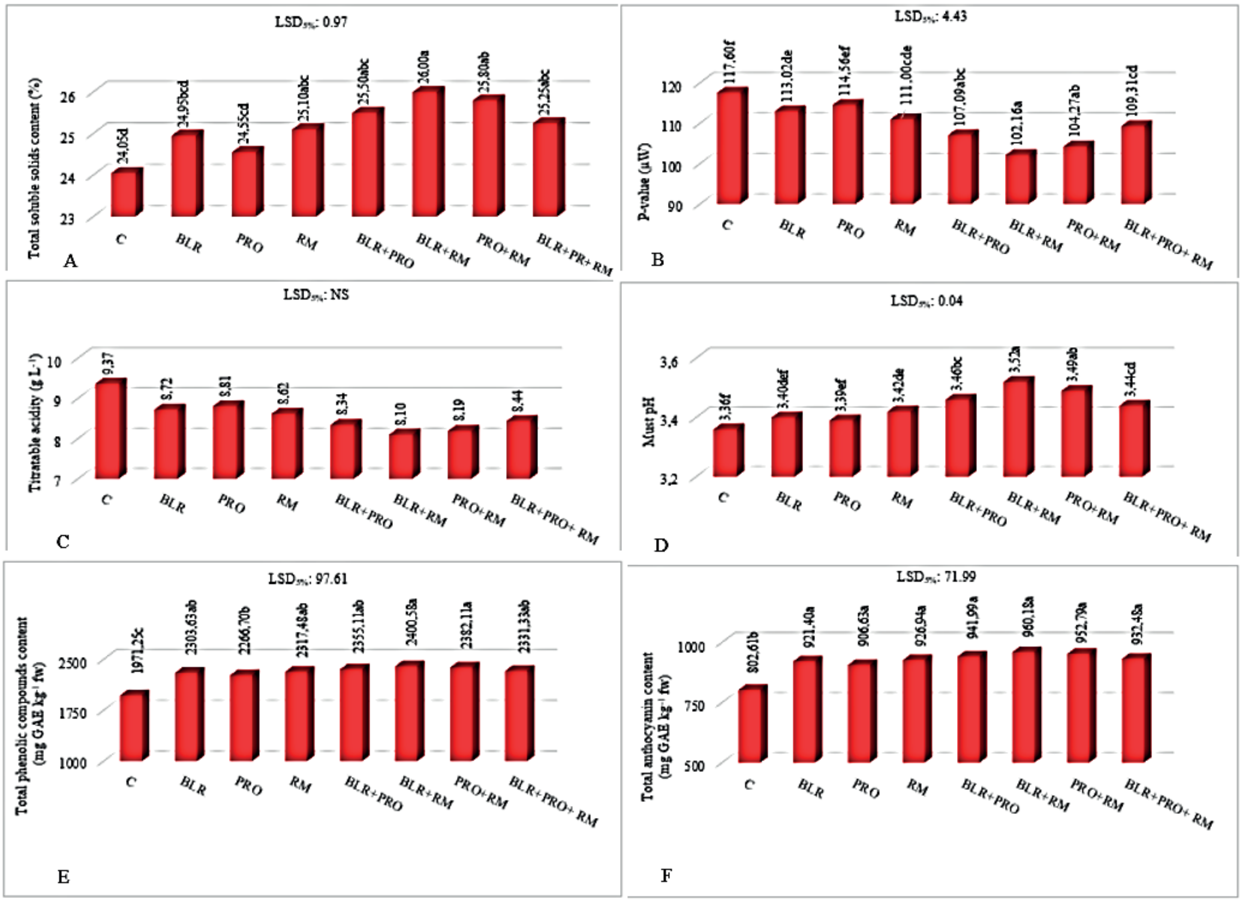

Fig. 1. Influences of different preharvest applications on quality characteristics of cv. Syrah

The must pH is a measure of the hydrogen ion concentration in grape and is commonly related to grape juice acidity [Christensen 2000, Kok and Bal 2017a]. Figure 1D indicated that must pH was significantly influenced by the preharvest applications ($P \leq 0.05$) and BLR+RM application resulted in the highest must pH (3.52) when the compared to C application (3.36).

Phenolic compounds are considerable for crop plants and there is a tremendous diversity of phenolic compounds determined in fruits and vegetables [Gomez-Plaza et al. 2017]. In the current study, preharvest applications significantly affected total phenolic compounds content ($P \leq 0.05$) and the applications of PRO+RM and BLR+RM resulted in the highest total phenolic compounds content (respectively, 2382.11 and 2400.58 mg GAE kg^{-1} fw) than C application (1971.25 mg GAE kg^{-1} fw) (Fig. 1E).

Anthocyanins are secondary metabolites synthesized in grape and their primary functions are grape skin coloration [Villegas et al. 2016]. In present study, significant differences in total anthocyanin content were observed in grapevines applied with the different preharvest applications ($P \leq 0.05$). As can be seen in Figure 1F, statistically significant higher total anthocyanin contents were obtained from applications of PRO (906.63 mg GAE kg^{-1} fw), BLR (921.40 mg GAE kg^{-1} fw), RM (926.94 mg GAE kg^{-1} fw), BLR+PRO+RM (932.48 mg GAE kg^{-1} fw), BLR+PRO (941.99 mg GAE kg^{-1} fw), PRO+RM (952.79 mg GAE kg^{-1} fw) and BLR+RM (960.18 mg GAE kg^{-1} fw) than for the C application (802.61 mg GAE kg^{-1} fw).

CONCLUSIONS

Wine grape quality may be modulated by numerous factors such as environmental stimuli and viticultural practices. In recent times, there has been growing interest in using reflective mulches, leading to increases in sunlight amount in bunch zone of grapevine and consequently enhance phenolic compounds and anthocyanins in wine grapes.

The study results indicated that applications of BLR+RM and PRO+RM caused the highest total phenolic compounds content. On the other hand, all applications also enhanced total anthocyanin content than C application. In conclusion, despite the fact that different preharvest combined applications were more effective on improving of yield and quality characteristics; reflective mulch application (RM) was found an especially useful practice among the alone preharvest applications for increasing of total phenolic compounds content and total anthocyanin content in cv. Syrah wine grape.

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