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Starch accumulation and agronomical performance of 'Syrah' under winter cycle: responses to pruning and ethephon management

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Summary

In the Brazilian Southeast, 'Syrah' grape harvested during the winter reaches better quality index than those from summer harvest. However, the management of annual double pruning to produce two grapevine growth cycles (vegetative cycle: spring – summer; reproductive cycle: autumn – winter) has increased the vineyard production costs and the vine reserve accumulation can be compromised. This study had two main objectives to improve the winter cultivation: i) to validate single pruning carried out only in summer (February) (experiment 1); to increase the reserve accumulation by ethephon (ethrel) sprayed two months before yield pruning (experiment 2). Both experiments were carried out in south of Minas Gerais State using three years old field-grown 'Syrah' grapevines grafted onto '1103 Paulsen' and trained in a vertical shoot position. The results from experiment 1 showed that grapevines pruned in summer also need to be pruned during the winter after grape harvest to avoid bud infertility during the following autumn-winter season. Single pruning reduced the starch contents in shoots, trunks and roots sampled before yield pruning and the commercial grape harvest was completely unavailable due to unfruitful shoots. On the other hand, the autumn-winter cycle was improved by ethephon sprayed in the previous vegetative growing cycle (summer cycle). During the autumn-winter cycle, sprayed grapevines showed higher starch content in trunks, high vegetative vigor and the yield and grape quality were not negatively affected.

K e y w o r d s : double pruning; *Vitis vinifera*; starch reserve; bud fruitfulness; vegetative vigor; grape quality.

Introduction

In the Brazilian Southeast, the growers of *Vitis vinifera* cultivars have adopted the double pruning management to harvest grape during the best ecological conditions in autumn-winter as compared to the summer. In this region, the low rainfall and high thermal amplitude of the autumn-winter season are favorable to sugar accumulation and synthesis of phenolic compounds in berries from grapevines grown under warm temperate and tropical conditions as already demonstrated by several authors (Favero *et al*. 2011, Mota *et al*. 2011, Regina *et al.* 2011, Dias *et al.* 2012).

Under this management the grapevines are first spur pruned at the end of winter (August or September) to develop the vegetative cycle where all clusters are removed. The reproductive cycle is started after second spur pruning, realized in January (or February), to allow grape harvest during the winter (July or August) (Favero *et al.* 2011, Regina *et al.* 2011). The management of double pruning to produce two grapevine growth cycles and only one grape harvest per year has increased the vineyard production costs. Until the moment, there is no information if double pruning is really necessary. The viability of the annual single pruning, realized only in January, needs to be investigated. Furthermore, in the absence of shoots, the carbohydrate reserves on permanent structures of grapevines are essential to support its initial vegetative growth (bud burst) (Williams 1996, Bates *et al.* 2002, Zapata *et al.* 2004). The knowledge about the impact of two growing seasons on those reserve materials is still inexistent.

On the other hand, it needs also to be considered that the second pruning is done during the summer season when the lignified shoots are still in active growth, not in dormant phase induced by low winter temperatures. Under temperate climate conditions, the grapevines are pruned after leaf fall when all carbohydrates have already mobilized from leaf to trunk and roots, the main organs of reserve storage (Bates *et al.* 2002, Zapata *et al.* 2004). Under double pruning management, the carbohydrate accumulation in these organs is probably reduced since all leaves are removed by summer pruning. In tropical viticulture where the vegetative growth is continuous, the use of growth inhibitors, such as ethephon (ethylene), is an alternative cultural practice to induce senescence and photoassimilates translocation before yield pruning (Fracaro and Pereira 2004). However, there is no scientific study to prove the benefits of ethylene application in vineyard

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under double pruning management, although some studies have shown reduction on development of shoot tips and lateral shoots under traditional vineyard management (Szyjewicz *et al.* 1984, Gonzalez *et al.* 2010). Moreover, some authors showed that although grapevine sprayed with ethylene increased the reserve and nutrients mobilization in the current season, the vegetative vigor was decreased in the following growing cycle (Schenato *et al.* 2007).

Despite the improvement of grape quality by double pruning, this technique still needs to be optimized to reduce costs and to improve the carbohydrate and nitrogen reserve accumulation. The objective of this work was to determinate how different management practices such as single pruning and ethylene application affect the reproductive cycle of grapevines growing during the autumn-winter season in the Brazilian Southeast.

Material and Methods

Experimental vineyard and double pruning management: Three year old 'Syrah' clone 747 – ENTAV-INRA grafted onto 1103Paulsen were studied in São Sebastião do Paraíso municipality, in the South of Minas Gerais State, at 20°55'S, 46°59'W and altitude of 973 m. The grapevines were planted in 2010, spaced 1.5 m between vines and 2.5 m between rows, north-south oriented, trained on a vertical shoot position and spur pruned with two spur nodes. The double pruning management was applied to allow grape harvest during the autumn-winter cycle. Usually, the first pruning to induce the vegetative cycle is done in August (or September) and all clusters are removed at bunch closure. Five to six months later, in January (or February), the yield pruning is done in lignified shoots to promote the reproductive cycle during the autumn-winter season. The climatic conditions (monthly mean temperature and rainfall) during the experimental period (vegetative and reproductive cycle) are shown in Fig. 1. The meteorological data were collected from August 2013 until August 2014 using a Vantage Pro 2 weather station (Davis Instruments Corp., CA, USA). Two independent experiments, described below, were installed.

Pruning experiment: This experiment was installed to evaluate the effect of single pruning on vegetative and reproductive development of 'Syrah' grapevines growing during the autumn-winter cycle. Two treatments were compared: "double pruning" (actual management) – where the first pruning (winter pruning) was realized in early September 2013 and second (summer pruning), five months later, in February 2014 and "single pruning" – where there was no winter pruning and the summer pruning (yield pruning) was realized in February 2014, one year after previous production cycle. In the second semester, the shoots were trimmed at 30 cm above the third wire in both treatments (around 120 cm of canopy height). This trial was a randomized block design using three replicates of 40 grapevines per treatment. The ecophysiological and agronomical measurements, as detailed below, were realized from February to August of 2014, during the reproductive cycle.

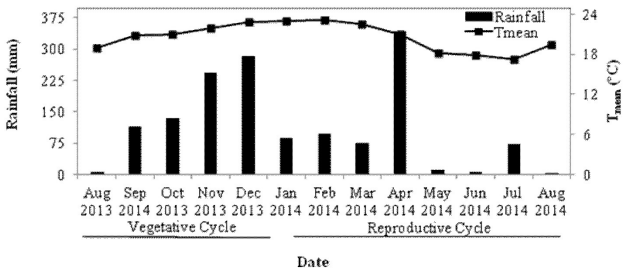

Fig. 1: Monthly mean temperature (°C) and rainfall (mm) during vegetative cycle (2nd semester of 2013) and reproductive cycle (first semester of 2014).

Ethylene experiment: This experiment was installed to evaluate the effect of ethephon (ethylene) spray application on vegetative and reproductive development of 'Syrah' grapevine submitted to actual double pruning management. Two treatments were imposed: "control" – no ethephon application and "ethephon" – vines sprayed with ethephon solution (200 mL Ethrel·100 L^{-1} H_2O = 1.44 g ethephon·L^{-1} H_2O) on 11th December 2013 and the yield pruning was done on 12th February 2014. A completely randomized design was used with 20 vines per plot and four plots per treatment. The physiological and agronomical measurements, as detailed below, were realized from February to August 2014, during the reproductive cycle.

Physiological measurements: In both experiments, the vegetative vigor was evaluated by leaf area and pruning weight per vine, the vine water status by stem water potential (Ψ_{stem}) and reserve carbohydrate status by starch contents in shoot, trunk and roots. The leaf area was estimated by non-destructive method (based on the length of the main veins) at the pea berry stage using eight vines per treatment according to Regina *et al.* (2000). The winter pruning weight was evaluated one month after grape harvest in the pruning experiment and in the ethephon experiment it was also evaluated at the yield pruning (summer pruning). All shoots per vine (eight vines per treatment) were pruned, all leaves were removed and only the shoots were dried in a forced air oven at 60 °C until constant weight was reached. During the ripening period, Ψ_{stem} was measured on eight vines per treatment (one leaf per vine) at midday using pressure chamber model 3005 (Soilmoisture Equipement Corp., Santa Barbara, CA, USA). Ψ_{stem} was measured on non-transpiring leaves near to the cluster zone that had been bagged with both plastic sheet and aluminum foil for at least one hour before measurements (Choné *et al.* 2001). The starch concentration was assessed on dried and powered samples of shoot, trunk and root taken from six grapevines per treatment before summer yield pruning at February. Shoot samples were collected from the first and second internode per vine (four samples per vine). The trunk samples were collected using a 5-mm drill bit. For each vine, holes were drilled at three positions per vine (one per arm and one from the middle of the trunk). The lateral root samples (around 2-5mm diameter) were taken at 20 cm distant from the trunk of each vine (around 30 cm depth). Sample of five roots per plant were collected from six grapevines per treatment. All samples were oven dried and stored until analyses. The starch

was extracted from 100 mg sample with 80 % (v/v) ethanol (80 °C, 20 min) and centrifuged (9,160 x g, 15 min). This process was repeated three times. The extracted pellet was dried overnight at room temperature and was hydrolyzed through incubation at 75 °C for 1 h with Termamyl® 120 L (diluted 1:500 in water), followed by incubation at 50 °C for 1 hour with Amyloglucosidase 300 L (28 unit mL^{-1}, in sodium acetate buffer, pH 4.8). The starch content was quantified from released glucose by colorimetrically method at 450 nm using glucose oxidase/peroxidase/ABTS assay (Bergmeyer 1974). Starch content was calculated as glucose multiplied by conversion factor of 0.9 (Cordenunsi and Lajolo 1995).

Agronomical measurements: During bunch closure, the bud fertility was evaluated only in the Pruning experiment by dividing the cluster number per shoot number per vine. At harvest, the number and weight of clusters and the yield per vine were measured on fifteen vines in the Pruning experiment whereas for the ethephon experiment twelve vines were used to measure yield components. Chemical analyses (soluble solids, pH and titratable acidity) were performed on the juice of pressed berries collected, at harvest, from all vines and representative of all cluster positions within the canopy and of all positions within the cluster. Soluble solids (°Brix) were determined using a handheld temperature compensated refractometer. The pH of undiluted juice of each sample was determined using a pHmeter and titratable acidity was determined by titration with 0.1 N NaOH to a phenolphthalein end point and expressed as $g{\cdot}L^{-1}$. Skins were weighed separately, frozen in liquid N_2 and stored at -20 °C until analysis. The anthocyanins and total phenolics in the berries were analyzed as described by Mota *et al.* (2011).

Statistical analyses: All data sets were subjected to analyses of variance (ANOVA). Tukey's HSD tests were carried out to determine differences between treatment means, using the STATISTICA software (ver. 5.0, Statsoft, Inc. Tulsa, OK, USA).

Results and Discussion

Effect of single *versus* double pruning on carbohydrate reserve, vegetative vigor, yield and berry quality of 'Syrah' growing under autumn-winter season: Before summer yield pruning on February 2014, the starch contents were quantified in shoots, trunk and roots of grapevines to evaluate the effects of both pruning treatments on grapevine reserve accumulation (Tab. 1). As expected, in both treatments, larger amounts of starch were accumulated in root and trunk than in shoot. As previously reported, the roots are the dominant storage organ for starch and nutrients (Bates *et al.* 2002, Zapata *et al.* 2004). However, the single pruning effects were less successful than expected since the starch content was significantly decreased in shoot, trunk and root of grapevines as compared to double pruning. During the spring, the increase of temperature and rainfall induced apical budburst from shoots of grapevines under single pruning, whereas its basal buds remained latent due to apical dominance. Although these new shoots were trimmed, they may have contributed for starch reduction in the perennial tissues since the leaves from previous cycle (from February 2013) were senescent and were not able to support the photoassimilates demand by new apical shoots. In contrast, after winter pruning, the shoot growth of grapevines under double pruning was supported by mature leaves reducing the starch mobilization from roots. It is well known in the literature that carbon assimilation by leaves at the onset of flowering becomes strong enough to support vegetative growth of grapevines (Mullins 1992, Williams 1996, Hellman 2003, Zapata *et al.* 2004). Moreover, during the vegetative cycle of grapevine subjected to double pruning management, the cultural practice of total cluster removal may have also contributed to increase the starch accumulation in shoot, trunk and roots, since the vine carbon partitioning varies considerably depending on the numbers of vegetative and reproductive sinks present (Hunter *et al.* 1995, Miller *et al.* 1996). In addition, some authors have also shown an increase of starch accumulation on leaves (Mota *et al.* 2010) and permanent structures of grapevines (Zufferey *et al.* 2012) promoted by cluster thinning.

Table 1

Effect of pruning management on starch content in shoot, trunk and root sampled before summer yield pruning of 'Syrah' grapevines. Same uppercase (for grapevine organs) and lowercase (for treatment) letters do not differ significantly as determined by Tukey's test ($p < 0.05$)

	Starch ($mg{\cdot}g\ DW^{-1}$)		
	Shoot	Trunk	Root
Double Pruning	54.3 ± 2.3 Bb	95.5 ± 6.6 Aa	103.6 ± 6.2 Aa
Single Pruning	41.7 ± 4.3 Ba	64.0 ± 4.7 ABb	73.5 ± 11.1 Ab

The single pruning induced the highest leaf area, but drastically decreased the bud fertility during the autumn – winter season (Tab. 2). This increased leaf area during the reproductive cycle induced by single pruning was due to the faster budburst and initial shoot growth (data not shown) probably caused by reduced cluster number (Tab. 3). Early reports showed that there is an increase on shoot growth and leaf area due to low crop load (Edson *et al.* 1995, Miller *et al.* 1996). On the other hand, the veg-

Table 2

Effect of pruning management on bud fertility, leaf area and pruning weight of 'Syrah' grapevines. Same letter do not differ significantly between treatments as determined by Tukey's test ($p < 0.05$)

Treatment	Bud fertility	Leaf area ($m^2{\cdot}vine^{-1}$)	Pruning weight ($kg{\cdot}vine^{-1}$)
Double pruning	1.00 ± 0.07 a	0.77 ± 0.08 b	0.08 ± 0.00 a
Single pruning	0.13 ± 0.04 b	1.64 ± 0.07 a	0.09 ± 0.01 a

Table 3

Effect of double pruning (DP) and single pruning (SP) on cluster number and yield per vine, pH, total soluble solids (TSS), tritatable acidity (TA), phenols and anthocyanin contents in 'Syrah' grape. Same letter do not differ significantly between treatments as determined by Tukey's test ($p < 0.05$)

	Yield and berry quality parameters						
Treat	Cluster number·vine^{-1}	Yield·vine^{-1} (kg)	pH	TSS (°Brix)	TA (g·L^{-1})	Phenols (g·berry^{-1})	Anthocyanins (g·berry^{-1})
DP	9.93 ± 0.58 a	1.01 ± 0.06 a	3.60 ± 0.03 a	21.30 ±0.26 b	6.02 ± 0.17 a	2.87 ± 0.21 a	1.12 ± 0.09 b
SP	2.27 ± 0.28 b	0.24 ± 0.03 b	3.66 ± 0.02 a	22.84 ± 0.35 a	5.73 ± 0.09 a	3.15 ± 0.17 a	1.52 ± 0.09 a

etative vigor measured by pruning weight was not affected by treatments (Tab. 2). The absence of differences between treatments in shoot weight at the end of the autumn-winter cycle, suggests that carbohydrate partitioning to shoot maturation was not affected by crop level. Some studies have also shown that grapevine photosynthesis may be incremented due to high demand for photosynthates caused by low leaf area:fruit ratio (Naor *et al.* 1997, Petrie *et al.* 2000) which may have increased the dry matter allocation to shoots of grapevines under double pruning. Furthermore, it also needs to be considered that shoot trimming realized during the experimental period may have contributed to decrease the differences between treatments.

The Ψ_{stem} was not affected by alterations on leaf area and crop level induced by treatments (Fig. 2). The lowest values of Ψ_{stem} (-0,5MPa) observed in both pruning treatments on June 25th were not indicative of water stress (Leeuwen *et al.* 2009). The highest amount of rainfall during the summer (Fig. 1) associated to high water holding capacity of the soil and low evaporative demand during the winter should have contributed to avoidance of vine water stress.

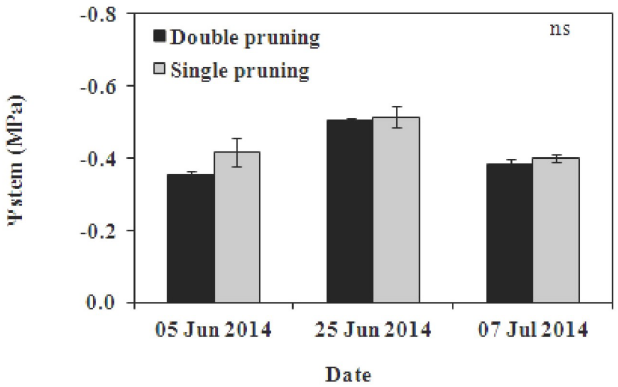

Fig. 2: Effect of pruning management on stem water potential (Ψ_{stem}) of 'Syrah' grapevines during autumn – winter cycle. Each bar is the mean ± standard error. ns = no significant differences ($p > 0.05$).

Due to unexpected unfruitfulness, the single pruning promoted a reduction of 77 % on cluster number and yield as compared to the grape production of grapevines submitted to double pruning management (Tab. 3). Under the lowest crop level, the grapes showed the highest soluble solids, total phenols and anthocyanins contents whereas the pH and total acidity were not affected by yield (Tab. 3). Although this increment on grape quality, the low yield (0.24 kg per vine) is not considered economically viable in viticulture. These results showed that a second pruning realized after grape harvest (August 2013) was really necessary to avoid the bud infertility in the following growing season (autumn-winter cycle of 2014).

The reasons of unfruitfulness in grapevines submitted to single pruning still remain unclear. In viticulture, it is well known that grape clusters of the current season are formed from inflorescence primordia developed within latent buds during the previous growing season (Carmona *et al.* 2008; Vasconcelos *et al.* 2009). During the autumn-winter cycle of 2014, the clusters from grapevines under double pruning originated from five month old latent buds (developed from September 2013 to February 2014) whereas the clusters from grapevines under single pruning were formed from one year old latent buds (developed from February 2013 to February 2014). Although the grapevines have been submitted to annual single pruning as usually done in traditional viticulture under temperate climate, in this study, the yield pruning was done in unpruned shoots that were submitted to two growing cycles (from February to August and from September to February). Under temperate conditions, there is no more shoot growth after grape harvest because they start the dormancy process caused by low winter temperature. In contrast, in the present study, after the previous harvest realized in August of 2013 (at the end of the winter), the lignified shoots of grapevines pruned only in February 2013 resumed their growth through budburst of apical buds due to favorable climate conditions whereas the grapevines submitted to second pruning in September 2013 resumed their normal growing cycle from spur pruned shoots as usually occurs in a traditional viticulture. However, the climatic conditions from previous period from the budburst to harvest (February 2013 to August 2013) are not affecting the inflorescence differentiation. In both treatments, the inflorescence primordia were formed within latent buds during that period. This bud fertility was confirmed by normal grape production during the vegetative cycle (where all clusters need to be removed) of grapevines pruned in September 2013 (double pruning treatment). The reduction on bud fertility of shoots pruned only in February 2013 (single pruning treatment) occurred from September 2013 to February 2014. The possible causes of unfruitfulness may be related to tendril formation (Yahyaoui *et al.* 1998) or to bud necrosis (Cox *et al.* 2012) promoted by reserve consumation and/or hormonal

Table 4

Effect of ethephon application in the summer growing season on starch contents in shoot, trunk and root sampled before summer yield pruning of 'Syrah' grapevines. Same uppercase (for grapevine organs) and lowercase (for treatment) letters do not differ significantly as determined by Tukey's test ($p < 0.05$)

	Starch (mg·g DW^{-1})		
Treatment	Shoot	Trunk	Root
Control	38.64 ± 2.63 Aa	64.33 ± 1.87 Ab	46.46 ± 8.39 Aa
Ethephon	38.39 ± 2.04 Ba	99.56 ± 9.93 Aa	53.37 ± 5.41 Ba

alterations caused by second growth cycle of apical shoots under single pruning management during spring and summer. Anatomical and molecular biological studies are necessary to investigate the development of latent bud in both consecutive growing seasons to explain what is happening during the second semester with inflorescence primordia formed in the first six months of the year.

Carbohydrate reserve, vegetative vigor, yield and berry quality of 'Syrah' growing under autumn-winter season after ethephon application in the previous season: Under double pruning management, the physiological and agronomical responses of 'Syrah' grapevines were investigated during their reproductive cycle (from February 2014 to August 2014) after exogenous application of ethephon at the end of the vegetative cycle (December 2013). The starch concentration was only increased by ethephon on trunk tissues sampled before yield pruning (February 2014) as compared to tissues from shoots and roots (Tab. 4). The trunk from ethephon sprayed grapevines also showed higher starch contents than shoots and roots. However, the shoot growth inhibition before yield pruning of sprayed vines was not detected by dry weight of pruned shoots (Tab. 5). The reasons for no growth inhibition by ethephon could be related to age-dependent response, since ethephon has more effect on younger than older tissues (Hirschfeld and Lavee 1980). In the present study, the ethephon solution was applied at the end of the previous growing season when the shoot growth is usually reduced and when most of the leaves are mature.

On the other hand, during the reproductive cycle, the sprayed grapevines showed the highest leaf area at pea size stage and pruning weight measured after grape harvest (Tab. 5). Fracaro and Pereira (2004) also demonstrated an increase in vegetative vigor of field-grown 'Niagara Rosada' in the following season induced by ethephon applied before yield pruning. This increment on vegetative vigor was possibly induced by the gain of starch accumulation in the trunk (Tabs 4 and 5) and also due to increased nitrogen mobilization by ethephon as shown by Schenato *et al.* (2007). In contrast, during the second growth cycle, the dry matter accumulation on shoots and leaves of rootstock SO4 growing under greenhouse conditions was reduced by ethephon application in the previous growing season as shown by Schenato *et al.* (2007). According to these authors, the reduced vegetative vigor was a result from increased competition for photoassimilates stimulated by the high shoot number from lateral buds of sprayed grapevines. Furthermore, the rootstock SO4 was completely defoliated by ethephon solution which may have also compromised the starch accumulation on permanent structure reducing the reserve mobilization to support the initial shoot growth. In our study, although the concentration of ethephon solution (1440 ppm) was higher than the concentration (72 ppm) used by Schenato *et al.* (2007) there was no vineyard defoliation and the trunk starch accumulation was favored. It should also be considered that the grapevine responses to ethephon can vary according to environmental conditions, pH solution, cultivar, concentration, timing and method of application (Hirschfeld and Lavee 1980, Szyjewicz *et al.* 1984).

Table 5

Effect of ethephon application in the summer growing season on leaf area and pruning weight of 'Syrah' grapevines growing under autumn-winter cycle. Same letter do not differ significantly between treatments as determined by Tukey's test ($p < 0.05$)

Treatment	Leaf area (m^2)	Pruning weight (kg)
Control	1.17 ± 0.14 b	0.08 ± 0.01 b
Ethephon	1.43 ± 0.13 a	0.12 ± 0.01 a

The vine water status during the ripening period was unaffected by ethephon as shown by Ψ_{stem} values (Fig. 3). Both treatments, in all dates, showed values higher than -0.5 MPa suggesting absence of water deficit in the vineyard (Leeuwen *et al.* 2009). There was no impact of ethephon on yield parameters (Tab. 6). The total phenols were increased in berries from sprayed grapevines whereas the total soluble solids, pH, total acidity and anthocyanins were not affected by ethephon treatment (Tab. 5). The phenol synthesis in berries from sprayed grapevines probably did benefit from increase in nitrogen mobilization since the biosynthesis of soluble phenolic begins with the aromatic amino acid phenylalanine, a product of the shikimate pathway (Conde *et al.* 2007, Schenato *et al.* 2007).

Conclusions

The success of winter growing cycle relies on double pruning management which needs a summer pruning realized on five to six months old shoots formed after the winter pruning, realized in the post harvest period. The

Table 6

Effect of ethephon application in the summer season on yield and berry quality parameters of 'Syrah' grapevines during the winter cycle. Same letter do not differ significantly as determined by Tukey's test ($p < 0.05$)

	Yield and berry quality parameters						
	Cluster number·vine^{-1}	Yield·vine^{-1} (kg)	pH	TSS (°Brix)	TA (g·L^{-1})	Phenols (g·berry^{-1})	Anthocyanins (g·berry^{-1})
Control	8.82 ± 0.80 a	0.80 ± 0.11 a	3.56 ± 0.03 a	20.73 ± 0.28 a	5.97 ± 0.08 a	3.43 ± 0.11 a	1.54 ± 0.04 b
Etephon	9.92 ± 0.83 a	0.97 ± 0.08 a	3.58 ± 0.02 a	21.48 ± 0.28 a	5.84 ± 0.09 a	4.125 ± 0.15 a	1.44 ± 0.05 a

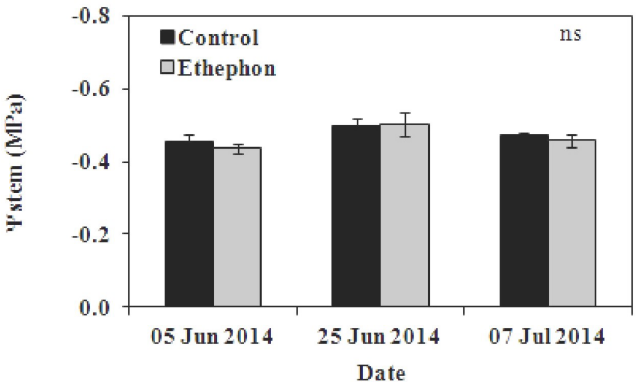

Fig. 3: Effect of ethephon application in the summer growing season on stem water potential (Ψ_{stem}) of 'Syrah' grapevines under autumn – winter cycle. Each bar is the mean ± standard error. ns = no significant differences ($p > 0.05$).

single pruning done, at summer, on one year old shoots (without winter pruning) induces bud infertility impeding the grape production during the winter season. The double pruning management can be improved by ethephon spray in the previous season. During the autumn-winter cycle, the ethephon sprayed grapevines exhibit a better vegetative vigor stimulated by trunk starch accumulation without negative effect on yield and grape quality of 'Syrah' cultivar.

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