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Website: AWRI-Metabolomics South Australia, 2019. 'Methods of targeted and non-targeted chemical analysis', Acedido a 20/2/2019, https://www.awri.com.au/research_and_development/metabolomics_facility

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A Comissão Científica

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Sugestão de *Template* do Manuscrito (a adaptar pelos autores em função da área científica da comunicação)

Effects of different deficit irrigation strategies on yield components and must quality of cv. Touriga Franca (*Vitis vinifera* L.) under mediterranean climate conditions in Douro Region

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Abstract:

Located in the northeast of Portugal, Douro Region is characterized by high temperatures and low rainfall during the vine growing season, which may jeopardize yield and must quality. In 2018, an irrigation trial was conducted in a plot of Touriga Franca variety. Three deficit irrigation regimes and a rain-fed modality were applied in three replicated blocks: R0 (no irrigation, Control), R25 (25 % of crop evapotranspiration (ETc)), R50 (50 % of ETc) and R75 (75 % of ETc). Water was supplied on a weekly (I8) or bi-weekly (I15) basis, since veraison until 15 days prior to harvest. Leaf water status, canopy evolution, microclimate conditions, yield and quality of musts were evaluated. In terms of climate, the year was characterized by higher precipitation and lower temperatures than the average at veraison and at the beginning of maturation, and hot and very dry conditions in the final period of maturation.

In this essay, no significant differences were found in total leaf area neither in most of the components of canopy density. The level of water deficit, assessed through the predawn leaf water potential (Ψ_{pd}), was higher in I15 than I8, being the lowest values obtained in R0 and R25 (-0.48 and -0.49 MPa, respectively). At harvest, the majority of I8 modalities showed higher values of probable alcohol (%), total acidity and anthocyanins (14.37 %; 4.37 g L^{-1} and 220.75 mg L^{-1}). On the other hand, values of berry weight, polyphenols and malic acid were, higher in I15 (1.92 g; 1003.68 mg L^{-1}; 186.42 mg L^{-1}). Significant differences were observed on probable alcohol content in I15 between R0 and R75 (13.47 and 14.38 % respectively). The number of clusters was affected by the frequency of irrigation, being the values in I15 higher than I8, with I15 showing significant differences in R75 in comparison to I8 (11.89 and 9.53). The yield was statistically higher in R50 between I15 and I8, with values of 2.41 kg $vine^{-1}$ and 1.47 kg $vine^{-1}$.

Keywords: Canopy growth, evapotranspiration, RDI, leaf water status, microclimate.

Resumo:

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Palavras-Chave: xxxxxxxxxxx, xxxxxxxxxxxxxxxxxxxxx, xxxxxxxxxxxxxxxxxxxxxxx, rrrrrrrrrrrrrrrrrrrr

Introduction

In Portugal, grapevine production occupies a substantial area, having an economic relevance. Since this culture is highly dependent on climatic conditions, such as temperature and rainfall and once there is a trend to climate change with an overall warming as well as a predicted increase in aridity (Schultz, 2000; Jones and Alves, 2011), irrigation might be an important tool in order to improve yield, wine quality and control vine vigour (Santos *et* *al*., 2007). However, a high amount of water supply can induce exaggerated vegetative growth and consequently denser canopies and lower fruit quality by reducing fruit exposure and increasing the incidence of diseases (Wheeler and Pickering, 2003). Additionally, the lateral shoot growth can also lead to a competition for photoassimilates and higher level of shading which delays berry maturation (Smart *et al*., 1990). In contrast, severe water deficit can lead to stomata closure, a natural mechanism to avoid water loss that affects the production of sugar and reduces yield and fruit quality (Ojeda *et al*., 2002). Finally, mild water stress, by controled deficit irrigation, helps reducing vine vigour and the competition for carbohydrates, promoting a shift in the use of photoassimilates by reproductive tissues and secondary metabolites and improving berry quality, namely total phenolic content (Smart and Robinson, 1991).

Regulated deficit irrigation (RDI) is being used as a tool to reduce water consumption, and is a common practice to manage water deficit during the final stages of grape development. The main objective of RDI is to reduce the irrigation input, improve canopy caractheristics and optimise fruit size and quality (McCarthy, 1997).

The goal of this study was to explore the effect of three irrigation regimes and a rain-fed modality, applied weekly or bi-weekly, in the most widely used red variety cultivated in Douro, Touriga Franca, in order to assess its impact in vine vigour, yield and quality parameters during the growing season of 2018.

Materials and methods

Site description and experimental design

The study was performed during 2018, in an eleven-year-old *Vitis vinifera* L. cv. Touriga Franca plot, located at Quinta da Cabreira (41˚ 03' 29''N, 7˚04' 02'' W), property of Quinta do Crasto S.A., Douro Superior sub-region, Portugal. The vines grafted on 110 Richter rootstocks were trained in a vertical shoot position system and pruned in Royat single cordon (10 buds per plant). Rows were oriented northwest-southwest, and vines were planted 1 m × 2.20 m, (4546 plants ha-1), in a steep hillside, with 45 % of slope, systematized in terraces with two rows of vines, and an average altitude of 240 m. The climate was Mediterranean, characterized by summers with hot to very hot days, fresh nights, and low levels of precipitation. The soil was schistic and predominantly acidic (pH between 4.2 and 5.5).

The experimental design was a split-plot, with three replicates. The treatments comprised irrigation applied every 15 days (I15) or every 8 days (I8) (level I), and irrigation modalities corresponding to 25 % ETc (R25), 50 % ETc (R50), 75 % ETc (R75) and a rainfed modality, the control (R0) (level II). Each experimental unit included 20 vines in I15 (4 modalities × 3 replicates × 20 vines) and 10 vines in I8 (4 modalities × 3 replicates × 10 vines), in a total of 360 vines. All plants of the irrigated modalities were drip-irrigated by irrigation pipes installed 0.4 m above the soil and with drippers spaced 1 m with a flow rate of 2 Lh^{-1} in R25; 4 Lh^{-1} in R50 and 6 Lh^{-1} in R75. Reference evapotranspiration (ET0) was estimated from weather parameters recorded at a meteorological station located in the plot (METOS®, Pssl Instruments, Weiz, Austria). ET0 along with constant crop coefficient (Kc = 0.8) were used to calculate the amount of water required by the plants (ETc) using the equation ETc = Kc × ET0. The constant Kc was chosen from previous records, considering the months where irrigation occurred, the vineyard characteristics and the values described by Prichard *et al*. (2004). Precipitation was deducted from ETc in each week.

The irrigation started in early August (August 1st), day of the year 213 (DOY 213). The decision of starting irrigation on that day was based on data provided by the soil probe (Aquagri, Oeiras, Portugal) installed in the field, when the value of pre-dawn leaf water potential (Ψ_{pd}) reached –0.4 MPa, and on the climatic conditions forecasts, that predicted a heat wave for the following days.

Field determinations

Pre-dawn leaf water potential (Ψ_{pd}) was evaluated on mature leaves (one per vine) of four representative vines per replicate (12 readings per modality) using a pressure chamber (PMS Instruments, Model 600, Albany, USA). This assessment was carried out throughout the growing season, from DOY 193 until DOY 255 and performed every 15 days, 2 hours before sunrise, in a total of 7 measurements.

Leaf area per shoot (12 shoots per modality) was assessed at DOY 225 during ripening in a non-destructive way, using the methodology proposed by Lopes and Pinto (2005).

Canopy density was assessed by Point Quadrat analysis at DOY 274, at the ripening stage, inserting a rod at regular intervals in the vegetation, as proposed by Smart and Robinson (1991). The total of horizontal insertions per modality were 48 (16 per replicate) and the parameters determined were: leaf layer number (LLN), percentage of gaps (% G), percentage of interior leaves (% IL) and percentage of exterior clusters (% EC).

Yield, berry composition and vine vigour

The harvest took place at DOY 277, being the number of bunches and the yield per vine registered. Berry samples were collected, by picking 100 berries per replicate, in a total of 300 berries per modality. Samples were evaluated according to berry weight (BW), probable alcohol (PA; %; Refractometer ATAGO®, Tokyo, Japan), pH (Crison®, CRISON Instruments, Barcelona, Spain), total acidity (TA; g L^{-1}), malic acid (MA; g L^{-1}), anthocyanins (A; mg L-1) and polyphenols (P; mg L^{-1}; Miura One®, Lisbon, Portugal), following the OIV recommendations (OIV, 2018).

In winter, the vigour was evaluated by measuring the pruning weight per vine, and the number of canes was registered.

Statistical analysis

All the data is presented as mean value ± standard deviation (SD) of the three replicates in each essay. The results were compared by one-way ANOVA analysis followed by Dunnett's multiple comparisons test using GraphPad Prism version 6.01 for Windows, (GraphPad Software, La Jolla California USA).

Results and discussion

When compared with the previous 5 years, the growing season of 2018 can be considered as an anomalous year, with a dry and cold winter followed by a cold and particularly rainy spring. These conditions remained during the beginning of summer, changing then to hot and extremely dry weather (ADVID, 2018), with temperatures equal or above 30 ˚C in more than 70 days, and a 5-days heat wave, reaching temperatures above 39 ˚C, at the beginning of August.

Table 1 summarizes the volume of water applied (mm) and the days when the irrigation was performed. Water was supplied since DOY 213 (August 1st) in both treatments. A total of 5 days of irrigation were performed in I8 and 3 times of irrigation were performed in I15 treatment. Regarding the first irrigation, the water amount to be applied was set considering the ETc of the previous 15 days. The last irrigation was performed in DOY 241 (August 8th) in I15 and in DOY 242 (August 9th) in I8 treatment (Table 1). The day with the largest amount of water supplied in I15 was in DOY 228, with a total of 44.10 mm of water. In I8, apart from the first watering in which the previous 15 days were considered (43.58 mm), the greatest supplied amount of water was on DOY 229 (26.22 mm; Table 1).

Table 1: Total quantity of water supplied per treatment (mm), modality and dates of irrigation.

Treatment	**I15**			**I8**			
DOY / Modality	**R25**	**R50**	**R75**	**DOY / Modality**	**R25**	**R50**	**R75**
213	7.26	14.53	21.79	**213**	7.26	14.53	21.79
				220	3.52	7.04	10.56
228	7.35	14.70	22.05	**229**	4.37	8.74	13.11
				236	3.11	6.23	9.35
241	6.40	12.79	19.18	**242**	3.22	6.44	9.66
Total	**21.01**	**42.02**	**63.02**	**Total**	**21.48**	**42.98**	**64.47**

In terms of water status, the Ψ_{pd} decreased during veraison, between the measurements DOY 193 and DOY 207 (Figure 1).

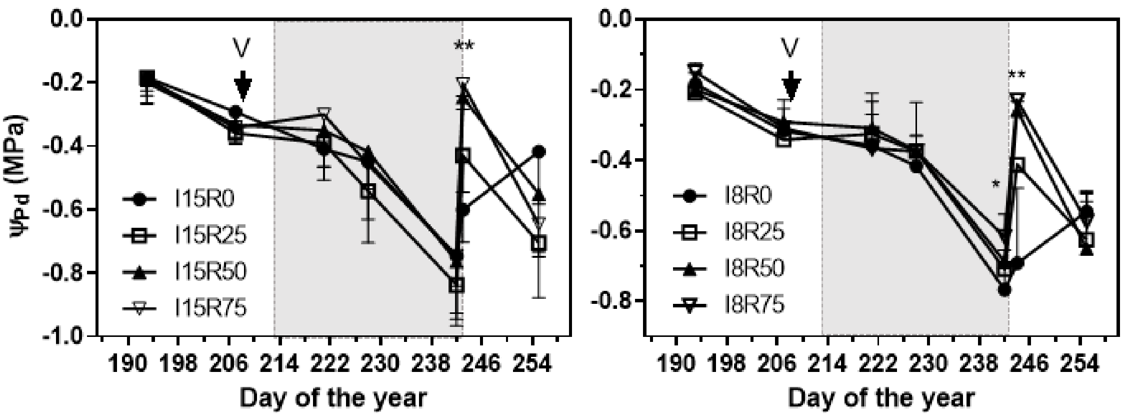

Figure 1: Evolution of pre-dawn leaf water potential (Ψ_{pd}) for all modalities of treatments I15 (left) and I8 (right) from DOY 193 until DOY 255. Each point represents the average of eight measurements with SD. V indicates veraison stage and the shadowed area indicates the irrigation interval. Asterisks indicate statistically significant differences: *$p<0.05$; **$p<0.01$.

However, no irrigation was applied once values were higher than –0.4 MPA, which is an indicator of moderate water stress (van Leeuwen *et al.*, 2009). The irrigation started at DOY 213 and no noticeable impact on Ψ_{pd} was observed in R75 - I15 and R25 - I8, while the others decreased. The values of Ψ_{pd} evidenced a decrease until DOY

242, with no significant differences between modalities, except in I8 between R75 and R0, which showed a severe water stress. Between DOY 228 and DOY 244, all vines presented severe water stress, with values below –0.4 MPa, reaching –0.8 MPa in some modalities in this last day. At the maturation stage, a measurement of Ψ_{pd} was performed immediately after irrigation, (DOY 242 in I15 and DOY 243 in I8). The results indicated that irrigation resulted in a hydric comfort in almost all irrigated modalities, with values above –0.5 MPa in I15 and I8, contrasting with non-irrigated vines (Figure 1). In this measurements, significant differences ($p<0.01$) were observed among R0 and R75, in I15 and I8. In the last measurement, on DOY 255, the observed values were, once again, below –0.5 MPa in all modalities both on I15 and I8 with no significant differences between plants, indicating moderate to severe water deficit, with I15 presenting the lowest values.

CONCLUSIONS

The results obtained in this trial, indicate that during 2018 season, the irrigation at 50 and 75 % ETc, when compared to 0 or 25 % ETc, resulted in higher levels of yield, when water was supplied every 15 days. It was also evidenced that irrigation performed every 15 days had generally beneficial impacts, not only in yield, but also resulting in higher number of clusters and berry weight as well as in some parameters of quality such as polyphenols and malic acid content. The 8 days' basis irrigation only had positive effects on probable alcohol and anthocyanins content although no significant differences were observed among the non-irrigated and the irrigated modalities. Therefore, we can conclude that, the strategy of irrigating every 15 days was the most efficient, particularly when the water was applied in highest amounts, in a percentage of 50 or 75 % of evapotranspiration, especially regarding the yield and must quality. However, further studies should be performed in order to corroborate these achievements, since these results are strongly influenced by climatic conditions.

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