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Research Note

Preveraison Water Deficit Accelerates Berry Color Change in Merlot Grapevines

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Abstract: In red varieties, color change of the berry from green to red is one of the first events associated to ripening and is often used as an indicator of veraison by viticulturists. Water deficit can accelerate the ripening process and increase the accumulation of pigments in the berry skin. The impact of water deficit on the timing and progression of berry color change in the vineyard was little investigated. Here we present the results of three years of observations (2011-2013) on the progression of color change in Merlot vines subjected to water deficit (WD) or irrigation (C) regimes. Water deficit did not affect the date when berries started changing color in 2011 and 2012, but pigmentation begun three days earlier in WD than in C vines in 2013. Water deficit accelerated the pigmentation process in all the years and WD berries completed color change five days before C on average.

Key words: anthocyanins, deficit irrigation, berry ripening, *Vitis vinifera* L.

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Introduction

In grapevine, berry development follows a double sigmoid growth curve divided into two growth phases (Stage I and III) separated by a lag phase (Stage II) during which expansion slows (Coombe 1992). The onset of berry ripening is commonly known as "veraison" and is associated with the transition from Stage II to Stage III (Coombe 1992) normally observed around 8–10 weeks after blooming. At this stage, significant physico-chemical changes occur in the berry, including softening, the resumption of growth, the decrease of organic acid concentration, and the accumulation of sugars and anthocyanins (in red-grape varieties). As a first noticeable sign of ripening, veraison is considered one of the major phenological stage. The date of veraison is usually recorded in commercial vineyards and used as a phenological reference for the application of several viticultural practices and for the prediction of the harvest period. As reviewed by Coombe (1992), within a vineyard, besides remarkably varying from year to year, veraison date varies between vines, between clusters, and between berries within each cluster. Differences in the timing of flowering and fertilization have been suggested as factors causing this asynchrony (Coombe 1992). However, Gouthu and Deluc (2015) recently reported that the seed weight-to-berry weight ratio also affects the timing of ripening initiation; with berries with a higher seed weight-to-berry weight ratio starting ripening later than berries with a lower ratio.

As a result of the berry to berry variability, veraison in a vineyard is often considered to occur when 50 % of the berries are exhibiting ripening signs such as softening and translucent color in white-grapes or red pigmentation in red-grapes. Indeed, in red-varieties, the change in color is observed when the berry is at 9 or 10 Brix (Keller 2010) and is a reliable indicator of the shift of the berry metabolism observed at the onset of ripening. The change in berry pigmentation at veraison can be from green to pink, red, purple, or blue hues accordingly with the profile and concentration of anthocyanins synthesized (Castellarin and Di Gaspero 2007); however, in this manuscript we will name any berry that has changed color from green to pink, red, purple, or blue as red berry, and we will refer to the berry color change from green to red as the pigmentation process.

Recent studies indicated that the blooming-veraison interval is strongly determined by the genetic background of the given variety (Costantini et al 2008). The reduction of the blooming-veraison interval through viticultural practices would be helpful to accelerate the entrance of the berries into the ripening phase, allowing the berries to have more time to ripe. This would be particularly valuable in viticultural areas characterized by a short growing season or a cool climate. Deficit irrigation treatments imposed from early stages of fruit development can accelerate sugar accumulation and advance harvest date (Shellie et al. 2006, Castellarin et al. 2007), promote the biosynthesis and concentration of anthocyanins in the berry skin (Castellarin et al 2007, Ollé et al. 2011). Also, observations made on berries of vines subjected to water deficit from fruit set to veraison, indicate that water deficit may induce an earlier beginning and an earlier end of the color change process (Hardie and Considine 1976, Castellarin et al. 2007), hence favoring a longer ripening period. Here we present the results of three years of observations (2011-2013) on the progression of berry pigmentation under water deficit (WD) and well-watered (C) conditions in a Merlot vineyard.

Materials and Methods

The experiment was conducted in 2011, 2012 and 2013 at the University of Udine experimental station "A. Servadei" (46°02' N, 13°13' E; elevation 88 m), in a 18 years-old

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vineyard of 'Merlot' grafted onto SO4 rootstock. The experimental site and design was described in detail in Herrera et al. (2015). Briefly, to maintain the vines under a fully-controlled water regime, four rows of 85 m in length were covered with an EVA (ethylene-vinyl-acetate) film using an open-side tunnel structure of 5 m in height. Only the central rows were included in the trial. Water was supplied by a sub-surface drip irrigation system and, with the exception of irrigation scheduling, vines were managed according to standard commercial practice that included inter-row cover-crop maintenance, weed removal, pesticide application, and nutrient management. An automated weather station, located 100 m from the experimental site, recorded maximum, minimum and average daily temperature, precipitation, relative humidity, wind speed and radiation.

Two water regimes were established from 31, 24, and 25 days after anthesis (DAA) in 2011, 2012, and 2013, respectively: i) Well-watered (C=Control), in which vines were irrigated weekly at 100% of ETc to maintain midday stem water potential (Ψ_{stem}) between −0.4 and −0.6 MPa; and ii) Water Deficit (WD), in which irrigation was withheld from 25-31 DAA and, when Ψ_{stem} was lower than −1.4 MPa, irrigation was managed to maintain Ψ_{stem} between −1.2 and −1.4 MPa until harvest. Each treatment was replicated four times in experimental plots of 10 vines each in a completely randomized design. Vine water status was estimated weekly using midday measurements Ψ_{stem} as in Herrera et al. (2015).

Monitoring berry pigmentation process

For monitoring the berry pigmentation process in the vineyard a tagging method was employed. In each experimental plot, ten clusters were randomly selected, tagged, and numbered at 40 DAA when all the berries were still green in color. Within each cluster, five berries were

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randomly selected, tagged, and numbered with progressive numbers; thus, a total of 40 clusters and 200 berries were considered for each treatment. These tagged berries were observed every 2 days from the start of berry color change (~50-55 DAA) until the day all tagged berries on all tagged clusters were red. At the first observed change in color from a green to a pink, red, purple, or blue hue the berry was categorized as red. The date when a given berry was classified as red was recorded as the veraison date for that berry. In parallel to the above described methodology, we performed a visual estimation of the percentage of berries that had changed color within each tagged clusters at each date of observation; in this case considering all the berries of the cluster and not only the five tagged berries. The results of this visual estimation were then compared with the results obtained considering the tagged berries.

Statistical Analyses

The effect of water deficit on the velocity of the pigmentation process in the population of berries was assessed using a survival analysis technique (Rich et al. 2010) performed with JMP® software (JMP 7.0, SAS Institute Inc., NC, USA). Survival analysis is commonly used in medicine and microbiology to study follow-up times from a defined starting point to the occurrence of a given event; for example, the time from the beginning to the end of a remission period or the time from the diagnosis of a disease to death. The survival function S(t) is defined as the probability of surviving at least to time t. In our case "surviving" equals to remain green, as the event of interest is the berry color change from green to red. The graph of S(t) against t is called the survival curve. The Kaplan–Meier method can be used to estimate this curve from the observed survival times without the assumption of an underlying probability distribution. We

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used this method to calculate the survival function in both, C and WD treatments and were tested for significant differences using the log-rank test ($p < 0.05$) (Rich et al. 2010).

Chi-square test ($p < 0.05$) was used to assess significant differences between the proportion of green and red berries in C and WD at each observation date.

Results

Climate, phenology and vine water status

Seasonal climatic conditions were different among the three years of experiments (Supplemental Table 1). Generally, the summers in 2012 and 2013 were warmer than in 2011 and the historical mean (1991–2013). However, monthly mean air temperatures during August (when veraison occurred) were warmer than in the 1991–2013 period in all three years and similar among years. Growing degree day (GDD) accumulation calculated from 1 April to 30 September were similar between 2011 and 2012 (1947 and 1935 GDD, respectively) and higher than 2013 (1785 GDD) and the historical average (1721 GDD).

Bud-break was observed on April 10 in 2011 and 2012 and on April 17 in 2013 (Table 1). Anthesis occurred earlier in 2011 (May 22) than in 2012 and 2013 (June 3 and June 7, respectively). Veraison (50% of red berries in the vineyard) was recorded 70, 60, and 65 DAA in 2011, 2012 and 2013, respectively. Grapes from C and WD were harvested on September 14 (115 DAA), September 18 (107 DAA), and September 25 (110 DAA) in 2011, 2012, and 2013, respectively.

The deficit irrigation treatment significantly reduced the midday stem water potential (Ψ_{stem}) of grapevines (Figure 1). In all the years considered, the Ψ_{stem} of C vines remained consistently higher than -0.60 MPa during the whole season, while it decreased progressively

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after irrigation was withheld in WD vines. WD Ψ_{stem} was lower than C Ψ_{stem} from 51, 40, and 38 DAA in 2011, 2012 and 2013, respectively; at these stages, WD Ψ_{stem} was recorded -0.70, -0.95, and -0.66 MPa. Differences were mainly related with the time when irrigation treatments were applied, as in 2011 the treatments were imposed 31 DAA, a week later than 2012 (24 DAA) and 2013 (25 DAA).

Impact of water deficit on berry pigmentation process

First colored berries in C vines were observed at 64, 55, and 59 DAA, and all the berries had changed color by 87, 71, and 77 DAA in 2011, 2012, and 2013, respectively (Figure 2). On the other hand, first colored berries in WD vines were observed 65, 55 and 56 DAA in 2011, 2012 and 2013, respectively, and all the berries had changed color by 81 DAA in 2011 and 68 DAA in 2012 and 2013. Hence, in two out of three years, there was no significant difference between irrigation treatments in the date of first color change. In 2013, first color occurred 3 days earlier in the WD than in the C irrigation treatment. Each year, the rate of berry color change was greater in vines under WD than C irrigation treatment. WD berries completed the pigmentation process 7, 3 and 6 days before C vines in 2011, 2012 and 2013, respectively (Figure 1). The survival analysis ($p < 0.05$) confirmed that this increase in the speed was significant in all three years (Supplemental Figure 1).

The same phenomenon described above was observed when pigmentation was assessed by visually estimating the percentage of red berries on the entire clusters (Supplemental Figure 2). A significant linear regression ($p < 0.001$) was observed between the percentages of red berries determined by observing the five berries that were tagged per cluster and the ones determined by observing the entire cluster (Figure 3).

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Discussion

In the three years of this study, C and WD vines had significantly different levels of water deficit prior to veraison, and the pre-veraison water deficit accelerated the rate of berry color change in the vineyard. When pre-veraison water deficit was milder (Ψ_{stem} = -0.7 MPa from 40 to 50 DAA in 2011), significant differences in the percentage of red berries between the irrigation treatments were observed later during development than when the deficit was more severe (Ψ_{stem} = -0.95 and -1.04 MPa from 40 to 50 DAA in 2012 and 2013, respectively). On the other hand, in 2013, when water deficit was more severe at pre-veraison stages than in the other two seasons, pigmentation started earlier in WD than in C vines, and differences in the percentage of red berries between the irrigation treatments were significant across the pigmentation process. Our results indicate that the earlier achievement of berry red pigmentation in Merlot vines subjected to water deficit is related to a faster transition from 100% green to 100% red berries rather than an earlier beginning of berry pigmentation process in the vineyard. In an experiment with potted 'Cabernet Franc' vines subjected to several irrigation treatments, Hardie and Considine (1976) reported that the berries of vines subjected to pre-veraison water deficit from 44 DAA to 76 DAA, began to change color five days earlier and completed the color transition in a shorter period than the berries of irrigated vines (control) and berries of vines subjected to early (from 22 DAA to 44 DAA) pre-veraison water deficit followed by restored irrigation prior to veraison. Authors hypothesized that an induction of a high sugar concentration through temporary shriveling might explain the early coloration of berries subjected to water deficit, however, in our study, no shriveling was observed in WD berries. Castellarin et al. (2007) reported that water deficit imposed from fruit set until the end of veraison (77 DAA), induced an earlier beginning and end of color change in Cabernet Sauvignon berries. Despite these studies were based on few

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observations within a single experimental season and did not report any detailed data on the progression of color change, the anticipation of the beginning of color change appeared to be the major driver of the earlier completion of the berry pigmentation. In this study, Merlot vines subjected to water deficit did not start the pigmentation process before than irrigated vines two out of three years. Interestingly, pigmentation started three days earlier in WD than in C in the season when water deficit was more severe before and at veraison, suggesting that the level of severity of water deficit might be critical for determining an earlier beginning of color change. Some authors showed that water deficit decouples the anthocyanin/sugar accumulation during ripening (Castellarin et al. 2007, Sadras and Moran 2012, Herrera et al. 2015, Shellie et al. 2015); although in this study we did not couple the observations on color change with sugar analysis on the same berries, our results suggest that the uncoupling observed in other works might be related to the accelerated berry color change observed here, and the faster pigmentation to an enhanced anthocyanin biosynthesis from the onset of berry pigmentation. The hormone abscisic acid (ABA) might play a critical role in regulating the acceleration of berry pigmentation under water deficit. ABA concentration in the berry increases remarkably at veraison (Owen et al. 2009) and several studies indicated that ABA stimulates the synthesis of anthocyanins in grapevine by promoting the expression of key biosynthetic genes (Jeong et al. 2004, Gambetta et al. 2010). Water deficit increases the ABA concentration in the berry (Hochberg et al. 2015) as well as the expression of ABA signaling genes at veraison, potentially involved in the regulation of ripening (Gambetta et al 2010).

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Conclusion

Our study quantified the impact of water deficit on the timing of the beginning of pigmentation in red grapes and showed that water deficit accelerates the transition of the berries from a green to a red hue. Overall these results indicate that water deficit generally hastens the beginning of ripening in the vineyard, favoring an extension of the ripening period than under well-water conditions. This extension possibly contribute in determining the different fruit composition often observed at harvest under water deficit that can translate into improved sensory features of the derived wines.

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Table 1 Dates of the major phenological stages recorded in the experimental vineyard in 2011, 2012, and 2013.

Phenology stage	2011	2012	2013
Budbreak	99[a]	100	106
Anthesis (50% capfall)	141	154	157
Veraison (50%)[b]	210	214	220
Harvest	256	261	267

[a] Dates are given as day of the year (DOY)
[b] Veraison stage is referred to the well-watered control (C) treatment.

Supplemental Table 1 Mean air temperature (°C) and cumulated growing degree days (GDD) in 2011, 2012 and 2013 in the experimental site.

	Mean air temperature (°C)				Cumulated GDD (°C)			
Month	2011	2012	2013	Mean 1991-2013	2011	2012	2013	Mean 1991-2013
Jan	3.2	3.0	4.3	3.7	0	0	0	0.3
Feb	5.2	2.3	3.9	4.5	0	0	0	0.8
Mar	8.7	11.6	7.3	8.6	22.8	62.2	0.9	20.0
Apr	15.0	12.1	13.8	12.7	172.3	136.0	123.5	109.5
May	19.1	17.6	15.8	17.6	455.8	371.1	292.9	344.7
Jun	21.2	22.3	21.0	21.1	793.0	740.3	617.8	671.8
Jul	22.0	24.4	25.6	23.0	1164.4	1185.8	1101.1	1075.0
Ago	24.0	24.8	23.6	22.9	1597.7	1643.1	1523.6	1474.5
Sep	21.7	19.7	18.7	18.2	1947.6	1935.1	1785.1	1721.6
Oct	12.9	14.4	14.8	13.7	2043.1	2082.0	1935.1	1843.1
Nov	8.4	10.6	9.9	8.8	2068.2	2119.6	1978.2	1870.6
Dec	5.0	3.5	5.8	4.4	2068.2	2119.6	1979.1	1871.8

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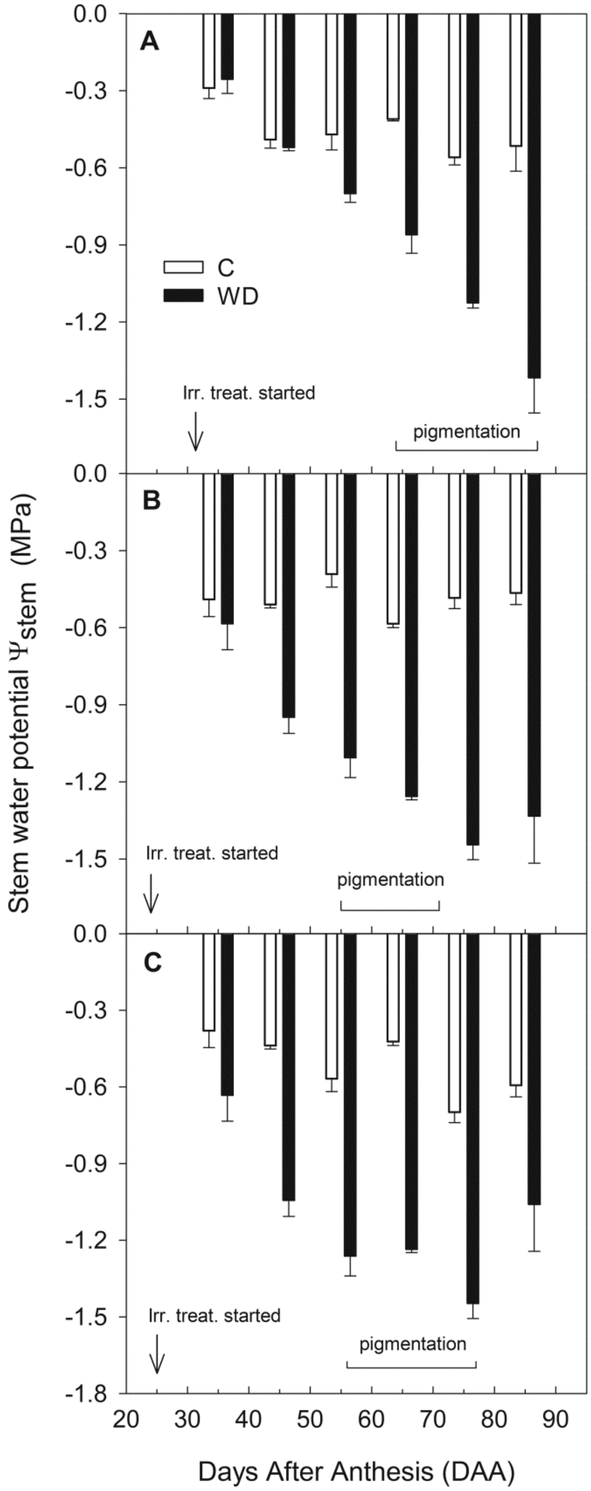

Figure 1 Midday stem water potential (Ψ_{stem}, MPa) of irrigated (C) and water deficit (WD) Merlot grapevines in (**A**) 2011, (**B**) 2012, and (**C**) 2013. Ψ_{stem} values are given as means and standard errors within the given period of time (DAA). Arrows indicate the date of imposed irrigation treatments. Pigmentation period indicate the time lapse between the first colored berry observed and 100% red berries, irrespective of the treatments.

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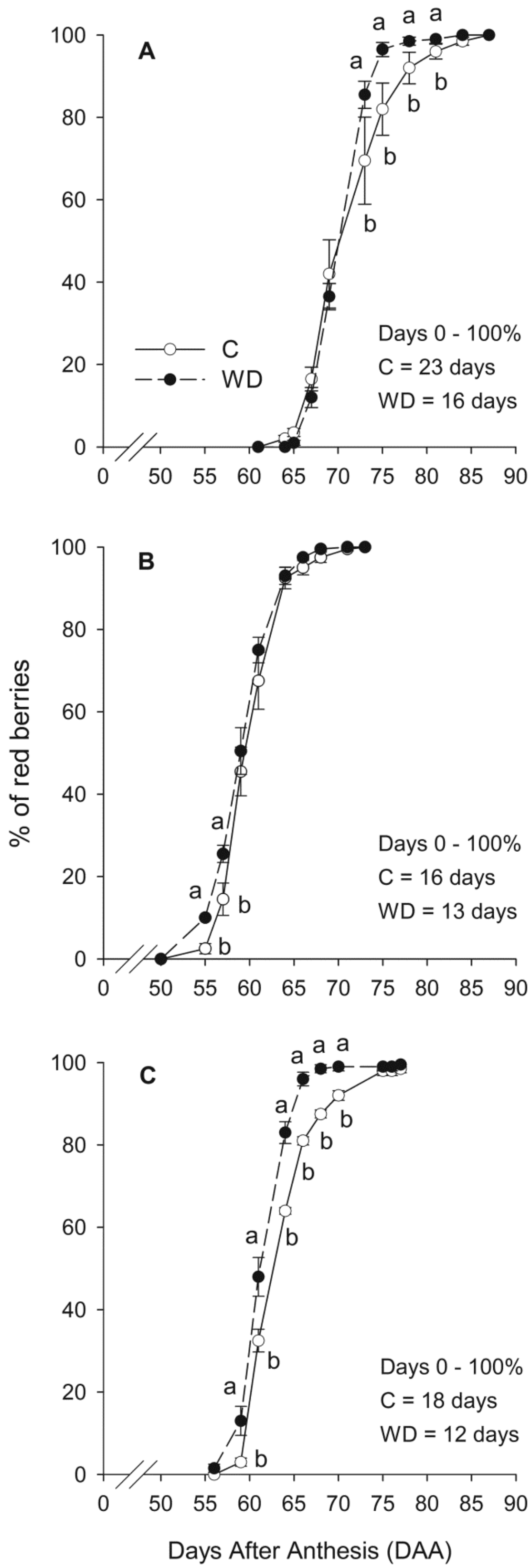

Figure 2 Effect of water deficit on the progression of berry pigmentation (% of red berries) assessed by observing tagged berries in (**A**) 2011, (**B**) 2012, and (**C**) 2013. Each point is the mean of four plots (50 berries each) at a given observation date. Bars represent the standard error (n = 4).

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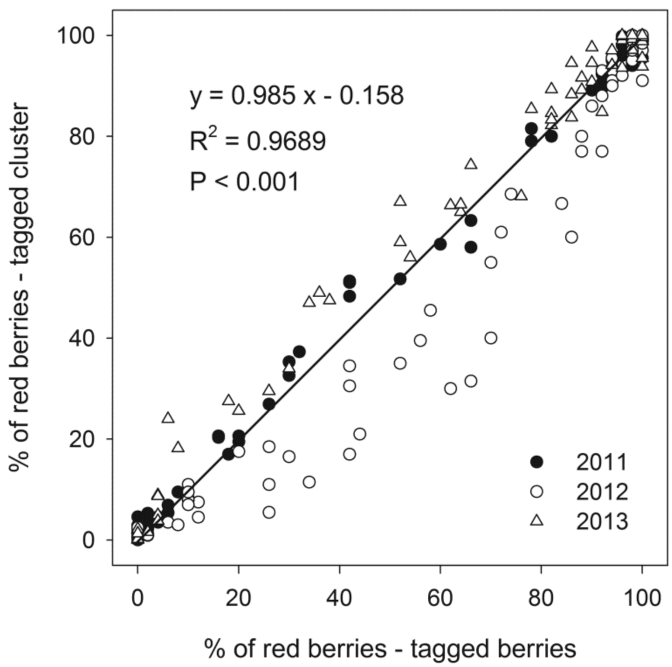

Figure 3 Relationship between the percentage of red berries determined by observing five tagged berries per cluster (200 berries per plot) and the percentage of red berries estimated by visually assessing pigmentation in tagged clusters (10 clusters per plot) at each sampling date in 2011, 2012, and 2013. Regression was performed considering the data from all the three years together.

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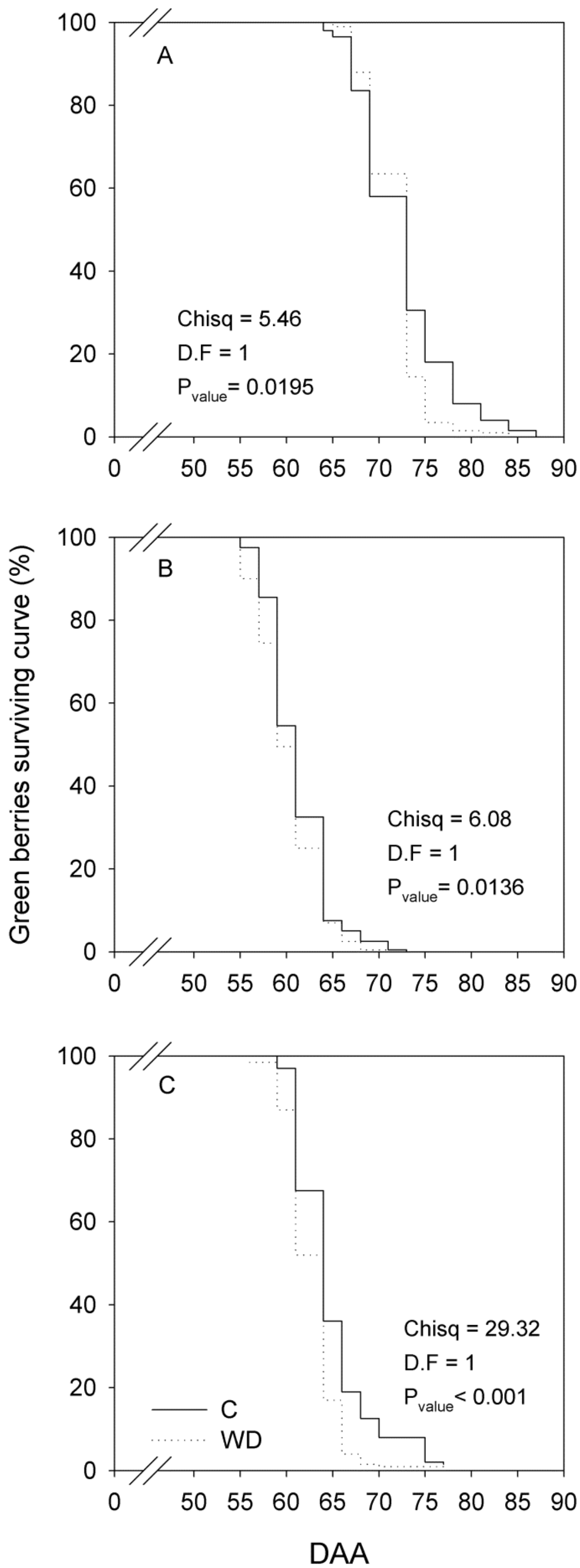

Supplemental Figure 1 Kaplan-Meier surviving curves in well-watered (C) and non-irrigated (WD) vines in (**A**) 2011, (**B**) 2012, and (**C**) 2013. The Log-Rank test parameters are shown in the graphs. $p < 0.05$ identifies a significant difference between C and WD survival curves.

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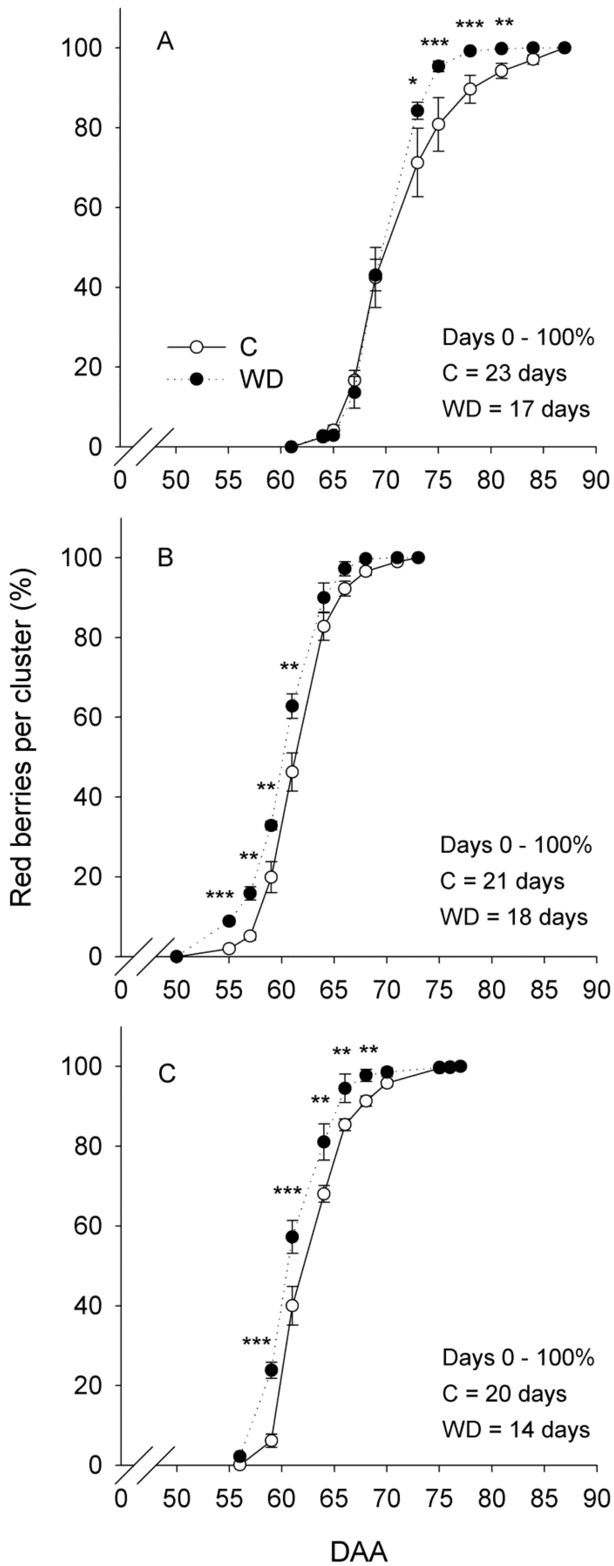

Supplemental Figure 2 Effect of water deficit on the progression of berry pigmentation (% of red berries per cluster) assessed by estimating the percentage of red berries in tagged clusters in (**A**) 2011, (**B**) 2012, and (**C**) 2013. Each point is the mean of four plots (10 cluster per plot) at a given observation date. Bars indicate the standard error (n = 4).