On the irrigation treatment

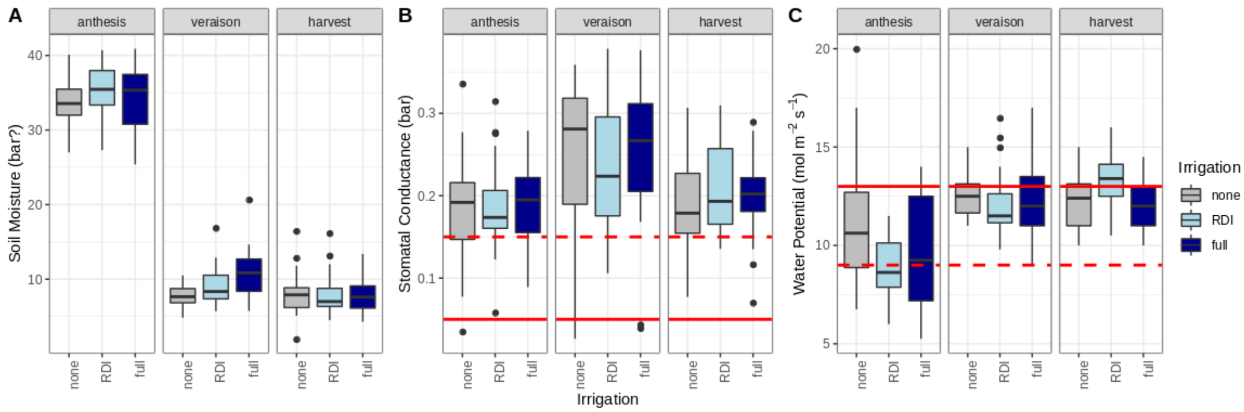
Note: This document has not necessarily been peer reviewed. This document details the methods by which we assessed the effect of the irrigation treatment for the manuscript currently hosted at https://www.biorxiv.org/content/10.1101/2020.11.10.376947v1. Data for this analysis are hosted on FigShare under two DOIs: Physiology (https://doi.org/10.6084/m9.figshare.13201016) and Weather (https://www.doi.org/10.6084/m9.figshare.13198682). The full analysis can be found as Jupyter Notebook in this repository or at https://github.com/PGRP1546869/mt_vernon_2017_leaf/blob/main/2017_weather_precip_data.ipynb.

The vineyard in Mount Vernon, MO features a block design randomized over irrigation treatment and the rootstock genotype to which a common scion (Chambourcin) is grafted (or ungrafted in the case of the control). The irrigation treatment comprises three experimental groups: full (replacing 100% of losses from evapotranspiration (ET)), reduced deficit irrigation (RDI; replacing approximately half of ET losses), and none (replacing 0% of ET losses). Estimated ET losses are calculated from an on-site weather station which measures daily precipitation, daily min/max temperature, wind speed, humidity, pressure, and total solar radiance, among others. These values compose an estimated short crop (reference) ET loss (Allen *et al.*, 1998)). Irrigation in the vineyard is then determined by combining that estimate, a grapevine-specific crop coefficient, and the efficiency of the irrigation system. The grapevine-specific crop coefficient (Kc) is the subject of much research (Doorenbos, 1977; Evans *et al.*, 1993; Williams *et al.*, 2003; Campos *et al.*, 2010; Marras *et al.*, 2016; Munitz *et al.*, 2019), but can be generalized to a polynomial function calculated by Grimes and Williams (Grimes & Williams, 1990), and mid-season maximum Kcs have been identified as ranging from 0.6 (describe) to 1.08 (describe). In this Note, we examine the impact of irrigation treatment in 2017 and develop a framework for handling irrigation in our statistical models.

We first examined three measures that should be associated with our irrigation regime assuming the vines were receiving variable amounts of water and/or were experiencing drought stress: 1) soil moisture, 2) water potential, and 3) stomatal conductance. We modelled each trait using the following model factors: Block, Phenology, Irrigation, Rootstock and all possible pairwise combinations (snTable 1). In cases where a trait showed a significant difference as modulated by irrigation (or an interaction with irrigation), we computed post-hoc mean comparisons. In short, we note that soil moisture is variable at veraison between the full and none irrigation treatments (t.ratio=4.03, padj=0.023). However this difference is not shown in comparisons of traits that potentially reflect water stress. In these cases, there is a difference in water potential at veraison between the RDI and none treatments (t.ratio=4.19, padj=0.013), and there is no difference based on irrigation in stomatal conductance. Despite the observed differences in water potential, the differences do not seem to be so severe that the irrigation treatment induced drought stress (snFig. 1). Van Leeuwen et al. (Van Leeuwen *et al.*, 2009) reported ranges of water potential indicative of stress in two categories: weak to moderate water deficit (-9 to -13 bar) and moderate to severe stress (-13 to -14 bar). Additionally, Flexas et al. (Flexas *et al.*, 2006) reported stomatal conductance ranges indicative of stress including moderate stress (< 0.15 mol/m^2/s) and severe stress (< 0.05 mol/m^2/s). While we certainly observed instances of water stress based on both of these categorizations, they do not seem to reflect the irrigation treatment.

snTable 1.

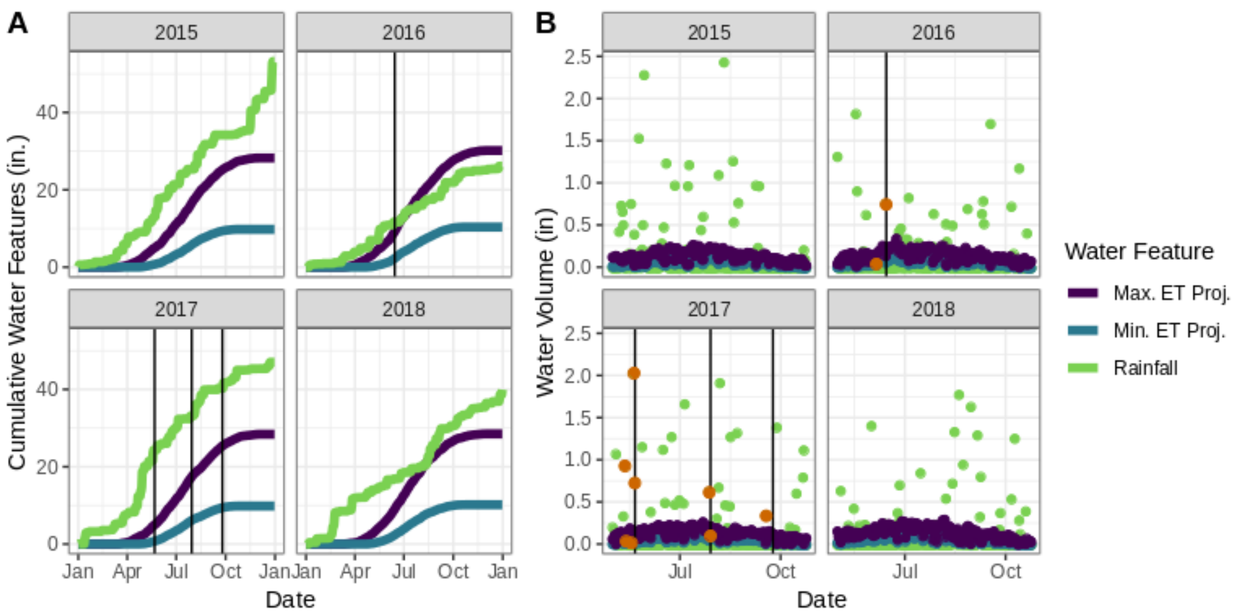
	Model Factor	SS	df	F	p
Soil Moisture	Irrigation	3.705298	2	0.245568	0.7825
Soil Moisture	Block	187.279664	2	12.4119301	**9.06E-06**
Soil Moisture	Rootstock	9.357492	3	0.4134442	0.7436
Soil Moisture	Phenology	4282.033527	2	283.7910935	**8.48E-56**
Soil Moisture	Irrigation:Block	40.437338	4	1.3399891	0.2570
Soil Moisture	Irrigation:Rootstock	65.420995	6	1.4452554	0.1999
Soil Moisture	**Irrigation:Phenology**	103.289907	4	3.4227611	**0.0101**
Soil Moisture	Block:Rootstock	40.368356	6	0.8918021	0.5022
Soil Moisture	Block:Phenology	200.998241	4	6.6605633	**5.15E-05**
Soil Moisture	Rootstock:Phenology	19.176968	6	0.4236502	0.8626
Soil Moisture	Residuals	1327.804005	176		
Stomatal Conductance	Irrigation	0.014462271	2	1.8917976	0.1539
Stomatal Conductance	Block	0.003624752	2	0.4741508	0.6232
Stomatal Conductance	Rootstock	0.01114674	3	0.9720639	0.4072
Stomatal Conductance	Phenology	0.062432311	2	8.1667187	**0.0004**
Stomatal Conductance	Irrigation:Block	0.022383646	4	1.463993	0.2152
Stomatal Conductance	Irrigation:Rootstock	0.024047769	6	1.0485562	0.3957
Stomatal Conductance	Irrigation:Phenology	0.005697191	4	0.3726224	0.8279
Stomatal Conductance	Block:Rootstock	0.033476007	6	1.4596562	0.1947
Stomatal Conductance	Block:Phenology	0.079669605	4	5.2107574	**0.0005**
Stomatal Conductance	Rootstock:Phenology	0.081232411	6	3.5419813	**0.0025**
Stomatal Conductance	Residuals	0.66891336	175		
Water Potential	Irrigation	29.537347	2	4.1984862	**0.0165**
Water Potential	Block	16.747156	2	2.3804678	0.0955
Water Potential	Rootstock	7.169907	3	0.6794282	0.5657
Water Potential	Phenology	102.149803	2	14.5197382	**1.46E-06**
Water Potential	Irrigation:Block	19.000741	4	1.350398	0.2532
Water Potential	Irrigation:Rootstock	22.971551	6	1.0884045	0.3711
Water Potential	**Irrigation:Phenology**	64.004491	4	4.5488509	**0.0016**
Water Potential	Block:Rootstock	26.274745	6	1.2449116	0.2856
Water Potential	Block:Phenology	87.96463	4	6.2517173	**0.0001**
Water Potential	Rootstock:Phenology	10.490926	6	0.4970657	0.8100
Water Potential	Residuals	619.10088	176		



snFig 1. Measured traits expected to respond to drought treatments. A) Soil moisture shown across the season and averaged across irrigation treatments. B) Stomatal conductance of leaves. The horizontal red lines indicate the maximal values for severe stress threshold (solid) and the moderate stress threshold (dashed) as reported by Flexas et. al (2006). C) Leaf water potential with horizontal red lines indicating the minimal values for moderate-to-severe stress (solid) and weak-to-moderate stress (dashed)

We then sought to understand why the irrigation treatment was not producing the anticipated stress responses. To do this, we downloaded four years of weather data from the on-site weather station comprising 2015 (the first complete year for which data exist) through 2018 (the last complete year for which data existed). For each year, we looked at how the cumulative rainfall was associated with the predicted estimates of grapevine evapotranspiration losses over a range of values derived from the literature (sources from Jupyter). In this analysis, we used the polynomial function derived by Grimes and Williams (1990) with adjusted y-intercepts to account for the range of literature derived values. Daily predicted ET losses were modified by multiplying those values by daily crop coefficient estimates. For each year but 2016, rainfall overshadowed the predicted ET losses across the entire year (snFig 2a). In 2017, we note a rapid accumulation of rainfall immediately prior to sampling at anthesis, a lack of rainfall leading up to sampling at veraison, and slightly longer lack of rainfall leading up to harvest.

Finally, we focused on the 2017 sampling window to see if sampling dates were prefaced by significant rainfall events, potentially "washing away" the effects of the irrigation treatments (snFig 2b). At anthesis, five of the ten days leading up to sampling experienced rainfall, three of which more than enough to fully replace evapotranspiration losses. Leading up to veraison, two days experienced rainfall following the second longest period of no rainfall in the sampling window. Leading to harvest, there was only one day of rainfall following the longest period of the sampling window without rainfall. Given that grapevines can typically recover from prolonged drought stress in a matter of days (Hochberg *et al.*, 2017), we propose that the vines were experiencing minimum stress throughout the season, with effects likely being apparent only during windows of decreased rainfall. However, both the anthesis and harvest sampling dates were sufficiently after rainfall events for the vines to have recovered or to have at least begun recovering from the drought and irrigation deficits. At veraison, there does not seem to have been enough time to overcome the prolonged dry period as evidenced by soil moisture, but the plants still do not exhibit signatures of stress as measured through water potential or stomatal conductance. It is possible, though, that while the vines are experiencing a lack of water, they are influenced by previous irrigation treatments, either from previous seasons, previous periods of prolonged rainfall reduction, or a combination of both. This is supported by our previous work where we showed that for a single pre-veraison, pre-irrigation treatment time point, the vines still showed signatures of the irrigation treatment in leaf shape, and leaf ion concentrations (Migicovsky *et al.*, 2019). This sampling event followed a short period of rainfall reduction and three years over which there were likely periods of decreased rainfall (like the window following ~harvest in 2015). Collectively, we have opted to only minimally include irrigation in the statistical models of this analysis. For each model fit, we included irrigation as a single, non-interacting fixed effect under the assumption that irrigation/ stress will have a minimal impact on the results of this study.



snFig 2. Rainfall as compared to projected ET losses at two scales. A) Cumulative rainfall as compared to cumulative ET loss projections from a modified form of Grimes and Williams estimated for all relevant years in which there was complete data. Dark vertical lines indicate sampling events for 2016 and 2017. B) Daily rainfall and predicted ET losses over an extended sampling window. Dark vertical lines indicate sampling events in 2017, and dark orange points indicate rainfall events up to ten days preceding vineyard sampling.

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