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Research Article

Cluster Thinning Does Not Improve Fruit Composition in Grapevine Red Blotch Virus-infected *Vitis vinifera*

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Abstract: The impact of grapevine red blotch virus (GRBV) on Vitis vinifera L. manifests predominantly as reductions in gas exchange, berry total soluble solids, and anthocyanins. Disease management is currently restricted by incomplete understanding of virus spread and is thus limited to vine removal. The present study investigated the potential of irrigation and cluster thinning to improve fruit quality in GRBV-infected Pinot noir vines. Two irrigation levels – grower standard and supplemental (2x grower standard) – were applied in a factorial combination with two cluster

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thinning levels – thinned to one cluster/shoot (at peppercorn-sized berries) and non-thinned (control) – on two different rootstocks: Riparia Gloire and 3309C. Vine growth, disease severity, and fruit composition were observed for three years in order to understand the potential effects of the treatments on GRBV-infected vines. Supplemental irrigation attenuated the proportion of red leaves, but thinning did not have a consistent effect. Supplemental irrigation increased yield by 16-23% and berry mass by 9-10% between rootstocks. Thinning clearly decreased yield, but it also increased berry mass by 4-11% between rootstocks. Supplemental irrigation increased gas exchange in 2020 yet thinning slightly reduced gas exchange. These impacts on gas exchange did not affect total soluble solids in the fruit at harvest. Increases in berry sugar content indicate that sugar import increased commensurately with berry size as a function of both increased irrigation and cluster thinning. Crop load (Ravaz index) exhibited a correlation with berry sugar for the Riparia Gloire rootstock only, suggesting that crop load adjustment has a limited impact on ripening for GRBV-infected vines. Neither irrigation nor thinning significantly impacted anthocyanin concentration and the impact on other secondary metabolites was inconsistent. The respective increase or decrease in yield may determine whether the limited improvements of supplemental irrigation and thinning on fruit quality in GRBV-infected vines are beneficial.

Key words: cluster thinning, crop level, irrigation, ripening, rootstock, virus

Introduction

Since 2008, Grapevine red blotch virus (GRBV) has emerged as an economically significant virus impacting winegrape production in the United States and other major winegrowing regions (Krenz et al. 2014, Al Rwahnih et al. 2015). GRBV impacts vines in a similar

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manner to the well-studied grapevine leafroll-associated viruses, which are also phloem-limited, cause leaf reddening, and impact critical fruit quality parameters for wine production (Maree et al. 2013). The most significant impact of the virus manifests as delayed ripening and ultimately results in a diminution of sugar and anthocyanin concentration in the fruit, both of which may reduce wine quality (Sudarshana et al. 2015, Girardello et al. 2020). The reductions in sugar and anthocyanins are a likely consequence of reduced gas exchange and carbon translocation, though the causal mechanism behind this is not well understood (Martínez-Lüscher et al. 2019, Bowen et al. 2020,). The economic cost of the virus has been estimated based on price penalties for reduced fruit quality, thus demonstrating the need for strategies to reduce the spread or incidence of the virus and reduce the impact of the resultant disease on fruit quality (Ricketts et al. 2017). Currently, removal of infected vines is the only recommended course of action, though vineyard floor management to reduce potential insect vectors has been preliminarily investigated (Bick et al. 2020).

To date, few viticultural practices have been thoroughly investigated in GRBV-infected grapevines. The common practice of deficit irrigation was recently shown to exacerbate the impacts of GRBV, suggesting that increasing vine stress is not appropriate for mitigating the impact of the disease (Levin and KC 2020). Conversely, increasing water supply may prove more appropriate for irrigation management in GRBV-infected vines (Copp and Levin 2021). Crop adjustment or cluster thinning has long been used to improve sugar accumulation in grapevine by adjusting the source:sink ratio, or the proportion of fruit to vegetative growth (Kliewer and Dokoozlian 2005). Cluster thinning has been shown to significantly improve sugar accumulation in leafroll-infected vines (Kliewer and Lider 1976). Cluster thinning was applied to GRBV-

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infected vines before the virus was well understood, though the improvement in sugar accumulation observed in that study was slight (Calvi 2011).

Selection of plant material is one of the first consequential decisions made in the management of a vineyard and thus may impact the effect of cultural practices or even the severity of disease expression. Some work has been conducted in GRLaV-infected vines showing that there is an interactive effect between disease status and rootstock on vine growth, but there are to date no such reports on the interaction of rootstock with GRBV (Golino et al. 2015). The influence of rootstock on vine response to water stress—one of the most prolific areas of grapevine rootstock study—may also consequentially impact the severity of GRBV on vine growth and fruit composition (Zhang et al. 2016). There are far fewer reports related to rootstock and cluster thinning, but one such study observed differences in photosynthetic response to cluster thinning on various rootstocks (Koblet et al. 1996).

The present study evaluates the efficacy of irrigation and cluster thinning practices to attenuate the negative effects of GRBD on vine physiology and fruit composition, and test the hypothesis that decreasing vine water stress and crop load may reduce the overall impact of the disease. This study also serves as a companion to another study investigating the impact of irrigation and fertilization practices on GRBV-infected vines (Copp and Levin 2021). Additionally, the present study observed responses of vines grown on two different rootstocks to the applied treatments, which may begin to address the interaction of disease expression and plant material in GRBV-infected vines. This study responds to previous reports related to the impact of irrigation management and cluster thinning on GRBV-infected vines and furthermore has the

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potential to inform vineyard management of GRBV by providing a more economical alternative to removal of infected vines.

Materials and Methods

Vineyard site. The study was conducted in a commercial vineyard block of V. vinifera L. cv. Pinot noir (Pommard clone) located in the Rogue Valley AVA near Ashland, Oregon (42.1946°N, 122.7095°W; 640 m asl). The study plot (0.90 ha) was comprised predominantly of Carney series clay soil with 5 – 20% slopes facing southwest. Soils were a fine, smectitic, mesic Udic Haploxerert. Vines were grafted on either 3309 Couderc (3309C; V. riparia × V. rupestris) or Riparia Gloire (RG; V. riparia) rootstock and planted in 2015. Rows were oriented NNW-SSE with a row spacing of 2.75 m, vine spacing of 1.22 m, and vine density of 2990 vines/ha. Vines were head trained and cane pruned to double Guyot with two 0.6 m canes of 6 to 8 buds each (12 to 16 buds per vine). Foliage was supported on a vertically shoot positioned (VSP) trellising system consisting of a fruiting wire at 0.9 m above the soil surface and three pairs of catch wires at approximately 1.2, 1.5, and 1.8 m above the soil surface. Pest, disease, and canopy management (e.g., shoot thinning and leaf removal) was conducted according to regional industry standards.

Treatments and experimental design. From 2018 to 2020, treatments consisted of grower control (CON) and supplemental (SUPP) irrigation and control (CON) and cluster thinned (THIN) treatments. SUPP irrigation treatments received twice the amount of irrigation as the grower control. The thinning treatments were applied at peppercorn-sized berries (E-L stage 29; Coombe, 1995). All clusters were retained for CON vines and THIN vines were thinned to one cluster per shoot by removal of distal cluster(s).

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The four experimental treatments were arranged in a randomized complete block design (RCBD) with a split-plot factorial treatment structure and four replications per rootstock. The main plots consisted of irrigation treatments and were imposed down the entire row. The thinning treatments were the split plots and were applied within the rows. Three vines per replicate were subsampled for all three years and the means of these subsamples were used for statistical analysis.

Climate data. Maximum and minimum air temperature, daily precipitation, and solar radiation data for 2018 were accessed from the Medford, Oregon AgriMet Weather Station (42.3311°N, 122.9377°W). Data in 2019 and 2020 were obtained from the Oregon IPM Center's Online Phenology and Degree-day Models tool (http://uspest.org/dd/model_app) using a weather station approximately 7 km from the study site.

Irrigation. Grower control irrigation treatments had two 2 L/hr. emitters per vine and supplemental irrigation treatments had four 2 L/hr. emitters per vine. Irrigation was scheduled by the grower and applied water amounts were quantified using in-line water meters. ETo was obtained from the Medford, Oregon AgriMet Weather Station (42.3311°N, 122.9377°W).

GRBV status. Vines were surveyed for symptoms of GRBD in 2017 and were tested for GRBV infection in early 2018 (February) using dormant cane tissue. The primer pairs CPfor/CPrev and Repfor/Reprev were used following the protocol of Krenz et al. (2014) for PCR-based diagnosis of GRBV with 16Sfor/16Srev used as an internal grapevine control. Originally, the treatments were intended to be replicated across GRBV-positive and GRBV-negative vines, but all data vines that tested negative for GRBV in spring 2018 tested positive in fall 2018 and were subsequently excluded from the study. The high incidence of GRBV symptoms (>97%) at

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the vineyard site along with prohibitive costs of additional testing precluded the identification and selection of replacement GRBV-negative data vines.

Vine water status. Stem water potential (ψstem) was measured throughout the 2019 and 2020 seasons to determine the effect of irrigation treatments on vine water status. Fully expanded photosynthetically mature leaves were covered with a foil bag for at least 10 min prior to determining ψstem with a pressure chamber (Model 615, PMS Instruments, Albany, OR) according to Levin (2019). Vine water status measurements were made on sunny days between 1300 and 1500 hr. on one leaf per replicate. Data are presented as means averaged across the treatment period—from treatment imposition to harvest—and reflect three sampling dates in 2019 and 2020 each.

Disease severity. The severity of GRBD symptom expression was quantified at harvest each year. Severity was estimated as the percent of symptomatic (interveinal reddening) leaves per vine at harvest on all three data vines per replicate. The Horsfall-Barratt scale was used to convert percentages to midpoint percentage values, which were ultimately used for analysis (Horsfall and Barratt 1945).

Canopy growth and leaf gas exchange. Pruning weights and shoot counts were recorded for each vine at the time of pruning in all three years. Leaf gas exchange was measured with a portable photosynthesis system (LI-6400XT, LI-COR Biosciences, Lincoln, NE) on one leaf per replicate five days postveraison in 2020. Data were obtained between 1100 and 1400 hr. on leaves similar to those used for ψstem determination. Chamber relative humidity and temperature were set to match ambient conditions. Flow rate was set at 400 µmol/s, chamber CO2 concentration was

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set in the reference cell at 400 µmol/mol, and irradiance was set at 2000 µmol/m2/s. Analyzers were matched every 30 min.

Yield and fruit composition. Plots were harvested within 24 hrs. of the contracting winery's decision based on desired technological maturity (23-25 ºBrix, depending on year). Total vine yield and cluster number per vine were recorded in the field at harvest each year and average berry mass was determined in the lab following harvest. Berries per cluster and cluster mass were calculated from the measured variables.

Berry chemistry and phenolics were determined at harvest each year. Additionally, in 2019, berry samples were harvested weekly from plots beginning one week prior to veraison through to the week prior to harvest. Harvested berry samples comprised 60 berries per replicate (20 randomly selected berries per data vine) and subsamples of 20 berries were stored at -20°C for later phenolic analysis. The remaining berries were juiced by hand and centrifuged at 15,000 × g for five min. Total soluble solids (TSS) was determined using a handheld digital refractometer (AR200, Reichert Analytical Instruments, Depew, NY). Juice pH was measured using a benchtop pH meter (Orion 3-Star, Thermo Fisher Scientific, Waltham, MA). Titratable acidity (TA) was measured by titration with 0.1 N NaOH using an autotitrator (T50, Mettler Toledo, Columbus, OH). Repeated samples from 2019 consisted of 20 berries per sample and were analyzed for TSS, pH, and TA as described above.

Secondary metabolites. The 20-berry subsamples from harvest were thawed, peeled, sorted into skin and seed fractions, dried, and extracted in 70% acetone for 24 hr. on an orbital shaker (VWR, Radnor, PA) at 100 rpm. Acetone was removed from skin and seed extracts (Syncore Analyst Polyvap, BUCHI Corporation, New Castle, DE). Tannins, iron-reactive

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phenolics, and anthocyanins were then quantified from the skin and seed extracts using the Harbertson-Adams assay (Harbertson et al. 2002, 2015, Heredia et al. 2006).

Data analysis. All statistical analyses were conducted, and figures generated using R statistical software (v. 4.0.3; www.R-project.org). Data associated with vine water status, gas exchange, disease severity, vegetative growth, yield, fruit composition, and wine composition were analyzed with a three-way Type III ANOVA for RCBD with a split-split-plot factorial treatment structure using the *lmerTest* package (v. 3.1.3; Kuznetsova et al. 2020) and the Kenward-Roger approximation of degrees of freedom. The main and split plots were irrigation and thinning treatments, respectively (as described above), and the split-split-plots were years. Rootstocks were not randomized in the field and the statistical analyses of data for each rootstock were thus conducted separately. Estimated marginal means (AKA least-squares means) were generated and compared using the *emmeans* package (v. 1.5.2.1; Lenth et al. 2020) with the Tukey-Kramer adjustment method for multiple comparisons. Transformation of data due to heteroscedastic variance was conducted when required, and presented data are backtransformed. Figures were generated using the *ggplot2* package (v. 3.3.2; Wickham et al. 2020).

Results

Environmental conditions, vine phenology, and treatment imposition. Differences in environmental conditions at the study site were largely related to precipitation (Table 1). For example, there was over a twofold increase in precipitation in 2019 compared to 2018. 2019 and 2020 were milder than 2018 with respect to GDD accumulation.

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Phenological dates were largely similar in all three years of the study. Bud break (50% leaf tips separated) was observed on 23, 16, and 16 April in 2018, 2019, and 2020, respectively. Bloom (50% cap fall) was determined on 3, 6, and 2 June in 2018, 2019, and 2020, respectively, and veraison (50% coloration of clusters) was determined on 10, 7, and 7 August in 2018, 2019, and 2020, respectively. Harvest dates were slightly more variable than other phenological events – fruit was harvested on 1 October, 25 September, and 17 September for 3309C and 1 October, 2 October, and 21 September for RG in 2018, 2019, and 2020, respectively. Maturity based on TSS was delayed in RG compared to 3309C in all three years of the study, even though they were harvested on the same date in 2018. Phenology by date and GDD accumulation are referenced in Table S1.

Total irrigation quantities were similar in 2018 and 2020, but approximately double in 2019 (Fig. 1A). Irrigation treatments commenced on 5 July, 12 June, and 2 June in 2018, 2019, and 2020, respectively. Considering the combination of applied irrigation and growing season precipitation, the water supply in 2019 was much greater than in 2018 or 2020. The crop for THIN treatments was adjusted to one cluster per shoot after fruit set on 21 June, 25 June, and 20 June in 2018, 2019, and 2020, respectively. In 2019, however, a late-season (three weeks postveraison; 27 August) thinning was conducted by the grower which essentially equalized clusters per vine across treatments (Fig. 1B-C).

Response of vine water status and leaf gas exchange. There was a significant effect of irrigation on ψstem for both rootstocks such that SUPP irrigation vines had higher ψstem (Fig. 2). For RG, however, there was an interaction between irrigation and thinning factors whereby water

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status was higher in vines that were thinned at CON irrigation level only. ψstem was on average higher in 2019 than in 2020.

Net carbon assimilation (Anet) and stomatal conductance (gs) were increased with SUPP irrigation in both rootstocks (Table 2). Anet increased by 38 and 102% for 3309C and RG, respectively, with SUPP irrigation, while gs¬ increased by 71 and 107% for 3309C and RG, respectively, with SUPP irrigation. THIN generally reduced both Anet and gs, though the trend was only statistically significant for Anet in RG. Additionally, there was a significant interaction between irrigation and thinning in the responses of Anet and gs in RG, whereby THIN reduced gas exchange more at the SUPP irrigation level. The vines that were not thinned and received SUPP irrigation consistently had the highest gas exchange values for both rootstocks. Averaged across all treatments, gas exchange values were ~33% lower for RG than for 3309C.

Yield and pruning mass. SUPP irrigation significantly increased yield for RG vines, but not for 3309C vines (Table 3). The significant interaction of irrigation and thinning for RG indicates that the difference in yield between thinning treatments was greater at the SUPP irrigation level. Averaged across treatments and years, yields for RG were only 5% higher than for 3309C. Yields decreased by 55 and 47% from 2018 to 2020 for 3309C and RG, respectively.

SUPP irrigation increased pruning mass by 27-70% and 28-47% in 3309C and RG, respectively (Table 3). Thinning had no significant impact on pruning mass in all three years. Averaged across years and treatments, pruning mass for RG vines was 46% lower than for 3309C vines. Thinning significantly reduced the ratio of yield to pruning mass (Ravaz index) in both rootstocks (Table 3). Ravaz index was 44-56% and 39-48% lower for vines that were thinned for 3309C and RG, respectively, in 2018 and 2020 when the late season thinning was not conducted.

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A significant interaction between year and thinning treatment for Ravaz index and yield is attributable to the late thinning conducted in 2019, which essentially equalized yields between thinning treatment plots. Despite this, Ravaz index values were still 48% lower in RG vines that were thinned early.

Disease severity. Disease severity was reduced by 12-25% and 11-21% by SUPP irrigation in 3309C and RG vines, respectively (Fig. 3). SUPP irrigation had a significant effect in 2018 for 3309C and in 2018 and 2019 for RG, though SUPP irrigation lowered disease severity generally in all three years. Disease severity for RG vines that were thinned trended higher, and the effect was statistically significant in 2019. Disease severity was 10% higher in RG vines compared to 3309C vines when averaged across years and treatments.

Berry growth and development. Berry mass was significantly increased by both SUPP irrigation and early thinning (THIN) for both rootstocks, but not in all years (Fig. 4). SUPP irrigation increased berry mass in 2018 and 2020 for 3309C, and in 2018 and 2019 for RG. THIN increased berry mass for 3309C only in 2019 regardless of irrigation level. For RG, THIN increased berry mass in all three years but only at the CON irrigation level. Notably, berry mass of THIN vines increased by ~9% for both rootstocks in 2019 despite the late thinning which equalized yields. Overall, berry mass was only 4% higher in RG compared to 3309C when averaged across years and treatments. In 2019, berry mass for 3309C was stable for approximately three weeks and started to decrease by harvest (Fig. 5). Both the thinning and irrigation treatments appear to have influenced berry mass for RG nearly six weeks before harvest in 2019, but the thinning effect on berry mass for 3309C did not manifest until harvest.

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There were no consistent nor significant impacts of the irrigation or thinning treatments on TSS at harvest in all three years of the study (Fig. 4). In 2019, there were however treatment effects on TSS that appeared during ripening but disappeared two or more weeks before harvest (Fig. 5). For example, both thinning and SUPP irrigation increased TSS for 3309C from early August to mid-September, at which point TSS values largely converged. For RG, only thinning had such an effect, and it was even more ephemeral than for 3309C. Fruit from 3309C vines reached higher TSS earlier and was thus harvested earlier than RG in 2019 and 2020.

The impacts of the treatments on sugar per berry were largely the same as for berry mass, whereby irrigation increased sugar per berry in 2018 and 2020 for 3309C and only in 2019 for RG (Fig. 4). THIN significantly increased sugar per berry only at the control irrigation level in 2019 for 3309C and in 2018 and 2020 for RG. Figure 5 shows that, in 2019, 3309C berries stopped accumulating sugar approximately three weeks prior to harvest at a sugar concentration of 22-23 °Brix while RG berries continued to accumulate sugar until harvest, albeit at a diminishing rate.

The relationships of TSS to both yield and Ravaz index were investigated for 2018 and 2020 in order to further interrogate any effect of the thinning treatments on sugar accumulation. However, TSS exhibited no significant relationship with either variable for 3309C in any year, and only exhibited a statistically significant but weak negative relationship with yield (R2 = 0.31, p = 0.014) and Ravaz index (R2 = 0.22, p = 0.037) for RG only in 2018. Similarly, the relationship of TSS to ψstem was analyzed for 2019 and 2020 to disentangle the effects of the irrigation treatments. The only noteworthy relationship was a very weak positive one between ψstem and TSS for RG in 2019 (R2 = 0.24, p = 0.057) for which TSS values ranged < 2 °Brix across the dataset.

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Finally, THIN raised juice pH consistently in both rootstocks, but SUPP irrigation only significantly reduced pH in 3309C vines in 2018 (Table 4). The most consistent trend in berry acid metabolism was higher juice TA values associated with the SUPP irrigation treatment. Though juice TA values trended lower in THIN vines, it was not statistically significant.

Secondary metabolites. Berry skin anthocyanin concentration (mg per gram fresh weight) was inconsistently affected by the treatments, though values did trend down with SUPP irrigation in 2019 for both rootstocks, and in THIN vines at the CON irrigation level for RG in 2020 (Fig. 6). Similarly, the effects of treatments on skin tannin concentration were neither consistent nor strong, and varied mostly by year. THIN generally increased skin tannins for 3309C ($p = 0.076$) and RG ($p = 0.002$) while SUPP irrigation reduced skin tannins for 3309C in 2019 yet increased skin tannins for RG in 2020. The responses of skin iron-reactive phenolics (IRPs) and seed-associated secondary metabolites were much more variable across treatments and years (Table S2).

Discussion

The present study sought to evaluate the potential of irrigation and cluster thinning for mitigating the effects of GRBV on vine physiology and fruit composition by reducing vine stress associated with water deficit and crop load. Additionally, the experiment was duplicated in two rootstocks to understand the differential impact of GRBV on vine physiology amongst rootstock phenotypes. In all three years, fruit from all treatments likely reached maximum sugar accumulation, thus it was difficult to delineate positive effects of supplemental irrigation or cluster thinning with respect to ripening. Increases in berry mass were consistent among supplemental

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irrigation and cluster thinning treatments, but this did not negatively impact the concentration of anthocyanins. Differences observed in gas exchange and ripening between 3309C and RG rootstocks are likely related to genetic differences in water relations and could be compounded by GRBV infection. Ultimately, the loss of yield and labor costs associated with cluster thinning may preclude it from being an effective strategy for producing better quality fruit from infected vines.

Thinning and supplemental irrigation do not improve TSS after sugar accumulation ceases in GRBV-infected vines. No treatment effects on TSS were observed in this study, due to the fact that the fruit likely reached maximum sugar accumulation. The TSS value at which sugar accumulation ceases may vary by variety and even virus status, but values for Shiraz have been reported around 20-22 °Brix (Coombe and McCarthy 2000). TSS values at harvest in the present study were above 22 °Brix—and in some cases up to 25 °Brix—irrespective of treatment. It is improbable that any cultural treatments would increase TSS once sugar accumulation has ceased. Normally, TSS would continue to increase through berry desiccation, but the data presented here for 2019 show that berry mass was rather stable up until harvest. Calvi (2011) suggested that additional hangtime may help fruit from GRBV-infected vines reach technological maturity, but the feasibility of this strategy would depend on climatic conditions near harvest and the length of the remaining growing season. Thus, the effectiveness of cultural practices to mitigate the effects of GRBV on sugar accumulation is likely useful only until the cessation of sugar accumulation, as demonstrated by the sugar accumulation curves. Accordingly, this strategy might be more effective on later-ripening cultivars whose berries are continuing to accumulate sugar right up until the very end of the season.

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Kliewer and Lider (1976) reported improvements in TSS for thinned, leafroll-infected vines, but TSS values were overall lower than what was observed in this study. In a companion study to the present one, supplemental irrigation did significantly improve TSS, but TSS values for the control irrigation treatment did not reach 22 °Brix over three years, and maximum sugar accumulation was likely not achieved for the control treatment (Copp and Levin 2021). Sugar per berry at harvest in the present study was commensurate with berry size such that thinned vines and supplemental irrigation vines yielded larger berries at a similar concentration of sugar. The phenomenon by which sugar import scales with berry size has been demonstrated in healthy vines, and is conserved even at various levels of water deficit (Roby et al. 2004). It is difficult to determine whether thinning or supplemental irrigation advanced sugar accumulation, but it ultimately did not matter as TSS values per treatment converged by harvest.

Economic estimates of Pinot noir production in Oregon indicate that cluster thinning to 50% could cost up to $17,661/ha or lead to a 58% reduction in revenue when factoring in the cost of both manual cluster thinning and lost revenues from reduced yield (Olen and Skinkis 2018). Ultimately, thinning fruit to achieve the same TSS as the other treatments would seem economically disadvantageous, especially if GRBV-infected fruit are already discounted due to potential wine quality concerns (Ricketts et al. 2017).

The late thinning of the CON thinning treatment vines in 2019 appeared to have little discernable effect on berry ripening or composition. The increase in berry mass observed in THIN vines for RG was conserved in 2019 despite a late thinning of CON vines in late-August (~3 weeks postveraison). The significant thinning effect on TSS for RG did disappear around the time of the late thinning, but there were no significant differences in TSS between thinning treatments in the

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other years either. For 3309C, the late season thinning of CON vines was concomitant with a slight decrease in berry mass and, by extension, sugar per berry at harvest, but this is likely due to sampling error. Moreover, the growth curve data demonstrate that neither early nor late thinning of clusters in infected vines improved ripening. The 2019 data confirm that any impact of thinning on ripening rate (i.e., Brix/day) is transient and largely disappeared by harvest. However, the results presented in herein cannot disprove the role that extended hangtime may have played in the convergence of TSS values across both irrigation and thinning treatments.

Despite increases in berry mass, thinning and supplemental irrigation are not necessarily deleterious for secondary metabolite concentrations. The significant increase in berry mass as a function of both thinning and supplemental irrigation was the most consistent effect of the treatments in this study, and even persisted despite a late thinning of the CON vines in 2019. This increase may, anecdotally, concern winemakers with respect to potential dilution of skin-associated secondary metabolites. However, berries from healthy Cabernet Sauvignon grapevines were demonstrated to show a relatively constant ratio of skin mass to flesh mass—irrespective of berry size—such that the concentration of skin-associated solutes (e.g., anthocyanins) would not decrease in the same way the proportion of surface area to volume decreases with increasing sphere size (Roby and Matthews 2004). This accounts in part for the lack of significant differences in anthocyanin concentration in berries and, arguably more importantly, in the resulting wines from the supplemental irrigation and thinning treatments.

Rootstock differences in water relations may impact ripening in GRBV-infected vines. The rootstocks in this study reflect two of the most commonly used rootstocks in Oregon winegrape production (Shaffer et al. 2004). Early work involving the drought resistance of

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different rootstocks classified 3309C as more drought resistant than RG based on a parameter integrating leaf area and stomatal conductance under restricted water supply (Carbonneau 1985). Data collected in this study demonstrate that gas exchange is lower in infected vines grafted to RG compared with infected vines grafted to 3309C at the same ψstem, though a rigorous statistical analysis of the respective rootstock responses was not possible in the present study due to lack of randomization. Nevertheless, the lower rates of gas exchange in RG likely resulted in delayed maturity compared to 3309C, which is inferred by a lower TSS at the same harvest date in 2018, and delayed harvest dates in 2019 and 2020.

Additionally, lower vegetative growth in RG compared with 3309C suggests that vines grafted on RG respond more dynamically to water deficit in order to conserve water. Though the main goal of this study was not to compare rootstock response to the treatments in a GRBV context, the data generally demonstrate that RG may ripen fruit later than 3309C under the virus and environmental conditions of the present study. This likely has limited utility for proactive disease management but rather indicates that the impacts of GRBV on ripening may be exacerbated in vines grafted to the lower vigor RG. More investigation is required to tease out the potentially differential responses of RG and 3309C (and other rootstocks) to GRBV infection, especially with respect to vine water relations.

Response of gas exchange and disease severity in RG to thinning elucidates the limitations of crop thinning for GRBV-infected vines. It is well-known that grapevine gas exchange adjusts to manipulations of crop load such that reductions in gas exchange accompany reductions in crop load (Downton et al. 1987, Koblet et al. 1996). Accrual of assimilates in source tissues beyond the demand from ripening fruit sinks may be exported to other non-reproductive

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sinks in healthy vines (Edson et al. 1993). In GRBV-infected vines grafted to RG, this response of gas exchange to crop thinning appears to be conserved, particularly when water supply is abundant (i.e., supplemental irrigation).

For RG, the reduction in gs may be partly responsible for the slight increase in ψstem in thinned vines. However, disease severity – a likely indicator of foliar sugar accumulation in GRBV-infected leaves – increased slightly in thinned vines grafted to both 3309C and RG at the supplemental irrigation level. This suggests that the GRBV-induced impairment of sugar export is not necessarily mitigated by thinning in a similar way that elevation of water status may improve sugar export from GRBV-infected leaves (Copp and Levin 2021). Thus, in GRBV-infected vines, leaf gas exchange adjusts to both feedback of reduced sink strength, like in healthy vines, and to accumulation of foliar sugar in a way that is unique to infected vines.

The increase in disease severity and reduction in gas exchange (in RG only) as a function of thinning did not ultimately impede import of sugar or concentration of soluble solids relative to the thinning control vines but does suggest that the impacts of GRBV on vine physiology are 1) a stronger function of source-mediated carbon export and partitioning rather than dependent on whole-vine source-sink balance, and 2) more sensitive to changes in vine water relations that would be strongly influenced by relative vigor conferred by plant material (e.g., rootstock * scion interaction effects). Further targeted investigation is required to test these new hypotheses.

Conclusions

While cultural practices like irrigation and cluster thinning appear to impact GRBV-infected vines in some ways like healthy vines, there are limitations to the effectiveness of these

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practices. Primarily, increasing vine water status and reducing crop load did not improve concentration of total soluble solids after sugar accumulation had apparently ceased. Secondarily, increased berry mass caused by supplemental irrigation and thinning did not significantly reduce concentrations of most secondary metabolites and even increased the concentration of some. The limited improvements to fruit composition may not justify the additional costs associated with crop thinning and increased irrigation, not to mention considerable reductions in yield (and revenue) with thinning. In other words, the increased costs due to thinning far outweigh those associated with increased irrigation. Finally, the relative differences among rootstocks to confer vigor to the scion may explain observed variation in effects of GRBV on vine physiology across vineyard locations and should be investigated further.

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Table 1 Evaporative demand and water supply. Growing degree days (GDD), reference ET (ET_o), and growing season precipitation are accumulated from April 1 to September 30. Dormant season precipitation is accumulated from October 1 of the prior year to March 31.

Year	GDD (base 10°C)	ET_o (mm)	Precipitation (mm)	
			Dormant season	Growing season
2018	1608	808	98	56
2019	1424	826	204	136
2020	1536	856	127	86
Mean	1523	830	143	93

Table 2 Response of photosynthetic rate (A_{net}) and stomatal conductance (g_s) to treatments and year for the rootstocks 3309 Couderc (3309C) and Riparia Gloire (RG) in 2020. Gas exchange data are means ± one standard error (n = 4) for one sampling date just after veraison.

Irrigation[y]	Thinning[z]	A_{net} (µmol $CO_2/m^2/s$)		g_s ($mol/m^2/s$)	
		3309C	RG	3309C	RG
CON	CON	11.5 ± 1.7 a[x]	5.8 ± 0.9 a	0.103 ± 0.022 a	0.057 ± 0.009 a
	THIN	11.1 ± 1.7 a	5.8 ± 0.9 a	0.096 ± 0.022 a	0.062 ± 0.009 ab
SUPP	CON	16.2 ± 1.7 b	13.0 ± 0.9 b	0.176 ± 0.022 b	0.134 ± 0.009 c
	THIN	14.9 ± 1.7 b	10.4 ± 0.9 a	0.165 ± 0.022 b	0.112 ± 0.009 b
	ANOVA				
		------------------------------ *p-values* ------------------------------			
	Irrigation	0.045	0.014	0.033	0.013
	Thinning	0.526	0.024	0.667	0.084
	I * T	0.738	0.023	0.909	0.016

[x] Means with different letters indicate statistically significant differences at $p < 0.05$.
[y] CON = Control (grower standard); SUPP = Supplemental (2x grower standard).
[z] CON = No thinning; THIN = one cluster per shoot.

Table 3 Response of vegetative growth to the treatments and year for the rootstocks 3309 Couderc (3309C) and Riparia Gloire (RG). Data are means ± one standard error (n = 4).

Year	Irrigation[y]	Thinning[z]	Yield (kg/vine)		Pruning mass (kg/vine)		Ravaz index	
			3309C	RG	3309C	RG	3309C	RG
2018	CON	CON	3.69 ± 0.26 bc[x]	2.88 ± 0.19 a	0.62 ± 0.10 a	0.31 ± 0.05 a	6.1 ± 0.9 c	9.5 ± 1.5 a
		THIN	2.36 ± 0.26 a	2.46 ± 0.19 a	0.69 ± 0.10 a	0.40 ± 0.05 ab	3.4 ± 0.5 ab	6.3 ± 1.0 a
	SUPP	CON	4.30 ± 0.26 c	3.89 ± 0.19 b	0.76 ± 0.10 ab	0.39 ± 0.05 ab	5.8 ± 0.9 bc	10.5 ± 1.7 a
		THIN	2.87 ± 0.26 ab	3.05 ± 0.19 a	0.90 ± 0.10 b	0.52 ± 0.05 b	3.3 ± 0.5 a	6.0 ± 0.9 a
2019	CON	CON	2.07 ± 0.26 a	2.34 ± 0.19 a	0.75 ± 0.10 a	0.31 ± 0.05 a	2.8 ± 0.4 a	7.8 ± 1.2 a
		THIN	2.20 ± 0.26 a	2.55 ± 0.19 ab	0.79 ± 0.10 a	0.46 ± 0.05 ab	2.7 ± 0.4 a	5.7 ± 0.9 a
	SUPP	CON	2.17 ± 0.26 a	3.08 ± 0.19 b	1.07 ± 0.10 b	0.56 ± 0.05 b	2.0 ± 0.3 a	5.6 ± 0.9 a
		THIN	2.12 ± 0.26 a	2.39 ± 0.19 a	1.12 ± 0.10 b	0.57 ± 0.05 b	1.9 ± 0.3 a	4.1 ± 0.6 a
2020	CON	CON	1.77 ± 0.26 bc	1.86 ± 0.19 bc	0.70 ± 0.10 a	0.42 ± 0.05 a	2.4 ± 0.4 b	4.5 ± 0.7 b
		THIN	0.75 ± 0.26 a	0.99 ± 0.19 a	0.69 ± 0.10 a	0.43 ± 0.05 ab	1.0 ± 0.2 a	2.2 ± 0.4 a
	SUPP	CON	2.27 ± 0.26 c	2.44 ± 0.19 c	1.17 ± 0.10 b	0.64 ± 0.05 c	1.9 ± 0.3 b	3.8 ± 0.6 ab
		THIN	1.14 ± 0.26 ab	1.25 ± 0.19 ab	1.19 ± 0.10 b	0.59 ± 0.05 bc	0.9 ± 0.1 a	2.1 ± 0.3 a
		ANOVA						
			-- *P-values* --					
		Irrigation	0.105	0.035	0.002	0.011	0.100	0.245
		Thinning	0.002	0.001	0.102	0.092	0.001	0.002
		Year	<0.001	<0.001	<0.001	0.004	<0.001	<0.001
		I * T	0.663	0.034	0.541	0.421	0.848	0.913
		I * Y	0.292	0.118	<0.001	0.301	0.328	0.277
		T * Y	0.002	0.013	0.379	0.121	0.002	0.311
		I * T * Y	0.991	0.454	0.881	0.408	0.903	0.850

[x] Means with different letters indicate statistically significant differences at $p < 0.05$.

[y] CON = Control (grower standard); SUPP = Supplemental (2x grower standard).

[z] CON = No thinning; THIN = one cluster per shoot.

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Table 4 Response of pH and titratable acidity (TA) to the treatments and year at harvest for the rootstocks 3309 Couderc (3309C) and Riparia Gloire (RG). Data are means ± one standard error (n = 8).

Year	Treatment	Level	pH		TA (g/L)	
			3309C	RG	3309C	RG
2018	Irrigation[y]	CON	3.87 ± 0.02 b[x]	3.80 ± 0.03 a	2.99 ± 0.19 a	2.82 ± 0.16 a
		SUPP	3.72 ± 0.02 a	3.76 ± 0.03 a	3.84 ± 0.19 b	3.40 ± 0.16 b
	Thinning[z]	CON	3.73 ± 0.02 a	3.73 ± 0.03 a	3.59 ± 0.19 a	3.21 ± 0.16 a
		THIN	3.86 ± 0.02 b	3.84 ± 0.03 b	3.25 ± 0.19 a	3.02 ± 0.16 a
2019	Irrigation	CON	3.52 ± 0.02 a	3.38 ± 0.03 a	4.96 ± 0.19 a	5.91 ± 0.16 a
		SUPP	3.47 ± 0.02 a	3.38 ± 0.03 a	5.52 ± 0.19 b	6.24 ± 0.16 a
	Thinning	CON	3.49 ± 0.02 a	3.34 ± 0.03 a	5.32 ± 0.19 a	6.19 ± 0.16 a
		THIN	3.51 ± 0.02 a	3.41 ± 0.03 b	5.16 ± 0.19 a	5.96 ± 0.16 a
2020	Irrigation	CON	3.41 ± 0.02 a	3.45 ± 0.03 a	6.13 ± 0.27 a	5.59 ± 0.16 a
		SUPP	3.41 ± 0.02 a	3.40 ± 0.03 a	7.06 ± 0.27 b	6.24 ± 0.16 b
	Thinning	CON	3.38 ± 0.02 a	3.38 ± 0.03 a	6.70 ± 0.19 a	5.94 ± 0.16 a
		THIN	3.45 ± 0.02 b	3.46 ± 0.03 b	6.49 ± 0.19 a	5.92 ± 0.16 a
ANOVA (*p*-values)		Irrigation	0.027	0.173	0.017	0.026
		Thinning	0.005	0.003	0.186	0.302
		Year	<0.001	<0.001	<0.001	<0.001
		I * T	0.290	0.220	0.964	0.605
		I * Y	0.008	0.518	0.615	0.538
		T * Y	0.041	0.629	0.893	0.794
		I * T * Y	0.167	0.695	0.796	0.800

[x] Means with different letters indicate statistically significant differences at $p < 0.05$.

[y] CON = Control (grower standard); SUPP = Supplemental (2x grower standard).

[z] CON = No thinning; THIN = one cluster per shoot.

TA = titratable acidity.

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Figure 1 Irrigation supply for the irrigation treatments (**A**) and cluster number of thinning treatments for 3309 Couderc (**B**) and Riparia Gloire (**C**). Cluster number data are means ± one standard error averaged across irrigation treatments (n = 8). Statistical significance at $p < 0.05$, 0.01, and 0.001 is represented by '*', '**', and '***', respectively. CON = no thinning; THIN = thinned to one cluster per shoot post berry set.

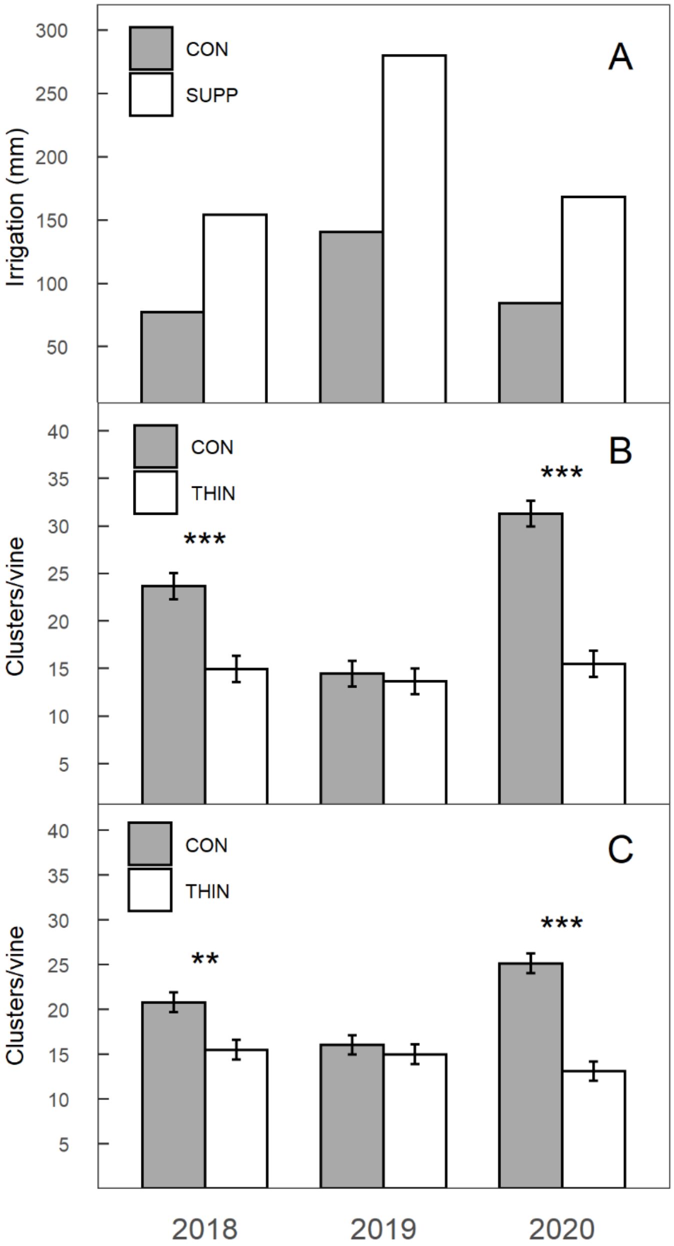

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Figure 2 Response of ψ_{stem} to the interaction of irrigation and thinning treatments for 3309 Couderc (**A**) and Riparia Gloire (**B**) in 2019 and 2020. Data are means ± one standard error (n = 4). Statistical significance for differences between irrigation treatments at $p < 0.05$, 0.01, and 0.001 is represented by '+', '++', and '+++', respectively. Statistical significance for differences between thinning treatments at each irrigation level at $p < 0.05$, 0.01, and 0.001 is represented by '*', '**', and '***', respectively. CON = control irrigation and no thinning; SUPP = 2x control irrigation; THIN = thinned to one cluster per shoot post berry set.

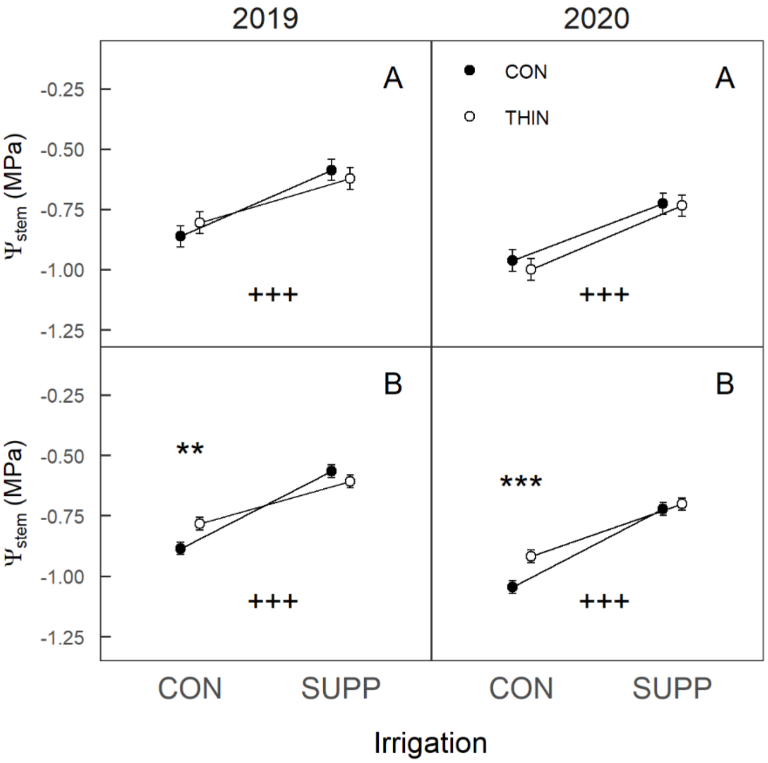

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Figure 3 Response of disease severity estimated as percent of symptomatic leaves per canopy to the irrigation (**A**, **B**) and thinning (**C**, **D**) treatments for 3309 Couderc (**A**, **C**) and Riparia Gloire (**B**, **D**). Data are means ± one standard error averaged across either thinning or irrigation treatments (n = 8). Statistical significance at $p < 0.1$, 0.05, 0.01, and 0.001 is represented by '.', '*', '**', and '***', respectively. CON = control irrigation and no thinning; SUPP = 2x control irrigation; THIN = thinned to one cluster per shoot post berry set.

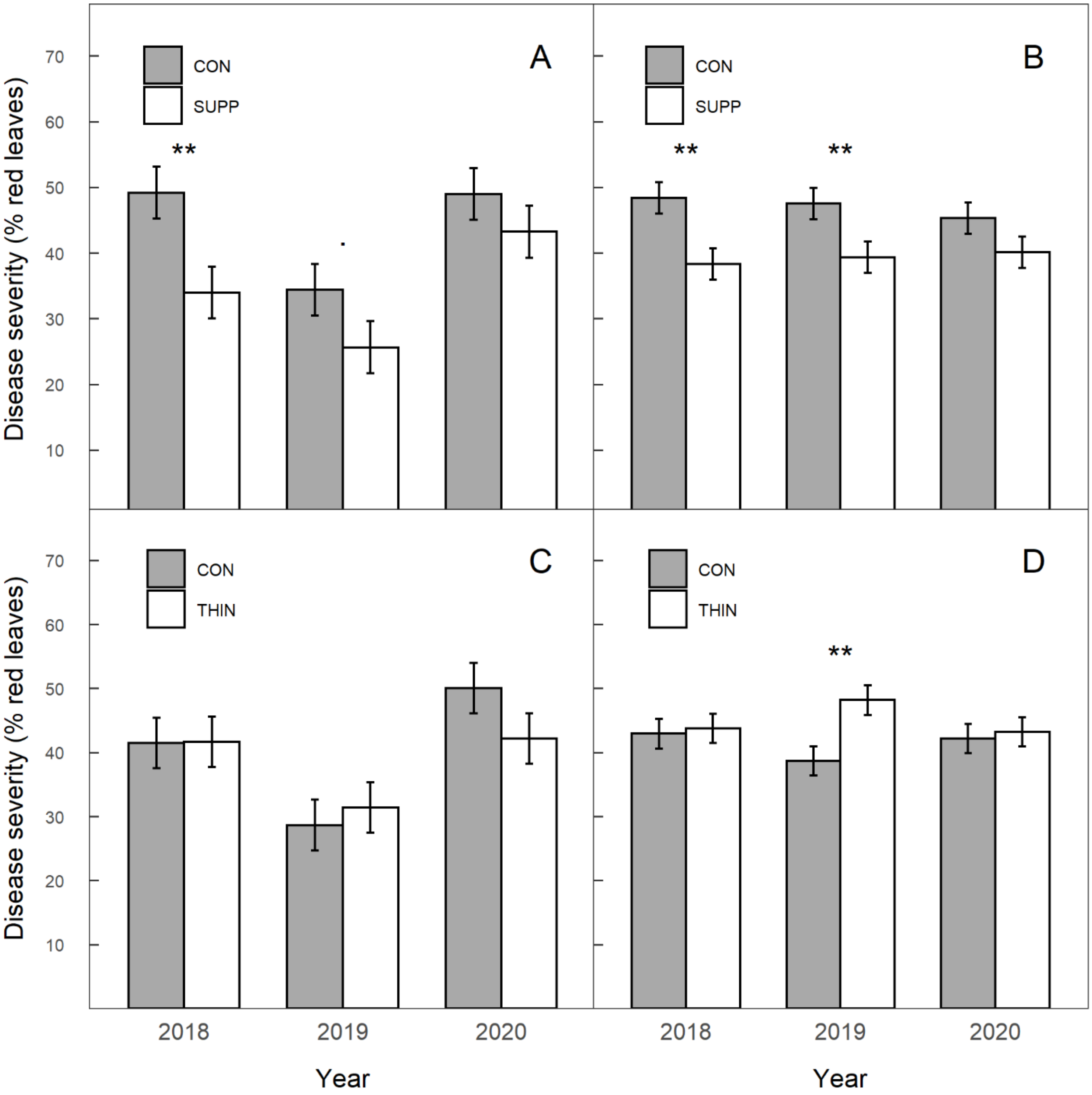

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Figure 4 Response of berry mass, total soluble solids, and sugar per berry to the interaction of irrigation and thinning treatments for 3309 Couderc and Riparia Gloire. Data are means ± one standard error (n = 4). Statistical significance for differences between irrigation treatments at $p < 0.05$, 0.01, and 0.001 is represented by '+', '++', and '+++', respectively. Statistical significance for differences between thinning treatments at $p < 0.05$, 0.01, and 0.001 is represented by '*', '**', and '***', respectively. CON = control irrigation and no thinning; SUPP = 2x control irrigation; THIN = thinned to one cluster per shoot post berry set.

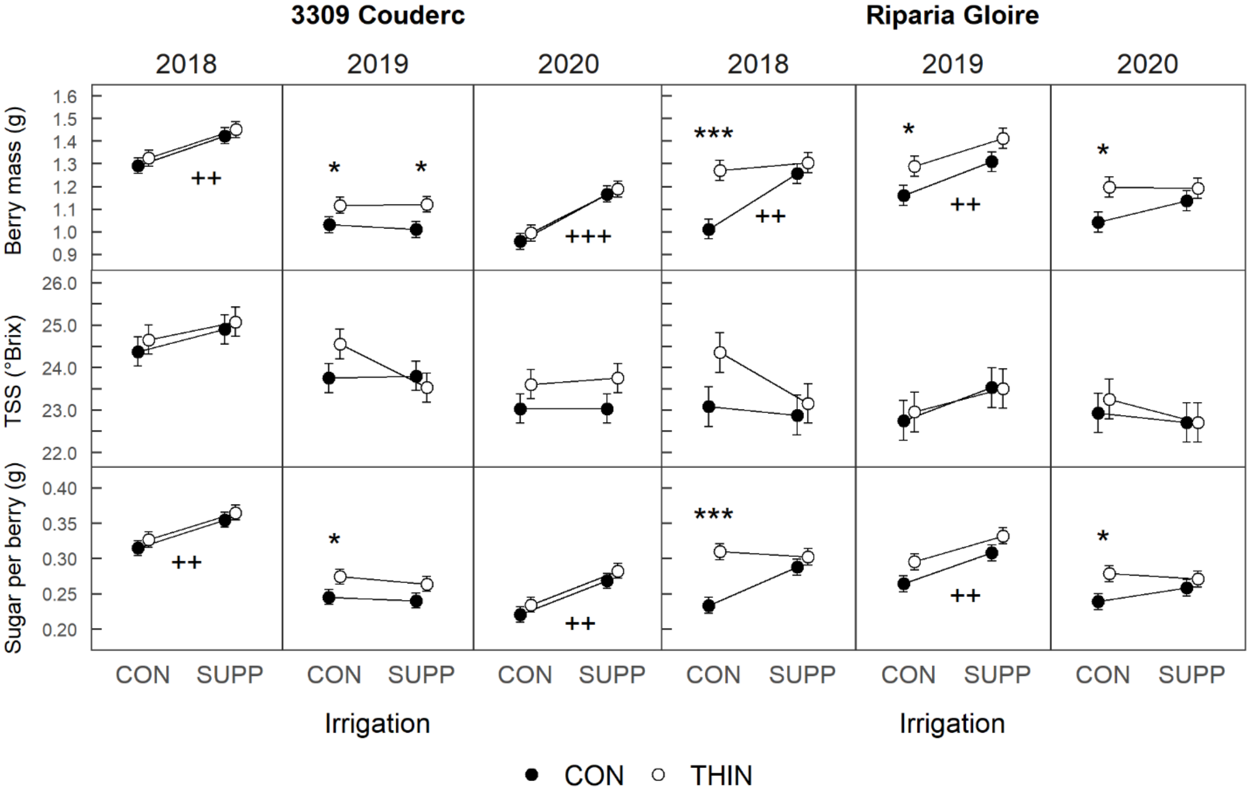

Figure 5 Berry growth and sugar accumulation in 2019 for 3309 Couderc and Riparia Gloire. Treatment labels consist of control (C) or supplemental (S) irrigation followed by control (C) or thinned (T) level. Data are means ± standard error (n = 4). Statistical significance for differences between irrigation treatments at $p < 0.05$, 0.01, and 0.001 is represented by '+', '++', and '+++', respectively. Statistical significance for differences between thinning treatments at $p < 0.05$, 0.01, and 0.001 is represented by '*', '**', and '***', respectively. CON = control irrigation and no thinning; SUPP = 2x control irrigation; THIN = thinned to one cluster per shoot post berry set.

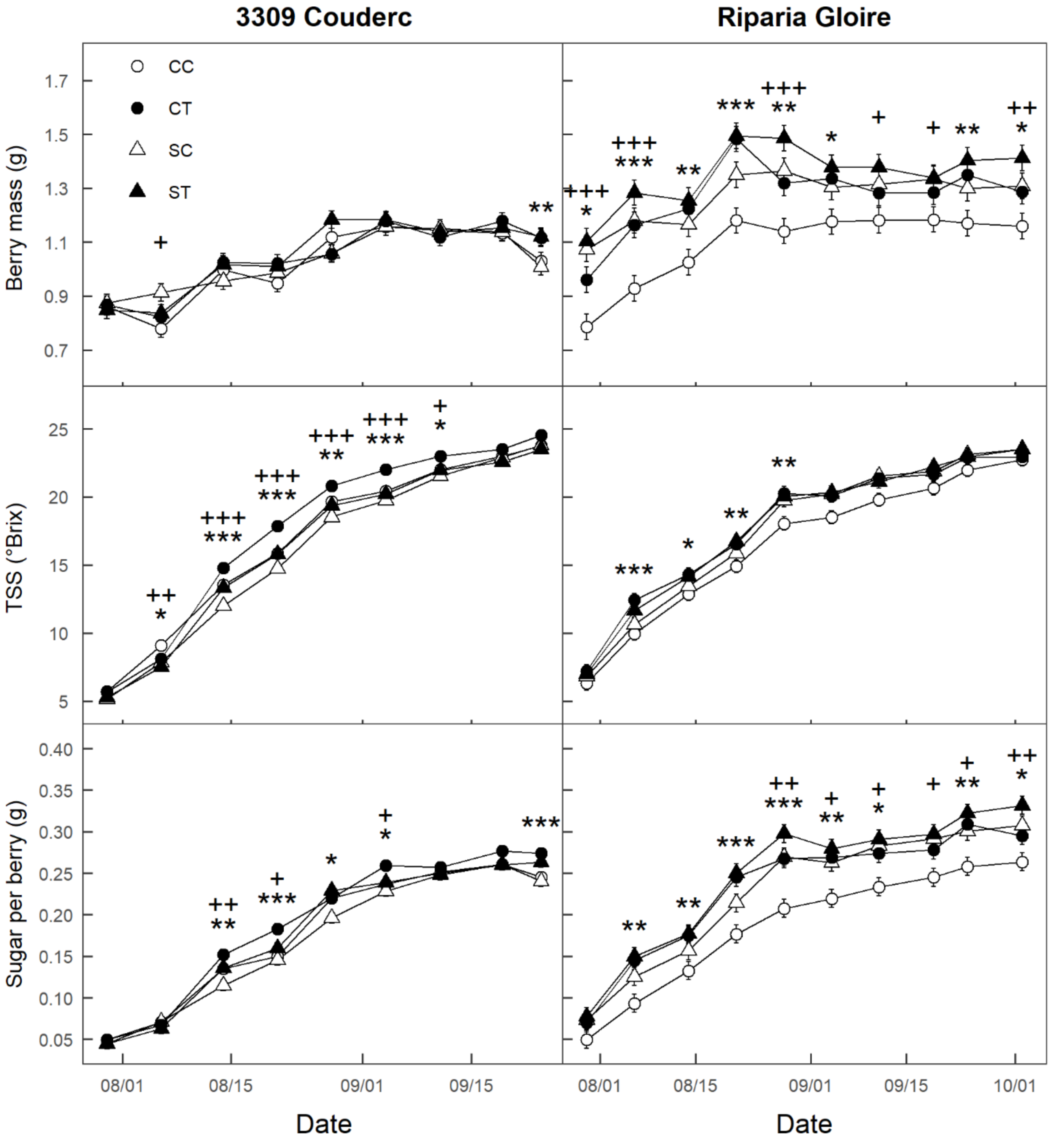

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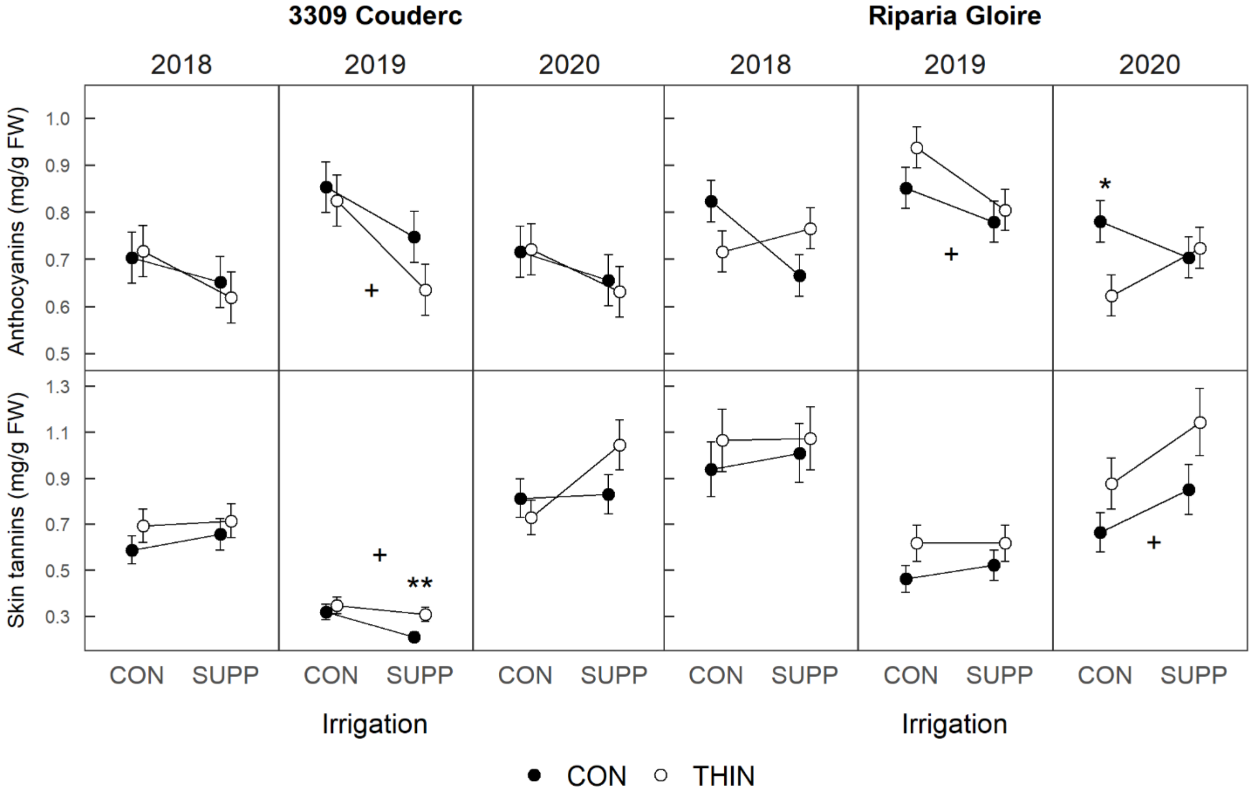
Figure 6 Response of anthocyanin and skin tannin concentrations to the interaction of irrigation and thinning treatments for 3309 Couderc and Riparia Gloire. Data are means ± one standard error (n = 4). Statistical significance for differences between irrigation treatments at $p < 0.05$, 0.01, and 0.001 is represented by '+', '++', and '+++', respectively. Statistical significance for differences between thinning treatments at $p < 0.05$, 0.01, and 0.001 is represented by '*', '**', and '***', respectively. CON = control irrigation and no thinning; SUPP = 2x control irrigation; THIN = thinned to one cluster per shoot post berry set.



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Supplemental Table 1 Phenology by date and accumulation growing degree days (GDD). GDD are accumulated from 1 April.

	Year	**Bud break**	**Bloom**	**Veraison**	**Harvest (3309C/RG)**
Date	2018	April 23	June 3	August 10	October 1
	2019	April 16	June 6	August 7	September 25/October 2
	2020	April 16	June 2	August 7	September 17/September 21
GDD (base 10°C)	2018	50	319	1143	1608
	2019	21	297	936	1408/1426
	2020	40	291	945	1423/1452

Supplemental Table 2 Treatment and year effects on secondary fruit composition per unit fresh weight at harvest for the rootstocks 3309 Couderc (3309C) and Riparia Gloire (RG). Data are means ± standard error (n = 4). CON = control irrigation and no thinning; SUPP = 2x control irrigation; THIN = one cluster per shoot.

Year	Irrigation	Thinning	Skin IRP (mg/g FW)		Seed IRP (mg/g FW)		Seed tannins (mg/g FW)	
			3309C	RG	3309C	RG	3309C	RG
2018	CON	CON	1.36 ± 0.14	1.85 ± 0.14	3.23 ± 0.20	3.05 ± 0.19	1.70 ± 0.11	1.55 ± 0.08
		THIN	1.66 ± 0.14	1.93 ± 0.14	3.74 ± 0.20	3.40 ± 0.19	1.88 ± 0.11	1.19 ± 0.08
	SUPP	CON	1.45 ± 0.14	1.74 ± 0.14	3.59 ± 0.20	2.91 ± 0.19	1.89 ± 0.11	1.48 ± 0.08
		THIN	1.93 ± 0.14	2.14 ± 0.14	3.36 ± 0.20	2.39 ± 0.19	1.92 ± 0.11	1.51 ± 0.08
2019	CON	CON	2.08 ± 0.14	2.16 ± 0.14	1.89 ± 0.20	1.73 ± 0.19	0.90 ± 0.11	0.80 ± 0.08
		THIN	1.98 ± 0.14	2.51 ± 0.14	2.19 ± 0.20	2.02 ± 0.19	0.89 ± 0.11	0.85 ± 0.08
	SUPP	CON	1.81 ± 0.14	2.17 ± 0.14	2.11 ± 0.20	2.07 ± 0.19	0.95 ± 0.11	0.90 ± 0.08
		THIN	1.76 ± 0.14	2.39 ± 0.14	1.94 ± 0.20	2.19 ± 0.19	0.85 ± 0.11	0.85 ± 0.08
2020	CON	CON	2.39 ± 0.14	2.61 ± 0.14	4.62 ± 0.20	3.42 ± 0.19	1.58 ± 0.11	1.14 ± 0.08
		THIN	2.39 ± 0.14	2.54 ± 0.14	4.51 ± 0.20	3.50 ± 0.19	1.49 ± 0.11	1.12 ± 0.08
	SUPP	CON	1.59 ± 0.14	2.33 ± 0.14	3.75 ± 0.20	3.23 ± 0.19	1.24 ± 0.11	1.05 ± 0.08
		THIN	1.73 ± 0.14	2.86 ± 0.14	4.07 ± 0.20	3.67 ± 0.19	1.36 ± 0.11	1.15 ± 0.08
		ANOVA						
		Irrigation	0.042	0.965	0.211	0.478	0.568	0.326
		Thinning	0.151	0.014	0.495	0.390	0.747	0.355
		Year	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
		I * T	0.481	0.119	0.397	0.434	0.960	0.157
		I * Y	<0.001	0.787	0.015	0.005	0.057	0.313
		T * Y	0.055	0.905	0.942	0.291	0.527	0.104
		I * T * Y	0.928	0.047	0.047	0.043	0.402	0.051