REPORT

Application of Fractions of Phyto-Cat Affects Carbon Partitioning of Grapevine Differentially in a Hot Climate

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Summary

Majority of viticulture regions are located in mid-latitudes characterized by weather variability and stressful environments relying on irrigation for mitigating environmental stress during the growing season and to ensure a profitable yield. The aim of this study was to characterize the response of grapevine (*Vitis vinifera,* L. cv. Cabernet Sauvignon) to different rates of Phyto-Cat application during two growing seasons with contrasting precipitation patterns. Phyto-Cat was initially marketed as an irrigation hose cleaning product. The experiment consisted of two Phyto-Cat treatments (2 ppm and 4 ppm) and an untreated control (0 ppm) applied to the irrigation stream based on the weekly 50 % of crop evapotranspiration. Grapevine water status decreased during the growing season reaching its lowest value (-1.5 and -1.2 MPa, respectively) at harvest in the more stressed vines (0 and 2 ppm treatments). Leaf gas exchange variables were measured during the two seasons and 4 ppm had the highest rates of photosynthesis (A_N), stomatal conductance (g_s) and better instantaneous water use efficiency (WUE); also resulting in higher leaf chlorophyll and carotenoid content. Mineral nutrient content for nitrogen and potassium increased linearly with the increase in applied Phyto-Cat. At harvest, no differences were observed in the number of clusters per vine; however, the Control had the lowest berry size and yield per vine with no difference in sugar content of berry. Conversely, sugar allocation to vegetative organs was highly affected by applied Phyto-Cat leading to different shoot to root biomass partitioning where shoot:root ratio, leaf non-structural carbohydrates, and photosynthetic pigments increased with greater applied Phyto-Cat. Likewise sucrose:N ratio and root non-structural carbohydrates decreased with the decreases in applied Phyto-Cat. Altogether, carbon allocation between source and sink organs likely controlled the response of grapevines to water deficit in a hot climate, and

replacing 50% of crop evapotranspiration with 2 ppm of Phyto-Cat was sufficient to sustain the grapevine in the hot climate.

1. Introduction

Within perennial crops, the grapevine (*Vitis vinifera* L.) is one of the most economically important fruit crop with more than 7.4 million cultivated hectares worldwide in 2016 (OIV, 2016). Many of the viticulture areas of the world rely on irrigation for consistent production (Wilson et al., 2020) and there is an increasing need for irrigation within the traditionally non-irrigated regions within the last decades (Costa et al., 2016; Martinez et al., 2020). By the middle of 21st century, climatic conditions are expected to change, potentially affecting key physiological and production variables (Hannah et al., 2013; Fraga et al., 2016). The increase in atmospheric CO_2 and other greenhouse gasses most certainly will increase the temperature of the planet ranging from 1.5 to 4.5 °C (IPCC, 2013). Furthermore, the incidence of extreme events, such as heat waves, is increasing with an associated risk for fleshy fruit crops (Fischer and Schär, 2010; Smith, 2011; Deryng et al., 2014; Martínez-Lüscher et al., 2017). Higher temperatures are associated with greater rates of water evaporation and therefore, higher global precipitation. However, these are unevenly distributed. Majority of viticultural regions are forecasted to experience a reduction in cloud coverage and rainfall and an increase in solar radiation reaching the earth's surface (Trenberth and Fasullo, 2009).

The theory of micro-irrigation indicated that it should be providing only the amount of water to meet the actual crop evapotranspiration without using the storage capacity of the soil, given the possibility of high frequency (Allen et al., 1998). Irrigation of vineyards would always introduce a predetermined deficit. Therefore, deficit irrigation has emerged as a potential strategy to allow grapevine to withstand water shortage during the growing season without yield loss and

maintaining the berry composition (Chaves et al., 2010; Costa et al., 2016). Severe limitations of applied water, in case of irrigation lines being clogged may accelerate sugar accumulation in grape berry in hot climates (Bonada et al., 2015; Zarrouk et al., 2016) and result in adverse effects on yield (Nelson et al., 2016) or fruit composition (Brillante et al., 2017) or wine composition (Yu et al., 2020). However, a sustained moderate water deficit in freely flowing water in irrigation lines has improved canopy microclimate, increased water use efficiency, modified source to sink ratio, and reduced berry size improving berry quality by means of enhancement of sugars and polyphenols in red wine grape (Cook et al., 2015; Santesteban et al., 2011; Zarrouk et al., 2012). Nevertheless, the final berry and consequent wine quality are highly dependent upon the proper control of carbohydrate partitioning, which balance the growth and metabolism of source: sink organs (Yu et al., 2020).

Non-structural carbohydrates (NSC) are responsible for providing energy and carbon for grapevine growth, being stored as reserves in grapevine perennial organs. The role of stored NSC in the early season is crucial until bloom when leaf photosynthesis becomes the primary source of carbon (Zapata et al., 2004). The grapevine's capacity for replenishment of these carbohydrate reserves increases at mid-ripening (Candolfi-Vasconcelos et al., 1994). In addition, sugars directly or indirectly control a wide range of physiological processes, including photosynthesis, sugar transport itself, nitrogen uptake, defense reactions, secondary metabolism, and hormonal balance (Smeekens et al., 2010; Dai et al., 2014). Not only sugar transport and partitioning play key roles in the regulation of plant development, but also they influence how grapevines response to biotic and abiotic factors (Meteier et al. 2019). Understanding how grapevines distribute the acquired resources among source and sink organs, which would be modifying grapevine growth, yield and berry chemistry is fundamental for their adaptation to the future constraints due to the dynamics

of NSC storage may be altered by both abiotic factors and internal competition for carbon in grapevine (Holzapfel and Smith, 2012). Therefore, to ensure sufficient vegetative growth, reproduction and acclimation to environmental stresses, grapevines must efficiently allocate available annual resources to both vegetative and reproductive tissues. Increased soil temperature due to the changing climate, especially before *veraison* strongly affect seasonal balance between shoot and root growth, bloom, plant water use, photosynthesis, and the availability of carbohydrate reserves (Field et al, 2020). In grapevines, water availability appears as the determining factor for cell growth and photosynthesis (Medici et al., 2014), and for the redistribution of carbohydrates between source and sink organs (Lemoine et al., 2013). Indeed, shifting in root-to-shoot biomass allocation in response to external resource availability allows plants to minimize imbalance in any critical resource that is growth limiting (Grechi et al., 2007). However, our understanding of NSC storage in different organs and consequently, the balance of the source and sink organ growth remains limited. Additionally, the sensitivity to water deficits brought on by irrigation distribution uniformity or lack of maintenance is particularly acute during reproductive development because of the competition for photo-assimilate allocation to newly established sinks such as flowers, seeds, and fruit, and roots under drought stress (Lemoine et al., 2013).

Therefore, the aim of this study was to characterize the primary metabolism response of grapevines to differing amounts of a commercial cleaner and supplement, Phyto-Cat® on grapevine physiology, as well as, to assess their effect on carbon partitioning among source and sink organs during two growing seasons.

2. Material and methods

2.1. Plant material and experimental design

The experiment was conducted during two consecutive seasons (2018-2019 to 2019-2020) in Oakville, CA (38.428 N, 122.409 W) with row orientation NW-SE. The vineyard was planted in 2011 with Cabernet Sauvignon (clone FPS08) on 110R rootstock at a spacing of 2.4 by 2.0 m (row × vine). The grapevines were trained to a bi-lateral cordon on a vertically shoot-positioned trellis with a cordon height of 96 cm above vineyard floor and pruned to 1-bud spurs. Plants were irrigated weekly with 2 drip emitters per vine with the capacity of deliver 4.0 liters of water per hour, respectively. The experiment was designed as a randomized block with a one-way arrangement of the following treatments of Phyto-Cat application: i) Control (0 ppm), ii) 2 ppm and iii) 4 ppm, with six replicates each consisting of 5 experimental units, 3 of which were used for data collection and the 2 on distal ends were treated as border plants.

2.2. Phyto-cat treatments

The treatments were applied by with the use of a 1200 L nurse tank that injected the following amounts 0 ppm, 2 ppm and 4 ppm of Phyto-Cat dilute solution into the irrigation stream. The irrigation application started in May of each year. Harvest commenced when the berry total soluble solids reached to *ca*. 24°Brix on 25 September 2019 (114 DAF) and 8 September 2020 (115 DAF), respectively.

2.3. Weather conditions

Weather data (Table 1) were obtained from the California Irrigation Management Information System, CIMIS, station (#77, Oakville, CA) located 160 m from the experimental vineyard (CIMIS, 2020). Number of days with temperatures above 30°C were counted for the 2018-19 and 2019-20 growing seasons. The growing degree days were calculated with a base of 10 °C with no upper limit in temperature maxima.

2.4. Plant water status and leaf gas exchange measurements

Plant water status was monitored by measuring the midday stem water potential (SWP) throughout both growing seasons every two weeks. A fully-expanded leaf from each treatment-replicate, sun-exposed and without sign of disease or damage from the Southwest side of the canopy was selected and measured Two hours prior to taking the measurements, foil-lined zip-top bags were placed on sun-exposed leaves and sealed before excising the petiole in order to suppress transpiration. SWP was then directly determined with a pressure chamber (Model 610 Pressure Chamber Instrument., PMS Instrument Co., Corvallis, OR, USA).

Leaf gas exchange was measured with a CIRAS-3 portable infrared gas analyzer system (PP Systems, Amesbury, MA, USA) featuring a broad-leaf chamber with a 4.5 cm^2 window. For each date and experimental unit, three measurements were made *ca.* solar noon (11:30 to 13:30 h) on a healthy leaf under light saturating conditions (>1500 μmol m^{-1} s^{-1}) and values were averaged. The cuvette was oriented perpendicularly to sunlight, which was always in saturating conditions (average of internal PAR = 1969 ± 135 μmol m^{-2} s^{-1}). Measurements were taken at 40% relative humidity, a CO_2 concentration of 390 μmol CO_2 mol^{-1}, and using a flow to the chamber of 300 mL min^{-1}.

To summarize the temporal information for plant water status and leaf gas exchange, integrals for all the parameters were calculated by using natural cubic splines for both years individually and collectively. The resultant values were divided by the number of the days between the first and the last measurements in each year and the first measurement in 2018-19 season and 2019-20 season to make the data comparable to each individual measurement.

2.5. Petiole mineral nutrient content

In June of each growing season (*ca.* anthesis) fifty leaves per treatment-replicate were collected, leaf blades were removed and petioles were dried at 70 °C in a forced air oven. Then, mineral nutrient analysis was carried out by Dellavalle, Inc., Fresno, CA, United States as reported elsewhere (Yu et al., 2020). Total nitrogen (N) was determined via automated combustion analysis (method B-2.20) while phosphorus (P), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), zinc (Zn), manganese (Mn), boron (B), iron (Fe), and cuprum (Cu) were analyzed via Nitric/Perchloric Acid Digestion (method B-4.20) as described by Gavlak et al. (1994).

2.6. Total chlorophyll (a + b) and carotenoid contents

At mid-ripening of the second growing season two leaves of each treatment-replicate were collected and 25 mg of tissue was used for determining total chlorophylls (a + b) and total carotenoids according to Sesták et al. (1971). Extraction was conducted by immersing samples of fresh tissue in 5 mL of 96% ethanol at 80 °C for 10 min. Absorbance of the extracts at 470, 649, 665 and 750 nm were determined with a spectrophotometer (Cary 100, Santa Clara, CA, USA). Then, total chlorophylls and total carotenoids were calculated by using the extinction coefficients and equations described by Lichtenthaler (1987) and expressed as $mg \cdot g^{-1}$ of dried weight (DW).

2.7. Yield components and total biomass of woody parts.

At harvest, sixty berries per treatment-replicate were randomly collected and weighed to determine berry mass. Then, in the 2019-20 season these berries were crushed for must sugars determination. Grapevines were harvested and clusters were counted and weighed on a top-loading balance. Total leaf area was calculated by defoliating one grapevine per treatment replicate after harvest (early November 2019 and October 2020, respectively), and using the regressive relationship between leaf dry mass and leaf area. A subsample of oven-dried leaves (30 mg) from each treatment-

replicate was collected for sugar and starch analysis. Then, leaf area to fruit ratio was calculated by dividing the leaf area by the yield per vine and reported as $m^2 \cdot kg^{-1}$.

Six weeks following the harvest of 2020 (15 to 19 October 2020), one grapevine per treatment-replicate was removed from the vineyard by using a mechanical spade. The grapevine was then portioned into trunk and cordon, roots and shoots. Then, each portion was weighed on a top-loading balance to obtain the fresh biomass of the portions. A sub-sample of shoots and fine roots was collected for organ dried biomass estimation and sugar and starch analysis.

2.8. Carbohydrate extraction and total soluble sugars and starch determination

Subsamples of leaves, shoots and roots were oven-dried at 70 ℃ to a constant weight. Dried tissues were ground with a tissue lyser (MM400, Retsch, Germany). Thirty mg of the resultant powder was extracted in ethanol:water (75:25) solution. Briefly, 1.5 mL was added to each sample and extracted for 10 min at 90℃ in a water bath. Then, they were centrifuged at 10000 rpm for a min, and supernatant was collected for sugar determination. The procedure was then repeated for starch determination in which the resultant pellet was used.

Total soluble sugars and individual sugars were determined in the shoot, leaf and root ethanolic extracts and in the diluted berry must samples (1:10). Samples were filtered with PTFE membrane filters (diameter: 13mm; 0.45 µm; Celltreat Scientific Products, Pepperell, MA, USA) and transferred into high performance liquid chromatography (HPLC) vials and subjected to reversed-phase HPLC analysis. Equipment consisted of an Agilent 1100 system coupled to a diode array detector (DAD) and an Infinity Refractive Index Detector (RID) (Agilent Technologies Inc., Santa Clara, CA, USA). The reversed-phase column was Luna Omega Sugar (150 × 4.6 mm, 3 µm particle size, Phenomenex Inc., Torrance, CA, USA) with a guard column of 5 mm. The

temperature of the column compartment was maintained at 40 °C and the RID flow cell was kept at 35 °C. The mobile phase system consisted in an isocratic elution with acetonitrile:water (v:v, 75:25) at a flow rate of 1.0 $mL \cdot min^{-1}$ with a run time of 22 min. Standard solutions of 10 mg/L of D-glucose, D-fructose, D-sucrose and D-raffinose were injected to obtain the retention time for each compound, and detection was conducted by RID. Sugar standards were purchased from VWR International (Radnor, PA, USA). Sugar concentration of each sample was determined by comparison of the peak area and retention time with standard samples curves.

Starch content of roots, shoots and leaves was conducted using the Starch Assay Kit SA-20 (Sigma-Aldrich, St. Louis, MI, USA) in accordance with the manufacturer's instructions. Briefly, pellets of different tissues were dissolved in 1 mL DMSO, and incubated for 5 min in a water bath at 100 ℃. Starch digestion commenced with adding 10 µL α-amylase and incubated in boiling water for another 5 min. then, the ddH2O was added to a total volume of 5 mL. Then, 500µL of the above sample and 500 µL of starch assay reagent were mixed and incubated for 15 min at 60 °C. Negative controls with the starch assay reagent blank, sample blank, and glucose assay reagent blank and positive controls with starch from wheat and corn were performed. Reaction started with the incubation of 500 µL of each sample and 1 ml of glucose assay reagent at 37 ℃ and was stopped with the addition of 1mL of 6M Sulfuric acid after 30 min. Reaction was followed with a Cary 100 Series UV-Vis Spectrophotometer (Agilent Technologies Inc., Santa Clara, CA, USA) and starch content expressed as percent of starch per tissue dried weight.

2.9. Statistical Analyses

Statistical analyses were conducted with R studio version 3.6.1 (RStudio: Integrated Development for R., Boston, MA, USA) for Windows. Seasonal integrals of SWP and gas exchange variables for each growing season and for both together were calculated by using the same software. All

data were subjected to Shapiro-Wilk's normality test. Data were normally distributed and subsequently was submitted to an analysis of variance (ANOVA) to assess the statistical differences between the different treatments. For seasonal integrals, a two ways ANOVA was applied to assess the effect of the growing season (year) and Phyto-Cat amounts on SWP and gas exchange parameters. For all data, means ± standard errors (SE) were calculated and when the F value was significant ($P \leq 0.05$), a Duncan's new multiple range *post hoc* test was executed using "agricolae" 1.2-8 R package (de Mendiburu, 2016). Percentage data were transformed according to the suggestion of the most likelihood test, into arcsine root square before ANOVA. Pearson correlation analysis were performed with the same software by using the 'corrplot' package (Wei and Simko, 2017).

3. Results

3.1. Grapevine mineral content, water status and gas exchange parameters of Cabernet sauvignon vines

Weather data shows that 2020 growing season was hotter and drier than 2019 (Table 1). Comparing to 2019 growing season, 2020 had 17 days more with temperature over 30℃, a maximum daily temperature of 1.1℃ higher and almost 800 mm less of precipitation, leading to an ET_o of 23 mm higher. On the other hand, the lower available water for grapevine growth, led to a lower canopy development decreasing the ET_c which explained the lower irrigation amount of 2020 comparing to 2019 (Table 1). Petiole mineral nutrients were not affected by Phyto-Cat in the 2018-19 growing season (Table 2). Conversely, total N increased in 4 ppm while K content in Control vines decreased in 2019-20 growing season.

The plant water status, declined throughout the season (Figure 1A, B). In 2019, the 4 ppm treatment had the greatest SWP while Control had the lowest as expected. Conversely, there were no significant differences though the 2020 season between treatments. Likewise, we measured significant differences between the different treatment amounts in g_s and A_N in both growing seasons (Figure 1C-F). We measured higher g_s and A_N in grapevines subjected to 4 ppm treatment from the second half of July, coinciding with the *veraison*, to harvest, compared to Control. The gs and A_N of 2 ppm was transiently lower than 4 ppm, but consistently greater than Control. The WUEi differed between treatment amounts at harvest in 2019 and at mid-ripening in 2020 with 4 ppm grapevines showing the highest WUE (Figure 1G, H). The enhancement of the photosynthetic performance in 4 ppm grapevines was accompanied by increased total chlorophyll and carotenoid contents in leaves (Table S1).

Calculation of the seasonal integral of SWP and gas exchange variables allowed to establish the seasonal-long trend for grapevine physiological response. Thus, SWP seasonal integrals ($_{si}$SWP) for both seasons were affected by the interaction between Phyto-Cat and year. During the 2019 growing season, no difference in the seasonal pattern was measured. However, in 2020 there was a significant increase of SWP with 100% and 2 ppm $_{si}$SWP compared to Control $_{si}$SWP (Figure 2A). On the other hand, seasonal integrals of g_s, A_N and WUE were significantly different between years. The A_N and WUE were significantly lower in 2020 compared to 2019 (Figure 2B-D).

3.2. Different Phyto-Cat amounts modulated yield components and vegetative growth of Cabernet sauvignon grapevines.

Cluster number was not affected by application of Phyto-Cat (Table 3). An increase in the yield per vine was observed in both seasons with a highly significant increase in yield per vine in 4 ppm treatment. Likewise, the linear increase in yield was evident from Control to 2 ppm as well

in both years. We also measured linear increases in leaf area to fruit ratio, berry size as the amount of Phyto-Cat increased from Control to 4 ppm.

Grapevine growth was monitored for different organs as shows Table 4 and Table S2. Leaf, shoot and roots fresh weights increased with increased Phyto-Cat amounts (2ppm and 4 ppm in 2019 and 4 ppm in 2020, Table S2). The biomass of leaf and root increased in the grapevines subjected to 4 ppm compared to 2 ppm and Control (Table 4). Although there was a likely trend of biomass increase in shoot and trunk, there was no significant difference amongst them in response to applied Phyto-Cat (Table 4).

3.3. Metabolism of carbohydrates in different grapevine organs was affected after two seasons of different Phyto-Cat amounts.

The starch and total soluble sugars (TSS) measured in different organs of the grapevine are presented in Figure 3. We measured a significant increase of TSS and starch content in leaves as affected by the applied Phyto-Cat amount (Figure 3A and 3B). This increase in leaf TSS was attributed to the increases in glucose, fructose and raffinose contents of leaves (Table 5). The total sugar and starch content of shoots were not affected by applied Phyto-Cat amount (Figure 3B, E). However, sucrose and raffinose in shoots increased in 2ppm and 4 ppm treatments compared to Control (Table 5). Root sugar content (Figures 3C and 3F and composition were not affected by treatments, with sucrose being the main soluble sugar found in root tissues (Table 5).

Our analysis of the different carbohydrates found in grapevine tissues indicated that starch was the main NSC in shoots and roots, which accounted for > 2 ppm regardless of applied Phyto-Cat affecting their proportions (Table 6). In leaves, starch content was the less abundant NSC but a significant effect of treatments was observed with the 4 ppm treatment reaching the highest

amount. Finally, proportions of sucrose and raffinose in shoots decreased in when Phyto-Cat appliation was restricted to Control (0 ppm) (Table 6).

Regarding must sugar composition, fructose and glucose were the main sugars found (Table 5), and their ratio ranged between 0.62 and 0.78 with no difference between treatments (data not shown). Finally, it is noteworthy that although TSS in berry must was not significantly affected by treatments, an increasing trend in TSS in the Control treatment was detected (Table 5).

3.4. Relationships between grapevine physiological response to different Phyto-Cat amounts and primary metabolism.

To analyze the carry over effect of Phyto-Cat amounts on grapevine growth and sugar metabolism a correlation analysis was conducted (Figure 4). Thus, strong relationships between the two growing season seasonal integrals of SWP ($_{si2018\text{-}20}$SWP) and gas exchange parameters ($_{si2018\text{-}20}g_s$, $_{si2018\text{-}20}A_N$, $_{si2018\text{-}20}$WUE) were shown. A higher grapevine water status ($_{si2018\text{-}20}$SWP) was positively related to an increased growth of roots, shoots and leaves. Similarly, leaf starch content was strongly correlated with $_{si2018\text{-}20}$SWP, $_{si2018\text{-}20}g_s$ and $_{si2018\text{-}20}A_N$ ($r = 0.74$ and $p \leq 0.0001$; $r = 0.51$ and $p \leq 0.05$; and $r = 0.50$ and $p \leq 0.05$, respectively). On the other hand, a significant relationship between increased leaf starch content and the yield per vine was observed ($r = 0.76$, $p \leq 0.0001$). Moreover, the increases in yield per vine were related with enhancements in leaf, shoot and root biomasses. Berry must TSS were positively correlated with $_{si2018\text{-}20}$WUE and negatively with $_{si2018\text{-}20}g_s$ ($r = 0.55$ and $p \leq 0.05$; and $r = -0.49$ and $p \leq 0.05$, respectively). Finally, increased vegetative growth of trunk, leaves and shoots negatively affected root TSS ($r = -0.64$ and $p \leq 0.01$; $r = -0.50$ and $p \leq 0.05$; and $r = -0.57$ and $p \leq 0.05$, respectively). Conversely, positive relationships between trunk, root, leaf and shoot biomasses with leaf starch content were recorded ($r = 0.52$ and $p \leq 0.05$, $r = 0.84$ and $p \leq 0.0001$; $r = 0.79$ and $p \leq 0.0001$; and $r = 0.88$ and $p \leq 0.0001$, respectively).

Furthermore, in order to delve into the effects of the Phyto-Cat application on grapevine physiology and metabolism, Pearson correlations between shoot:root ratio and petiole N content with the total biomass (BM) and primary metabolites were conducted (Figure 5). Shoot:root ratios of Cabernet Sauvignon vines were significantly correlated with the total BM, leaf and root NSC, photosynthetic pigments, plant sucrose to N ratio and N contents (Figure 5A-E, J) where increased shoot:root ratio showed higher total BM, leaf NSC, chlorophylls and carotenoids and N contents. Moreover, increased shoot to root ratio were related to decreased root NSC contents and low sucrose to N ratios (Figure 5D-E). On the other hand, the petiole N content was positively correlated with the total BM, leaf NSC, and photosynthetic pigments (Figure 5F-H) again, with 4 ppm vines reaching the highest values of all the above-mentioned parameters. The petiole N content also showed a significant relationship with the the yield per vine (Figure 5I).

Conclusion

Our results provided evidence that differences in carbon partitioning and allocation between the source and sink organs of the grapevine explained the response of grapevines to Phyto-Cat. Therefore, grapevines that were not supplied with Phyto-Cat showed a reduced rate of photosynthesis, and water status, less photo-assimilates in source (leaves) available for new growth and exported to sinks, and a lower plant BM due to the water restriction. Conversely, 4 ppm showed the highest photosynthetic performance and water status, which led to increased contents of soluble sugars and starch in leaves and greater yield. Finally, our data revealed that in 2 ppm treatment, the enhancement of sugar transport, mainly sucrose and raffinose, could slow down the detrimental effect of water deficits on yield. Finally, an important role of sugar and nitrogen was suggested due to their significant relationship with biomass partitioning.

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Figure captures

Figure 1. Stem water potential (SWP, A and B), stomatal conductance (g_s, C and D), leaf net carbon assimilation (A_N, E and F) and intrinsic water use efficiency (WUE, G and H) from Cabernet sauvignon grapevines (clone FPS08), subjected to different Phyto-Cat application (Control, 2 ppm and 4 ppm) and collected through 2018-19 and 2019-20 growing seasons in Oakville, CA. Values represent means ± SE (n = 6). At each time point, different letters indicate significant differences ($p \leq 0.05$) between treatments according to one-way ANOVA followed by Duncan's new multiple range test. *, and *** indicate significance at 5%, and 0.1% probability levels, respectively.

Figure 2. Seasonal integrals of stem water potential ($_{si}$SWP, A), stomatal conductance ($_{si}g_s$, B), leaf net carbon assimilation ($_{si}A_N$, C) and water use efficiency ($_{si}$WUE, D) from Cabernet sauvignon grapevines (clone FPS08), subjected to different Phyto-Cat application (Control, 2 ppm and 4 ppm) for 2018-19 and 2019-20 growing seasons in Oakville, CA. Values represent means ± SE (n = 6). Different letters indicate significant differences ($p \leq 0.05$) between treatments and year according to two-way ANOVA followed by Duncan's new multiple range test. ns and * indicate non-significance or significance at 5% probability level, respectively.

Figure 3. Leaf, shoot and root total soluble sugar (TSS, A, B and C) and starch (D, E and F) contents of Cabernet sauvignon grapevines (clone FPS08), subjected to different Phyto-Cat application (Control, 2 ppm and 4 ppm) during two growing seasons (2018-19 and 2019-20) and collected in October 2020 in Oakville, CA. Values represent means ± SE (n = 6). Different letters indicate significant differences ($p \leq 0.05$) between treatments according to one-way ANOVA followed by Duncan's new multiple range test. *, and *** indicate significance at 5%, and 0.1% probability levels, respectively.

Figure 4. Correlation matrices among seasonal integrals for two seasons of stem water potential ($_{si2018\text{-}20}$SWP) and gas exchange parameters ($_{si2018\text{-}20}g_s$, $_{si2018\text{-}20}A_N$ and $_{si2018\text{-}20}$WUE), yield, vegetative growth (total biomass, BM), total soluble sugars (TSS) and starch of different organs from Cabernet sauvignon grapevines (clone FPS08), subjected to different Phyto-Cat applications (Control, 2 ppm and 4 ppm) during two growing seasons (2018-19 and 2019-20) and collected in October 2020 in Oakville, CA. Circle size and color represent R values for the Pearson's correlation analysis. *, **, and *** indicate significance at 5%, 1%, and 0.1% probability levels, respectively.

Figure 5. Relationship between shoot to root ratio and total biomass (BM, A), leaf non structural carbohydrates (NSC, B), photosynthetic pigments (C), root NSC (D) and sucrose:N ratio (E) and between N content and total BM (F), leaf NSC (G), photosynthetic pigments (H), yield per vine (I) and shoot to root ratio (J) from Cabernet sauvignon grapevines (clone FPS08), subjected to different Phyto-Cat application (Control, 2 ppm and 4 ppm) during two growing seasons (2018-19 and 2019-20) and collected in October 2020 in Oakville, CA. Dashed line represent regression curves for the Pearson's correlation analysis. *, **, and *** indicate significance at 5%, 1% and 0.1% probability levels, respectively.

Table 1: Weather conditions during the growing seasons of 2018-2019 and 2019-2020. Weather data were obtained from the CIMIS weather station #77 (Oakville, CA) located at the research site.

	Month												
	October	November	December	January	February	March	April	May	June	July	August	September	
Year	**Mean daily temperature (°C)**												Mean
2018-19	15.8	11.4	9	9.7	7.5	11	15.4	14.6	19.7	19.6	20.8	19.2	14.5
2019-20	15.4	11	9.5	8.8	11.4	10.7	14.6	17.4	19.7	19.2	21.1	20	14.9
	Minimum daily temperature (°C)												Mean
2018-19	7.2	3.7	3.5	4.7	2.7	4.9	8.8	8.4	11.2	11.1	12.3	9.7	7.4
2019-20	4.9	3.3	5.7	3.5	3.7	4.4	7.1	8.8	10.4	10.1	12.3	11.1	7.1
	Maximum daily temperature (°C)												Mean
2018-19	26.4	21.3	15.3	15.9	12.9	17.5	23.3	22.4	29.2	29.9	31.2	29.4	22.9
2019-20	26.6	20.8	14.3	15.4	20.6	17.6	23	26.2	29.5	30.2	31.8	31.4	24.0
	Days with temperature over 30 °C (no)												Total
2018-19	2	2	0	0	0	0	3	0	11	13	19	13	63
2019-20	7	0	0	0	0	0	3	8	11	14	17	20	80
	Precipitation (mm)												Total
2018-19	36.3	135.0	77.5	248.5	422.2	145.6	12.5	88.9	0.0	0.2	0.0	1.5	1168.2
2019-20	0.2	24.4	66.0	58.5	1.0	29.8	25.9	26.1	0.2	0.2	1.6	0.3	234.2
	Reference ET (ET_o, mm)												Total
2018-19	97.3	53.1	38.0	35.3	40.1	82.1	133.65	189.46	190.28	189.4	174.9	138.5	1362.1
2019-20	115.3	56.8	23.8	37.5	81.6	84.1	132.9	163	197.3	194.0	169.1	126.7	1385.1

Table 2. Petiole mineral content of Cabernet sauvignon grapevines (clone FPS08) subjected to different Phyto-Cat application (Control, 2 ppm and 4 ppm), collected in Oakville, CA in 2018-19 and 2019-20 seasons.

	N %	P %	K %	Zn mg/kg	Mn mg/kg	Na %	Ca mg/kg	B %	Mg %	Fe mg/kg	Cu mg/kg
2019											
Treatments											
Control	0.69 ± 0.02	0.53 ± 0.04	1.25 ± 0.07	113.0 ± 1.5	31.7 ± 2.3	0.018 ± 0.003	2.23 ± 0.19	68.00 ± 1.52	0.69 ± 0.03	30.67 ± 2.60	8.67 ± 0.34
2 ppm	0.69 ± 0.02	0.59 ± 0.01	1.27 ± 0.01	119.7 ± 3.9	30.3 ± 0.9	0.010 ± 0.004	2.38 ± 0.09	67.77 ± 1.67	0.68 ± 0.03	31.67 ± 1.86	9.33 ± 0.34
4 ppm	0.73 ± 0.03	0.59 ± 0.02	1.41 ± 0.13	121.0 ± 7.2	30.7 ± 1.8	0.013 ± 0.003	2.45 ± 0.13	69.00 ± 1.53	0.68 ± 0.01	30.67 ± 1.45	10.00 ± 0.57
ANOVA	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2020											
Treatments											
Control	0.82 ± 0.03 b	0.52 ± 0.03	2.92 ± 0.15 b	101.0 ± 2.0	36.8 ± 2.8	0.007 ± 0.002	1.73 ± 0.14	44.20 ± 0.70	0.68 ± 0.05	36.00 ± 1.91	12.00 ± 0.63
2 ppm	0.90 ± 0.02 b	0.49 ± 0.02	3.11 ± 0.10 a	99.8 ± 2.0	36.0 ± 1.5	0.008 ± 0.002	1.89 ± 0.10	46.50 ± 1.18	0.68 ± 0.03	33.67 ± 2.22	12.17 ± 0.54
4 ppm	1.18 ± 0.06 a	0.46 ± 0.02	3.36 ± 0.19 a	94.4 ± 6.2	39.2 ± 4.6	0.006 ± 0.002	1.73 ± 0.09	47.80 ± 2.92	0.64 ± 0.07	36.60 ± 2.64	11.40 ± 0.75
ANOVA	***	ns	*	ns	ns	ns	ns	ns	ns	ns	ns

Values represent means (n = 6) separated by Duncan test (P≤ 0.05). Different letters within line, indicate significant differences as affected by the different treatments. ns, *, and *** indicate non-significance or significance at 5% and 0.1% probability levels, respectively. Values are expressed as % or mg of the mineral per kg of petiole dry weight.

Table 3. Yield components of Cabernet sauvignon grapevines (clone FPS08) subjected to different Phyto-Cat application (Control, 2 ppm and 4 ppm), collected in Oakville, CA in 2018-19 and 2019-20 seasons.

	Clusters per vine (no)	Yield kg/vine	Leaf area to fruit ratio (m^2/kg)	Berry mass (g)
2019				
Treatments				
Control	56 ± 1	6.78 ± 0.45 c	472.67 ± 21.22 b	1.08 ± 0.04 c
2 ppm	58 ± 1	8.83 ± 0.44 b	409.26 ± 29.70 b	1.20 ± 0.04 b
4 ppm	59 ± 1	10.35 ± 0.38 a	726.04 ± 24.19 a	1.37 ± 0.03 a
ANOVA	ns	***	***	***
2020				
Treatments				
Control	55 ± 1	4.80 ± 0.31 c	498.85 ± 36.83 b	0.85 ± 0.05 b
2 ppm	53 ± 1	6.26 ± 0.32 b	340.99 ± 12.78 c	1.08 ± 0.02 b
4 ppm	53 ± 1	9.14 ± 0.25 a	837.90 ± 47.46 a	1.15 ± 0.02 a
ANOVA	ns	***	***	***

Values represent means (n = 6) separated by Duncan test ($P \leq 0.05$). Different letters within line, indicate significant differences as affected by the different treatments. ns, and *** indicate non-significance or significance at 0.1% probability level, respectively.

Table 4. Total biomass (DW) of trunks, leaves, shoots and rootstocks of Cabernet sauvignon grapevines (clone FPS08) subjected to different Phyto-Cat application (Control, 2 ppm and 4 ppm) during two growing season (2018-19 and 2019-20) and harvested in Oakville, CA in November 2019 and October 2020, respectively.

	Leaves kg/vine	Shoots kg/vine	Trunk kg/vine	Roots kg/vine	Shoot:root ratio
Treatments					
Control	0.39 ± 0.03 b	0.42 ± 0.06	2.52 ± 0.19	0.97 ± 0.04 b	0.48 ± 0.08
2 ppm	0.47 ± 0.04 b	0.57 ± 0.06	2.89 ± 0.17	1.13 ± 0.10 ab	0.50 ± 0.04
4 ppm	0.81 ± 0.07 a	0.84 ± 0.20	3.09 ± 0.22	1.35 ± 0. 13 a	0.65 ± 0.03
ANOVA	***	ns	ns	*	ns

Values represent means (n = 6) separated by Duncan test (P≤ 0.05). Different letters within line, indicate significant differences as affected by the different treatments. ns, *, and *** indicate non-significance or significance at 5%, and 0.1% probability levels, respectively.

Table 5. Individual sugars (mg/g of dry weight) determined in leaves, shoots, roots and berry must (g/L) of Cabernet sauvignon grapevines (clone FPS08) subjected to different Phyto-Cat application (Control, 2 ppm and 4 ppm) during two growing season (2018-19 and 2019-20) and harvested in Oakville, CA in October 2020.

	Leaves				Shoots				Roots				Berry must			
	D-Fructose	D-Glucose	D-Sucrose	D-Raffinose	D-Fructose	D-Glucose	D-Sucrose	D-Raffinose	D-Fructose	D-Glucose	D-Sucrose	D-Raffinose	D-Fructose	D-Glucose	D-Sucrose	TSS
Treatments																
Control	19.17 ± 1.73 b	25.41 ± 2.91 b	14.32 ± 4.17	10.41 ± 0.72 b	8.84 ± 0.81	12.59 ± 1.17	1.45 ± 0.13 b	0.91 ± 0.10 b	2.52 ± 0.32	3.10 ± 0.18	19.20 ± 2.31	1.88 ± 0.11	229.2 ± 55.1	348.3 ± 69.6	0.75 ± 0.10	578.3 ± 124.7
2 ppm	21.55 ± 1.09 ab	24.55 ± 0.92 b	8.69 ± 1.24	10.82 ± 0.94 b	9.97 ± 0.74	13.25 ± 0.80	4.28 ± 0.48 a	1.62 ± 0.22 a	3.09 ± 0.45	3.78 ± 0.40	19.40 ± 1.48	2.29 ± 0.21	187.3 ± 36.5	246.9 ± 47.7	0.54 ± 0.10	434.7± 79.9
4 ppm	25.56 ± 2.32 a	34.01 ± 2.89 a	8.91 ± 1.23	14.08 ± 1.37 a	8.58 ± 1.17	12.07 ± 1.37	3.96 ± 0.44 a	1.93 ± 0.11 a	2.06 ± 0.25	3.16 ± 0.37	15.30 ± 0.73	1.78 ± 0.17	179.6 ± 40.2	284.6 ± 54.2	0.65 ± 0.09	464.8 ± 94.1
ANOVA	*	*	ns	*	ns	ns	*	**	ns	ns	ns	ns	ns	ns	ns	ns

Values represent means (n = 6) separated by Duncan test (P≤ 0.05). Different letters within line, indicate significant differences as affected by the different treatments. ns, *, and ** indicate non-significance or significance at 5% and 1% probability levels, respectively.

Table 6. Percentage of total non-structural carbohydrates (NSC) in leaves, shoots and roots of Cabernet sauvignon grapevines (clone FPS08) subjected to different Phyto-Cat application (Control, 2 ppm and 4 ppm) during two growing season (2018-19 and 2019-20) and harvested in Oakville, CA in October 2020.

	% of total NSC				
	D-Fructose	D-Glucose	D-Sucrose	D-Raffinose	Starch
Leaf					
Control	26.91 ± 1.77	35.59 ± 3.10	19.06 ± 4.86	14.58 ± 0.54	3.86 ± 0.73 c
2 ppm	30.38 ± 1.34	34.61 ± 0.88	12.32 ± 1.84	15.24 ± 1.17	7.45 ± 0.91 b
4 ppm	26.96 ± 1.29	35.79 ± 1.40	9.53 ± 1.38	14.80 ± 0.79	12.92 ± 1.67 a
ANOVA	ns	ns	ns	ns	***
Shoot					
Control	15.99 ± 0.63	22.79 ± 0.80	2.69 ± 0.24 b	1.65 ± 0.11 b	56.89 ± 1.24
2 ppm	16.05 ± 0.85	21.42 ± 0.95	6.94 ± 0.76 a	2.60 ± 0.33 a	52.99 ± 1.24
4 ppm	14.45 ± 1.62	20.35 ± 1.68	6.72 ± 0.65 a	3.31 ± 0.25 a	55.16 ± 2.87
ANOVA	ns	ns	***	***	ns
Root					
Control	3.35 ± 0.43	4.14 ± 0.34	24.82 ± 2.01	2.52 ± 0.23	64.98 ± 2.38
2 ppm	3.99 ± 0.80	4.77 ± 0.61	24.11 ± 1.02	2.96 ± 0.46	64.16 ± 2.23
4 ppm	2.83 ± 0.37	4.31 ± 0.48	20.91 ± 0.66	2.43 ± 0.22	69.51 ± 1.14
ANOVA	ns	ns	ns	ns	ns

Values represent means (n = 6) separated by Duncan test (P≤ 0.05). Different letters within line, indicate significant differences as affected by the different treatments. ns, and *** indicate non-significance or significance at 0.1% probability level, respectively.

Table S1. Total chlorophylls and total carotenoids in leaves of Cabernet sauvignon grapevines (clone FPS08) subjected to different Phyto-Cat application (Control, 2 ppm and 4 ppm) during two growing season (2018-19 and 2019-20) and harvested in Oakville, CA in October 2020.

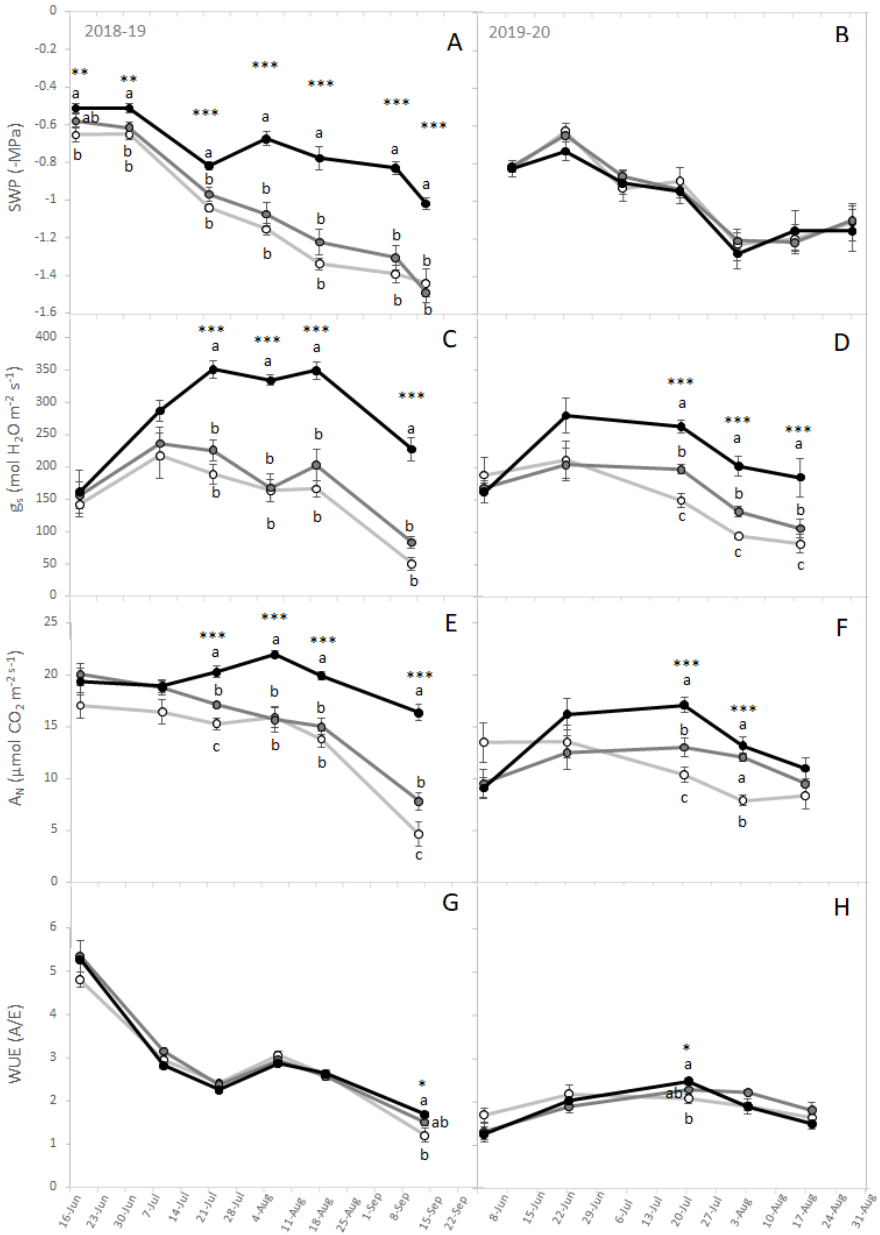
Treatments		Total chlorophylls mg/g DW	Total carotenoids mg/g DW
	Control	2.36 ± 0.70 b	1.15 ± 0.18 b
	2 ppm	2.58 ± 0.47 b	1.29 ± 0.16 b
	4 ppm	5.59 ± 0.27 a	2.05 ± 0.10 a
	ANOVA	***	**

Values represent means (n = 6) separated by Duncan test (P≤ 0.05). Different letters within line, indicate significant differences as affected by the different treatments. ** and *** indicate significance at 1% and 0.1% probability levels, respectively. DW, dried weight.

Table S2. Total biomass (FW) of trunks, leaves, shoots and roots(kg/vine) of Cabernet sauvignon grapevines (clone FPS08) subjected to different Phyto-Cat application (Control, 2 ppm and 4 ppm) during two growing season (2018-19 and 2019-20) and harvested in Oakville, CA in November 2019 and October 2020, respectively.

	Leaves	Shoots	Roots
2019			
Treatments			
Control	1.30 ± 0.04 b	1.22 ± 0.10 b	ND
2 ppm	1.99 ± 0.20 a	1.56 ± 0.17 ab	ND
4 ppm	2.55 ± 0.09 a	1.89 ± 0.19 a	ND
ANOVA	**	**	
2020			
Treatments			
Control	1.26 ± 0.17 b	0.71 ± 0.08 b	1.92 ± 0.20 b
2 ppm	1.42 ± 0.10 b	0.98 ± 0.10 b	2.24 ± 0.11 ab
4 ppm	2.47 ± 0.22 a	1.74 ± 0.17 a	2.64 ± 0.21 a
ANOVA	***	***	*

Values represent means (n = 6) separated by Duncan test (P≤ 0.05). Different letters within line, indicate significant differences as affected by the different treatments. *, ** and *** indicate significance at 5%, 1% and 0.1% probability levels, respectively. ND, non-determined.



4 ppm

2 ppm

0 ppm

Figure 1.

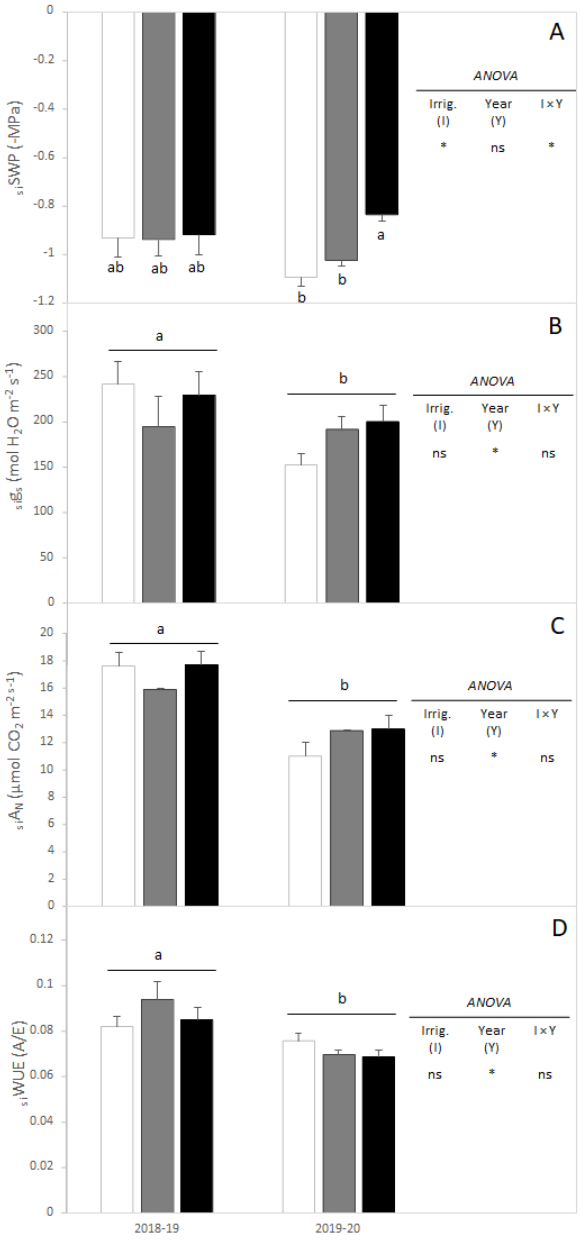


Figure 2.

- 4 ppm
- 2 ppm
- 0 ppm

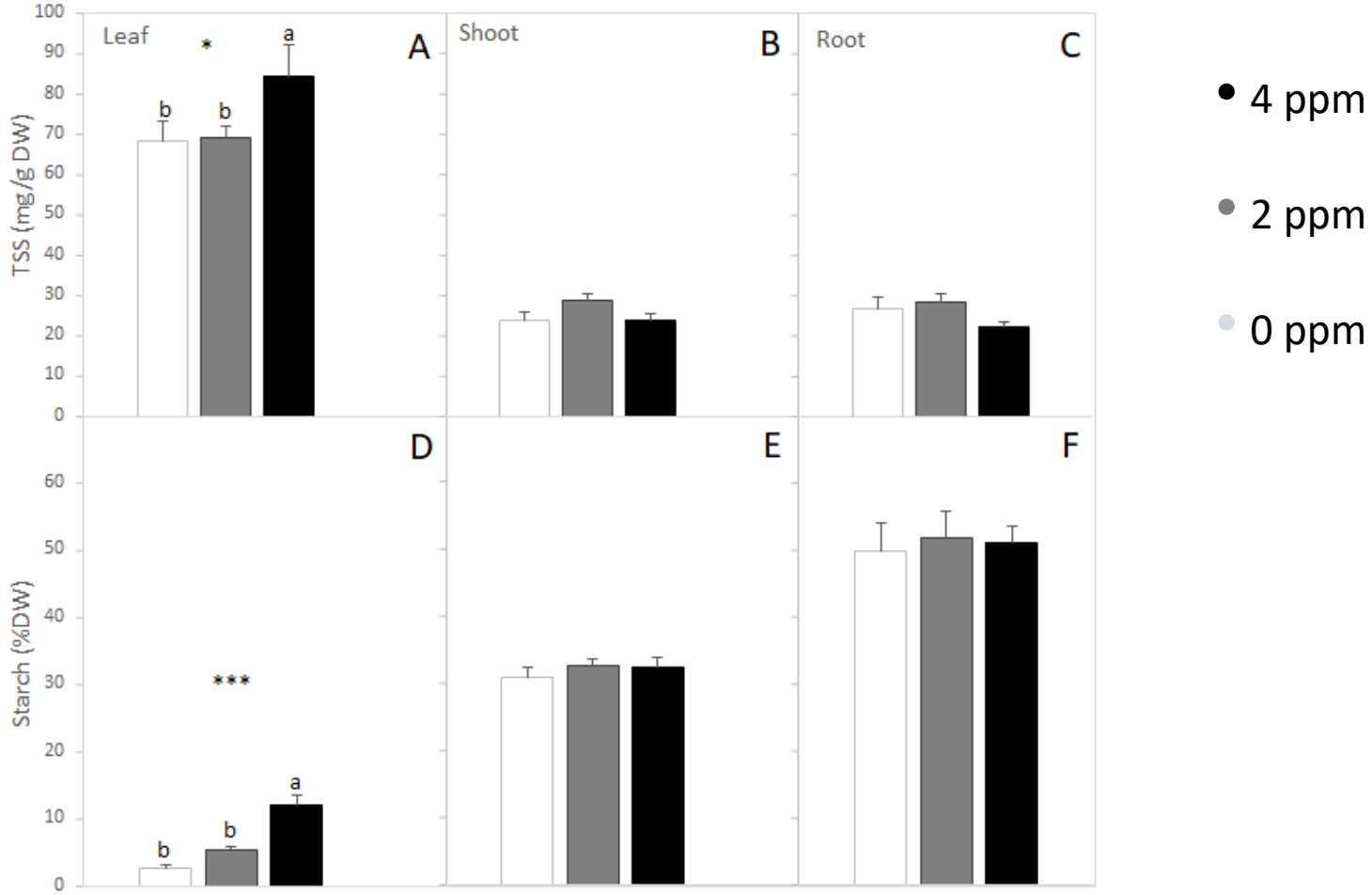

Leaf
Shoot
Root
A
B
C
D
E
F
TSS (mg/g DW)
Starch (%DW)
*
***
a
b
b
b
b
a
4 ppm
2 ppm
0 ppm

Figure 3.

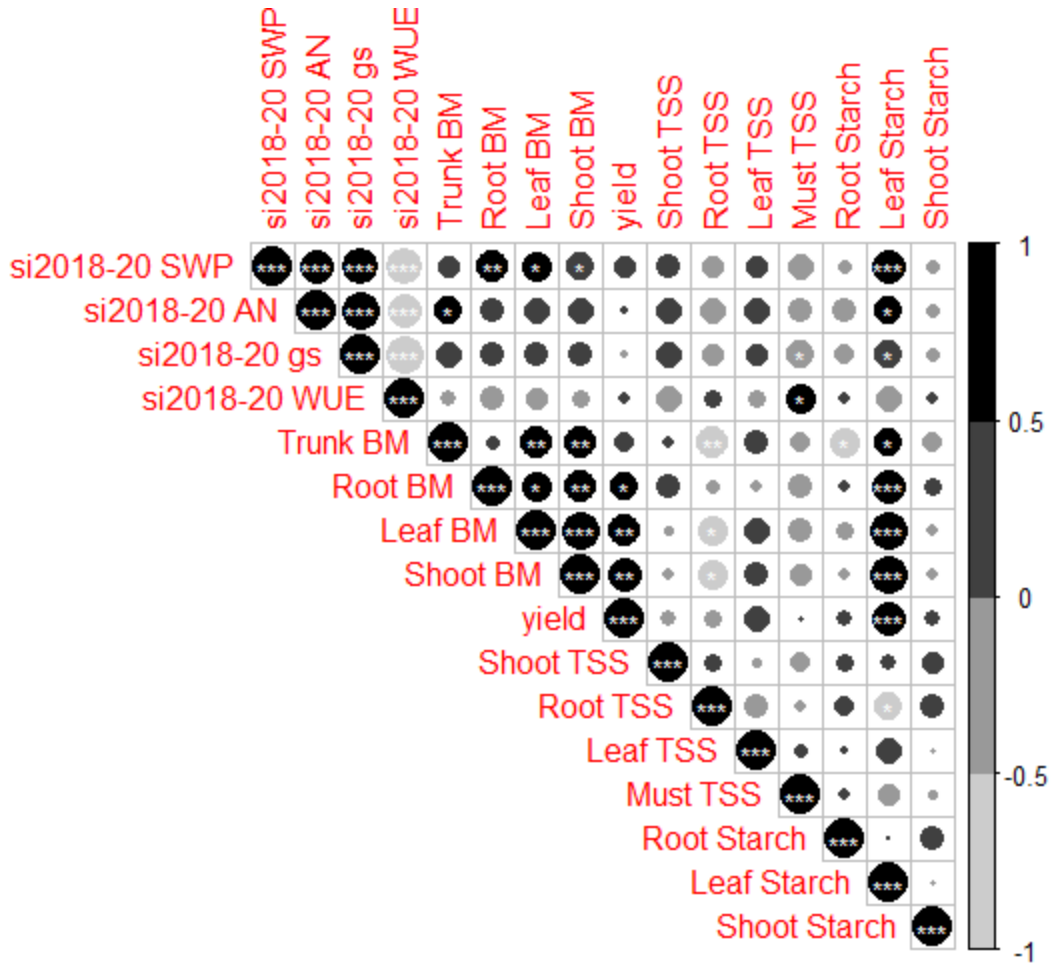

si2018-20 SWP
si2018-20 AN
si2018-20 gs
si2018-20 WUE
Trunk BM
Root BM
Leaf BM
Shoot BM
yield
Shoot TSS
Root TSS
Leaf TSS
Must TSS
Root Starch
Leaf Starch
Shoot Starch
1
0.5
0
-0.5
-1

Figure 4.

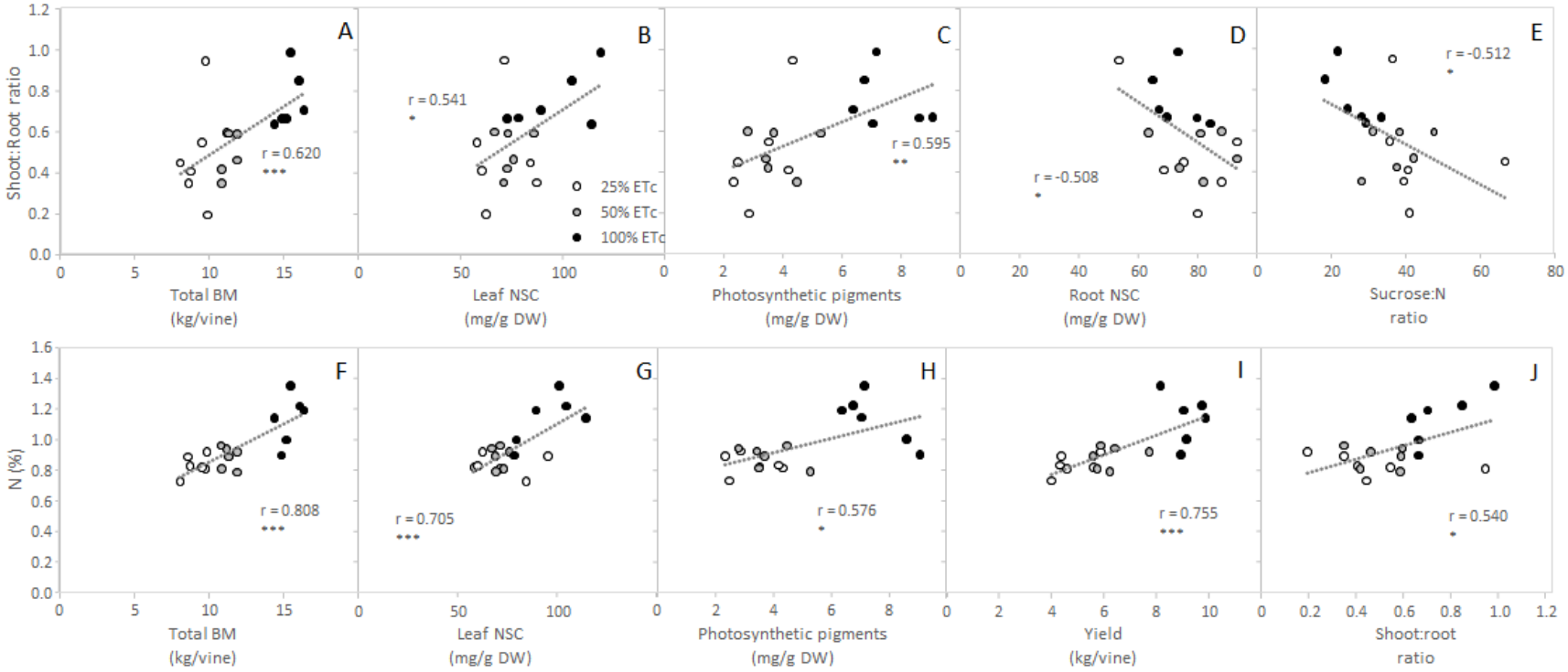

A
B
C
D
E
F
G
H
I
J
Shoot:Root ratio
N (%)
r = 0.620
r = 0.541
r = 0.595
r = -0.508
r = -0.512
r = 0.808
r = 0.705
r = 0.576
r = 0.755
r = 0.540
25% ETc
50% ETc
100% ETc
Total BM (kg/vine)
Leaf NSC (mg/g DW)
Photosynthetic pigments (mg/g DW)
Root NSC (mg/g DW)
Sucrose:N ratio
Yield (kg/vine)
Shoot:root ratio

Figure 5.