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Project number 002200

Project Title: Mitigation of Grapevine Red Blotch Virus and Climate Change through the use of Abscisic Acid Analogs

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Executive Summary

This project is a continuation of research projects concerning ABA analogs (OGWRI #001700) and linked to the AAFC grape cluster project, specifically Activities 3 and 7. It is a collaboration with 3 different scientists of varying expertise (viticulturist, plant hormone chemist, and virologist) to understand how ABA analogs can be used as a mitigation strategy by grape growers to deal with Grapevine Red Blotch virus and freeze injury.

Abscisic acid (ABA) is a plant hormone that is involved in many plant processes. One of the key roles of ABA is mediating the adaptation of plants to stress (drought, salinity, freeze). Some of its key functions include leaf abscission, induction and maintenance of dormancy, growth control, berry development, as well as regulation of water loss in the plant. Exogenous ABA applications to grapevines can hasten fruit maturation, improve yield and fruit quality, and induce leaf abscission/dormancy to name a few. The overall goal of this study is to use exogenous ABA and ABA analogue applications to mitigate the effects of Grapevine Red Blotch Virus (GRBV) and climate change with respect to fruit quality and cold hardiness while gaining further insight on how ABA analogs, GRBV, and their interactions impact grapevine physiology.

Detailed Description of the Project

The proposed research will focus on synthesizing and field-testing ABA analogs to mitigate the detrimental effects of Red Blotch virus on fruit maturation as well as to accelerate dormancy, improve cold hardiness acclimation and optimize hardiness throughout dormancy. The project will include synthesis of long lasting analogs of ABA, testing their efficacy in the field, developing practical methods of application in order to achieve the goal of commercializing an agrochemical product for grower operations. In addition, hormone profiling will be performed on vines of different virus status and treatments to determine residue levels in grape tissues over time, identify metabolites of bioactive analogs and how ABA, ABA analogs and other related hormones and metabolites change and impact key performance and quality attributes.

a) Objectives and Project input

Objective

The proposed research will focus on mitigation of grapevine red blotch virus and climate change impacts through the use of ABA analogs. The project will include measuring ABA concentrations in grapevine tissue as well as synthesizing and field- testing ABA and ABA analogs to mitigate the detrimental effects of GRBV on grape production as well as accelerate and prolong dormancy in grapevines and optimize hardiness throughout dormancy.

The specific objectives include:

1. Investigate how exogenous ABA and ABA analogs may mitigate the effects of Red Blotch virus on fruit composition

2a. Elucidate impacts of exogenous ABA and ABA analog applications on vine performance of Red Blotch virus infected vines

2b. Develop and test an ABA antagonist to confirm ABA as the main hormone impacted by Red Blotch virus

3. Determine how ABA analogs may improve and/or maintain dormancy in healthy and Red Blotch virus infected vines

4. Investigate ABA and analog treatments to improve freeze tolerance and extend dormancy of Merlot and Marquette vines.

This project involved the PIs, Dr. Jim Willwerth and Dr. Sud Poojari at CCOVI and Dr. Suzanne Abrams at ABAzyne/UofS. Dr. Willwerth coordinated the project along with research technicians, Alex Gunn and Stephanie Bilek and OEVI undergraduate honour's students Katrina Kastelic, Ryan MacIntyre-Newell and Connor Book. Dr. Abrams coordinated synthesis of the ABA analogs at U of S and Dr. Poojari coordinated the virus diagnostic testing for the Red Blotch component of the research. In-kind contributions were provided by Huebel Grape Estates and Funk Farms for vineyard management and fruit donations from the experimental Cabernet franc block (Huebel only). Cash financial support was provided by OGWRI for the project along with a portion from AAFC through the CAP activity.

b) Project Activities and Outputs

A. Synthesis of ABA analogs and ABA antagonist

The long-lasting, biologically active ABA analog (+)-8'-acetylene ABA was synthesized according to methods developed and optimized in the Abrams lab. The acid form of the analog was produced in large scale to provide material for testing in the field. An ABA antagonist recently developed by the Abrams' lab was also synthesized. These products will be formulated to increase uptake and transport within the plant. Natural ABA was provided through Valent BioSciences.

B. Red Blotch Virus Diagnostic testing

Cabernet franc grapevines were tested for viruses and confirmed to have infection with Red Blotch Virus. Further testing will be done using droplet digital -PCR developed by co-PI will be used to measure GRBV titers in infected vines. These data will be used to elucidate any relationships between amount of virus titer in the grapevine to vine performance and fruit quality as well as level of mitigation by exogenous ABA and ABA analog applications.

C. Field testing of exogenous ABA applications for improving cold hardiness

Two different genotypes of *Vitis* were used for ABA field experiments. These include a "cold tender" *V. vinifera* variety (Merlot) and a *V. riparia*-based variety (Marquette) which is susceptible to early deacclimation and spring frost. Cabernet franc was used for Red Blotch experiments as described under subheading 'D'. 'Industry standard' practices were used for vineyard management. Randomized complete block design plots were established in mature vineyard blocks. ABA treatments of different formulations and concentrations were applied at different times prior to dormancy. In 2019, only Marquette vines were used as Merlot vines were not harvested prior to a frost and canopies were not suitable for ABA and ABA analog applications.

Table 1: Experimental treatments for hardiness experiments (5 vines/treatment x 3 replicates)

Treatment	Rate	Timing
Control – surfactant (Agral 90)	0.05%	Post-harvest
S-ABA	5g/L + surfactant	Post-harvest
(+)-8'Acetylene ABA	0.5g/L + surfactant	Post-harvest
(+)-8'Acetylene ABA	0.25 g/L + surfactant	Post-harvest

Bud cold hardiness were evaluated beginning 4 weeks post-veraison and continue throughout the dormant period using differential thermal analysis (DTA) on 2-3 week intervals. Bud survival was assessed late winter and initiation of bud break was monitored. Periderm formation was assessed and measurement of cold hardiness-related plant metabolites (carbohydrates, dehydrins) in bud tissue will also be conducted. Vine performance in the following growing season will be performed as described under subheading 'D' and ABA will also be measured as described under subheadings 'F' and 'G'.

D. Field testing of exogenous ABA applications and ABA antagonist on Red Blotch-infected vines

ABA, ABA analog and ABA antagonist treatments were applied to Cabernet franc vines infected with Red Blotch virus to investigate if these plant growth regulators can mitigate the negative effects of the virus on vine performance and fruit quality. The ABA antagonist will essentially be used as another control to determine if ABA is the hormone largely responsible for Red Blotch virus' impact on fruit maturation. Applications occurred at veraison and included separate full canopy and fruiting zone applications to determine optimal application procedures. The experiment included a randomized complete block design that was completed after virus diagnostic testing to ensure positively infected grapevines. Based, on results from the 2019 experimental year, it was decided to add 4 additional treatments with higher rates of both the ABA analog and ABA antagonist (see Table 3).

Table 2: Experimental 2019 treatments for Red blotch virus experiments (5 vines/treatment x 3 replicates)

Treatment	Rate	Timing	Target area
Control – surfactant (Agral-90)	0.05%	Veraison	1. Canopy 2. Fruit
S-ABA	400 mg/L + surfactant	Veraison	Fruit*
(+)-8' Acetylene ABA	40 mg/L + surfactant	Veraison	1. Canopy 2. Fruit
ABA antagonist	50 mg/L +surfactant	Veraison	1. Canopy 2. Fruit

*Fruit applications only due to phytotoxic effects

Table 3: Experimental 2020 treatments for Red blotch virus experiments (5 vines/treatment x 3 replicates)

Treatment	Rate	Timing	Target area
Control – surfactant (Agral-90)	0.05%	Veraison	1. Canopy 2. Fruit
S-ABA	400 mg/L + surfactant	Veraison	Fruit*
(+)-8' Acetylene ABA	40 mg/L + surfactant	Veraison	1. Canopy 2. Fruit
(+)-8' Acetylene ABA	100 mg/L + surfactant	Veraison	1. Canopy 2. Fruit
ABA antagonist	10 mg/L +surfactant	Veraison	1. Canopy 2. Fruit
ABA antagonist	10 mg/L +surfactant	Veraison	1. Canopy 2. Fruit

*Fruit applications only due to phytotoxic effects

4. Project Outcomes

1. Grapevine Red Blotch Mitigation Experiment

Materials and Methods

The 2019/20 study was conducted between May-December 2019 at a vineyard site in Queenston, Niagara-on-the-Lake, Ontario (43°09' N, 79°03' W). Three rows of *Vitis vinifera* Cabernet franc grapevines were used as treatment material for exogenous applications of ABA. The experimental design was a randomized block of seven treatments (see Table 2) where each block was a section of a vineyard row (Appendix B). Analysis and laboratory work were performed at the Cool Climate Oenology and Viticulture Institute (CCOVI) at Brock University. The 2020 study was conducted at the same vineyard site in Niagara-on-the-Lake, ON with a similar experimental design using eleven treatments as described in Table 3 below.

Grapevine Virus Testing

Collection of Plant Material

A leaf sample was collected for 125 vines on August 2, 2019 to determine optimal treatment arrangement for the ABA project. Following CCOVI's Grapevine Virus Testing Procedure, four representative mature

leaves with intact petioles were collected from each vine. It was ensured that leaves were sampled from the bottom portion of the canopy and leave

heavily damaged or dead were to be avoided. Samples were bagged separately, transported to the lab on ice packs and stored at -20 °C until further tested. The grapevines used for the experiments in 2020 were sampled on July 8-9, 2020 and tested for GRBV using the same procedure.

Nucleic Acid Extraction and Detection of GRBV

Extraction bags and microcentrifuge tubes were labelled with corresponding vine codes. Petioles were cut using sterile razor blades to achieve approximately 0.250g. To avoid contamination gloves were changed for each sample. Cut petioles were then added to extraction bags with 5 mL Grapevine Extraction Buffer (GEB) on ice. Using the Homex 6 homogenizer (Bioreba, Reinach, Switzerland), petioles were ground until a smooth consistency was achieved. Approximately 1mL of the sample was collected into a microcentrifuge tube. Samples were kept in -80 °C until further tested.

Six microliters of sample were added to 50uL of GES buffer with 1% mercaptoethanol to avoid degradation of RNA. DNA extraction occurred at 95 °C for 10 minutes using the C1000 Touch Thermal Cycler (BioRad, Hercules, California). Extract samples were stored in -80 °C until further tested.

Three microliters of the extracted DNA sample were added to 22uL of Mastermix (14.25 uL H2O, 2.5 uL Buffer, 2.5 uL Sucrose, 0.25 uL Taq polymerase, 0.5 uL dNTP, 20m, 1uL Primer 1 (20mM), 1 uL Primer 2 (20mM)) for endpoint PCR. Diagnostic primers specific for the GRBV gene, 5'GTAGATTGAGGACGTATTGG'3 (forward) and 5'CGCAAGAATACCACGTACCATGGG'3 (reverse) were used to amplify a 557bp DNA fragment (Poojari et al., 2013). Thermocycling followed the protocol: 94 °C for 3 minutes to lyse whole cells and activate polymerase, followed by 36 cycles at 94 °C for 30 seconds to melt the template, 54 °C for 45 seconds to anneal the primers and 72 °C for 1 minute for extension. A final extension of 72 °C for 10 minutes and storage at 4 °C until further tested.

PCR amplified samples were visualized using QIAxcel Advanced capillary electrophoresis (QIAGEN, Hilden, Germany). The QIAxcel DNA Fast Analysis Kit with QXDNA Size Marker 50 bp-1.5 kb and 15 bp/3 kb alignment markers were run alongside the PCR fragments. The DM 80 v2.0 method with the following parameters was used: sample injection voltage at 15 kV, sample injection time at 8 s, separation voltage at 15 kV and separation time at 80 s. The separated samples were then visualized using QIAxcel ScreenGel Software.

A second leaf collection occurred for treatment specific vines on October 24, 2019 followed by nucleic acid extractions as explained above. Both extracted samples from August 2, 2019 and October 24, 2019 were kept in -80°C for further quantification of virus titer using ddPCR following the Tai et al. protocol (2018) with the QX200 Droplet Digital PCR System (includes the QX200 Droplet Reader and the QX200 Droplet Generator) (BioRad,Hercules, California).

ABA Treatments

Solution Preparation

ABA solutions were made using synthesized ABA compounds from Dr. Suzanne Abrams lab at the University of Saskatchewan (Saskatoon, Saskatchewan). Each treatment consisted of 0.05% Agral 90 as the surfactant. 4L per treatment were prepared to ensure full coverage among 3 blocks of Cabernet franc. Canopy applications of S-ABA were not implemented due to the likelihood of phytotoxic effects.

Table 3: Treatment rates and target applications for exogenous applications of S-ABA, ABA analog and ABA antagonist on GRBV infected Cabernet franc.

Treatment	Rate	Target Area
Control	0.05% surfactant (Agral 90)	Canopy and Fruit
S-ABA	400 mg/L + surfactant	Fruit
(+) 8' Acetylene ABA	40 and 100 mg/L + surfactant	Canopy and Fruit
ABA Antagonist	50 mg/L + surfactant	Canopy and Fruit

Application Day Conditions
Treatment vines were approximately 50% véraison. Each treatment contained four vines with two buffer vines on either side. Treatments were sprayed until complete coverage was achieved on either side of the vinesIn 2019, the exogenous applications were applied on September 5, 2019. The applications occurred from 10:00-12:00 with a temperature between 16.9-20℃; relative humidity between 64.6-81.5%; wind between 6-9 km/hr (Vine & Tree Fruit Innovations, 2019).

In 2020, the exogenous applications were applied on August 26, 2020. The applications occurred from 09:30-12:00 with a temperature between 15.8-20.1℃; relative humidity between 65-725%; wind between 7-8 km/hr. The fruit was sampled to be at 12.2 Brix at the time of spray applications.

Grape Berry Sampling
Berry collection occurred biweekly for basic chemical analysis of ABA treatments from September to harvest in October. 150 berries were collected for each treatment (composite sampling within 4 vines). Samples were brought back to the lab, weighed and then divided for separate analysis purposes. All analyses were done in duplicates. 90 berries were analyzed for total soluble solids, pH, and titratable acidity. 50 berries were analyzed for anthocyanin and total phenolics. Anthocyanin and total phenolic samples were placed in -25°C until further testing.
Ten berries were collected for hormone profiling. Hormone profiling samples were frozen immediately in liquid nitrogen and stored in -80°C prior to being sent out for analysis (Owen et al., 2009). Along with sampling, weekly vine performance observations were made. Harvest occurred on November 4th with a final per vine berry collection for the same analyses as above with additional yield data collection.

Basic Grape Berry Composition
90 berries per treatment were tested the day of berry collection at room temperature. Berries were immediately juiced using the Model 9000 Omega Centrifugal Juicer (Omega, Harrisburg, Pennsylvania). For total soluble solids, the ATAGO 3810 Pocket Refractometer (Atago, Tokyo, Japan) was used. Approximately 3 drops of water were added to the prism surface using a plastic pipette and was zeroed for calibration. Prism surface was then washed with sample and wiped using lint-free tissue before sample measurements. Soluble solids were measured by pressing “start”.
pH and titratable acidity were measured using the Mettler Toledo Titrator T50 (Mettler Toledo, Colombus, Ohio) with LabX Titrator software procedure. The pH meter setup, calibration, titrant setup and standardization were performed before analyses. To measure pH and TA successively, the 3c pH and TA by endpoint method was selected, requiring 15mL of sample. Titration was measured by titration with 0.1N NaOH to an endpoint of pH 8.2. Titratable acidity was expressed in tartaric acid equivalents.

Whole Berry Anthocyanin and Total Phenolic Extraction
Anthocyanins and total phenolics were determined using the Iland method (Iland *et al*., 2004). 50 berries from each treatment were collected biweekly. Samples were weighed in 125 mL plastic containers and stored in -25℃ until analysis. For analysis, the samples were partially thawed before homogenising (thawed to less than 10 °C). Berries were homogenised at 24000 rpm using the 850 Homogenizer with the 10mm x 115 mm probe generator (Thermo Fisher Scientific, Waltham, Massachusetts) on ice until the mixture of flesh, skins and seeds were of smooth consistency to ensure for a representative sample (approximately 6 minutes). 1 gram
(g) of the homogenate was collected and weighed into a 50 mL centrifuge tube. For extraction of anthocyanins, 10 mL of 50% v/v ethanol adjusted to pH 2.0 was pipetted into the centrifuge tube with the homogenate. Contents were mixed every 10 minutes for an hour. The samples were then centrifuged at 3500 rpm for 5 minutes using the Thermo Scientific CL2 benchtop centrifuge (Thermo Fisher Scientific, Waltham, Massachusetts). 0.5 mL of the extract was then pipetted into 10 mL 1M HCl (21x dilution). After 3 hours, the absorbance of the samples was read at 700 nm, 520 nm and 280 nm using the Agilent Technologies Cary 60 UV-Vis Spectrophotometer (Agilent Technologies, Santa Clara, California). The measurement of absorbance at 280 nm was measured in quartz cuvettes. Anthocyanins were calculated as malvidin-3-glucose equivalent.

Results to date

Viral Detection

Primer pairs successfully amplified a 557 bp fragment of GRBV in 124 samples of 125 samples. All GRBV positive vines exhibited dark bands, suggesting a high virus titer in the vines. From the detection of the virus, the treatment layout between blocks could be organized appropriately.
In 2020, all samples were tested positive for GRBV.

Fruit Maturation

Composite berry samples were collected biweekly after the exogenous applications of ABA. Vine performance was observed weekly to note any significant differences between treatments. Visual observations focused on foliar health, colour development and the overall variation of ripening within clusters.

Harvest Composition

Primary Fruit Chemistry

2019

Basic chemical compositions of harvested Cabernet franc are displayed in Figures 1-2. Figure 1 evaluates the basic chemical composition between canopy applied treatments with (+)-8'-acetylene ABA and the ABA antagonist. No differences were found compared to the control in both soluble solid and pH results. The highest soluble solid reading was found in the (+)-8'-acetylene ABA treatment and the lowest was found in the ABA antagonist treatment. The titratable acidity of the ABA antagonist treatment was significantly lower than the control by 1.50 g/L. (+)-8-acetylene ABA treatment had the highest titratable acidity at 10.96 g/L. Figure 2 evaluates the basic chemical composition between fruiting zone applied treatments with S-ABA, (+)-8'-acetylene ABA and ABA antagonist. S-ABA and (+)-8'- acetylene ABA had significantly greater soluble solid results from the control at 20.8 °Brix and 20.4 °Brix, respectively. No significant differences were found from the control with respect to pH and titratable acidity results. However, the lowest titratable acidity to the control was (+)-8'- acetylene ABA at 9.46 g/L.

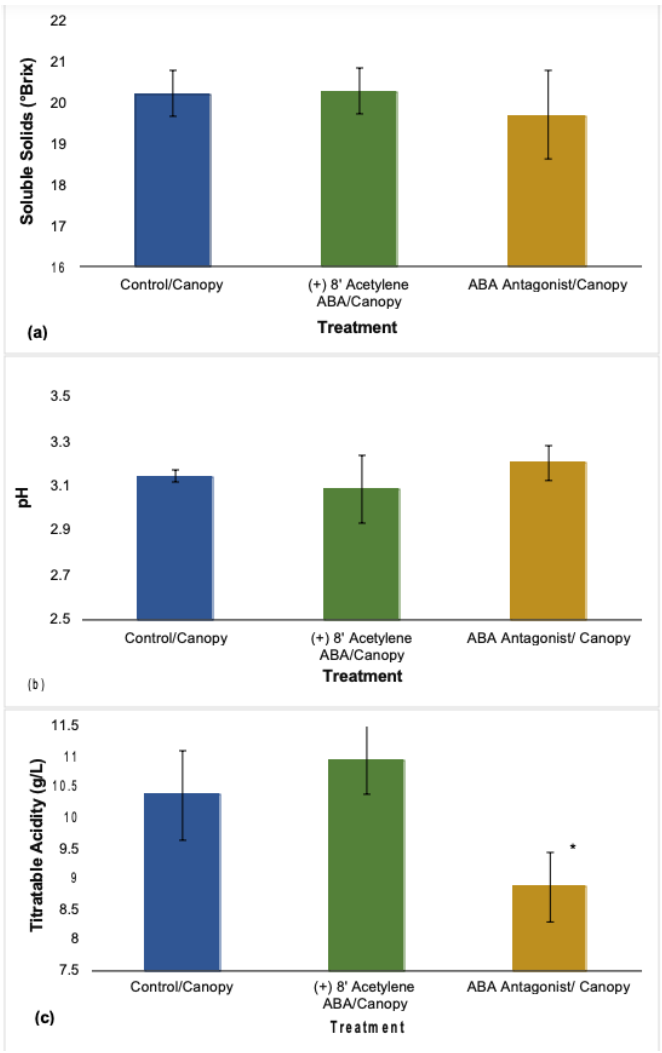


Figure 1: Basic chemical composition of (a) soluble solids; (b) pH; (c) titratable acidity from harvested Cabernet franc ABA canopy treatments. * indicates statistical differences within the same application area against the control treatment using Dunnett's Test ($p<0.05$).

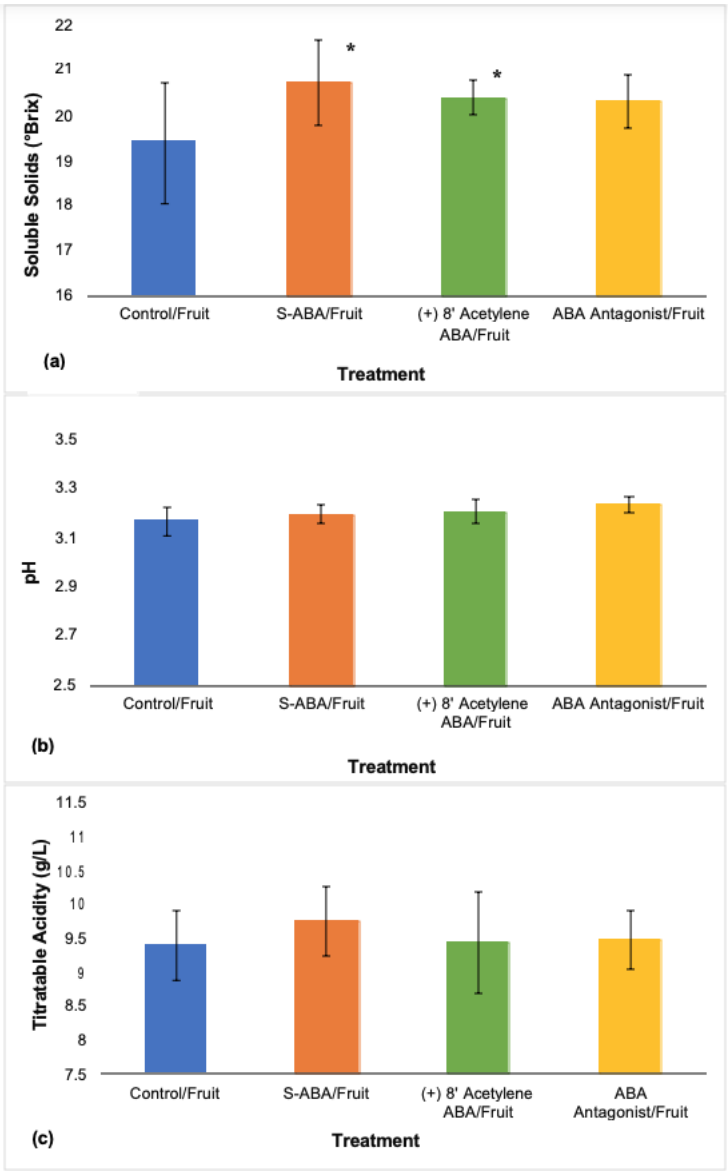
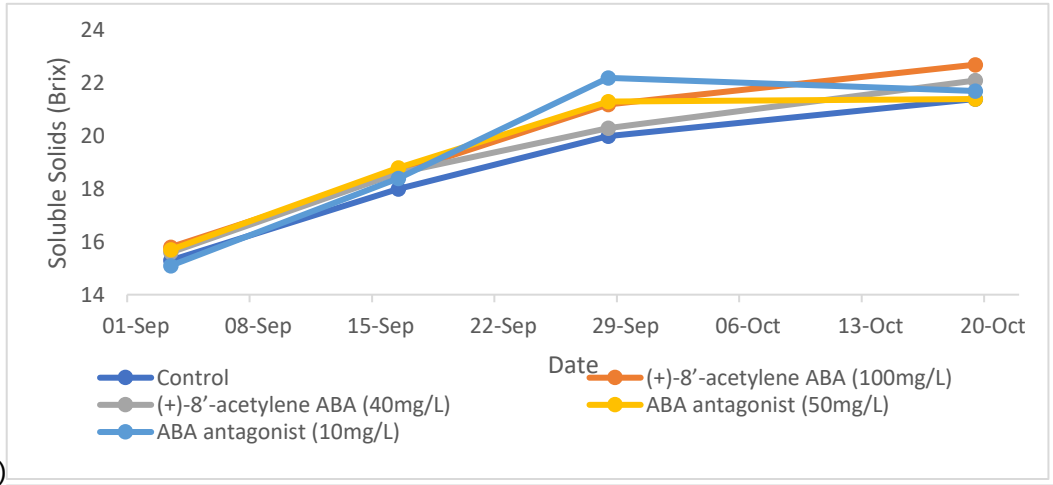
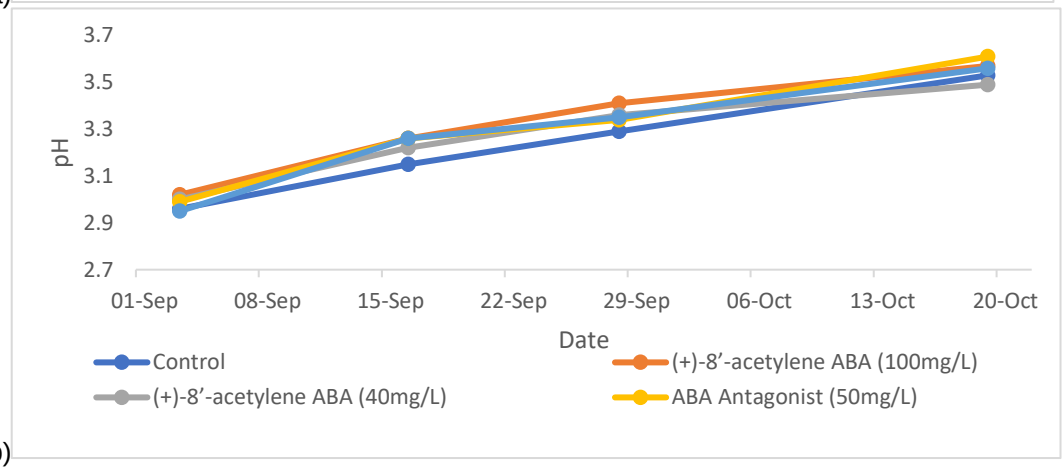


Figure 2: Basic chemical composition of (a) soluble solids; (b) pH; (c) titratable acidity from harvested Cabernet franc ABA fruiting zone treatments. * indicates statistical differences within the same application area against the control treatment using Dunnett's Test ($p<0.05$).

2020



a)



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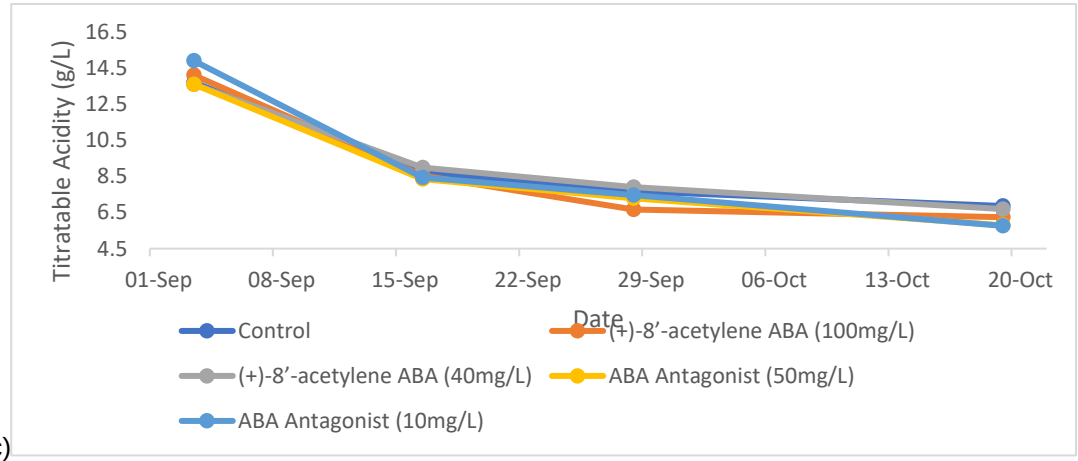
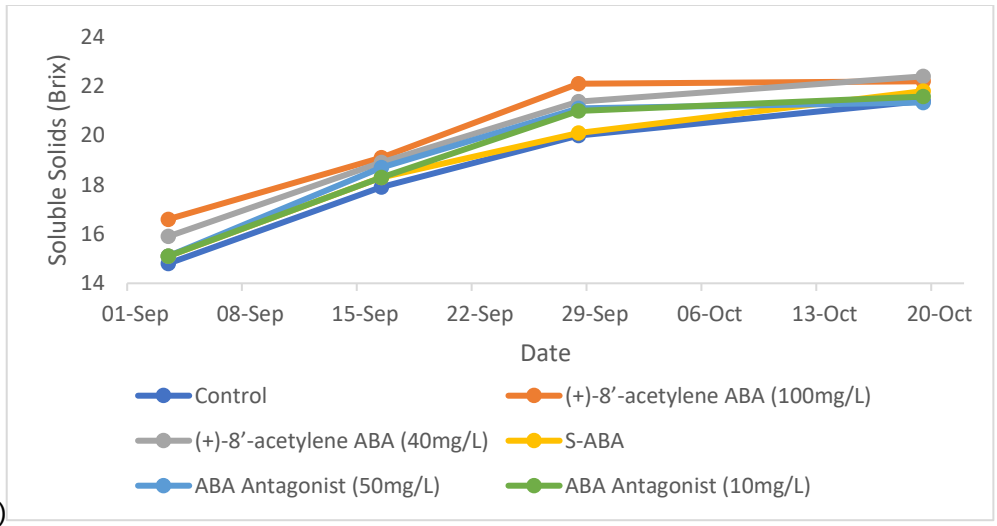


c)

Figure 3: Berry maturation in Cabernet franc canopy applied exogenous ABA treatments in terms of (a) soluble solids, (b) pH and (c) titratable acidity. * indicates statistical differences within the same application area against the control treatment using One way ANOVA ($p<0.05$)



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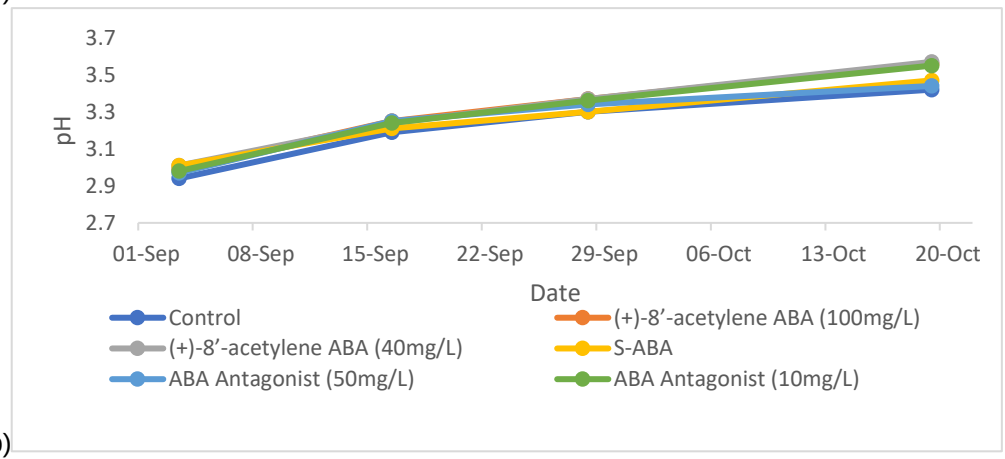

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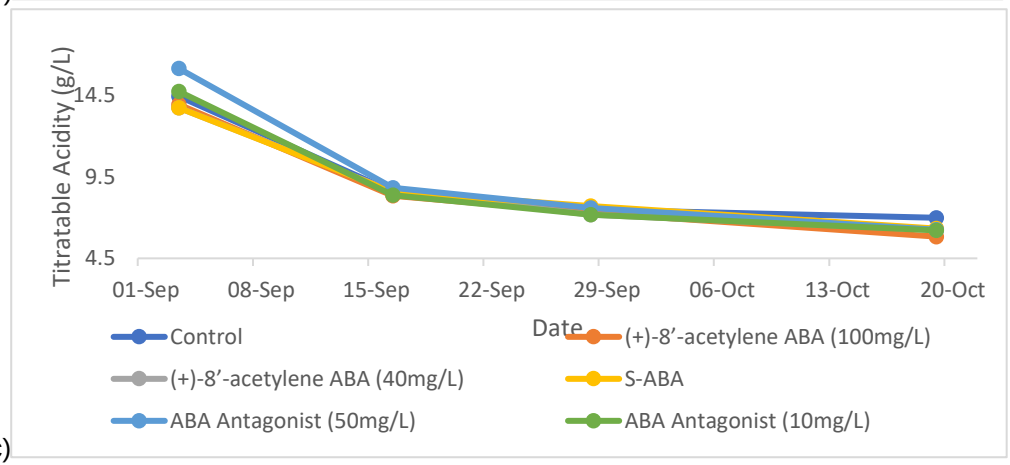


c)

Figure 4: Berry maturation in Cabernet franc fruiting zone applied exogenous ABA treatments in terms of (a) soluble solids, (b) pH and (c) titratable acidity. * indicates statistical differences within the same application area against the control treatment using One way ANOVA ($p<0.05$)

Basic chemical compositions of harvested Cabernet franc are displayed in Figures 3-4. Following analyses, there were some fruit quality improvements with increases in soluble solids with all of the (+)-8'-acetylene ABA applications. At some timepoints during fruit maturity, Brix increases were 1-2° Brix higher in these treatments. The high rate application of the ABA antagonist had the lowest soluble solid content. Titratable acidity did not vary much among

treatments however the high rate of the ABA antagonist applied to the canopy had much higher titratable acidity for the first two weeks post-application. These does support previous work that this particular analog will act as an ABA antagonist and it may delay fruit maturity. Further, fundamental research will need to be done through future research studies to determine how Red Blotch is impacting fruit maturation but based on these data, it does suggest that ABA signaling and related pathways are being impacted by red blotch and that some of these effects may possibly be overcome through ABA analog applications.

Anthocyanin and Total Phenolics

2019. Anthocyanin and total phenolic compositions from fruit application treatments are visualized in Figure 4. Anthocyanins ranged between 0.74-1.01 mg/g berry weight. S-ABA had a significantly higher colour accumulation compared to the control treatment. Total phenolics from entire berries (skins, pulp, seeds) were further assessed. There were no significant differences from S-ABA, (+)-8'-acetylene ABA and ABA antagonist from the control treatment. Total phenolics ranged between 1.66-1.95 absorbance units (au) per berry.

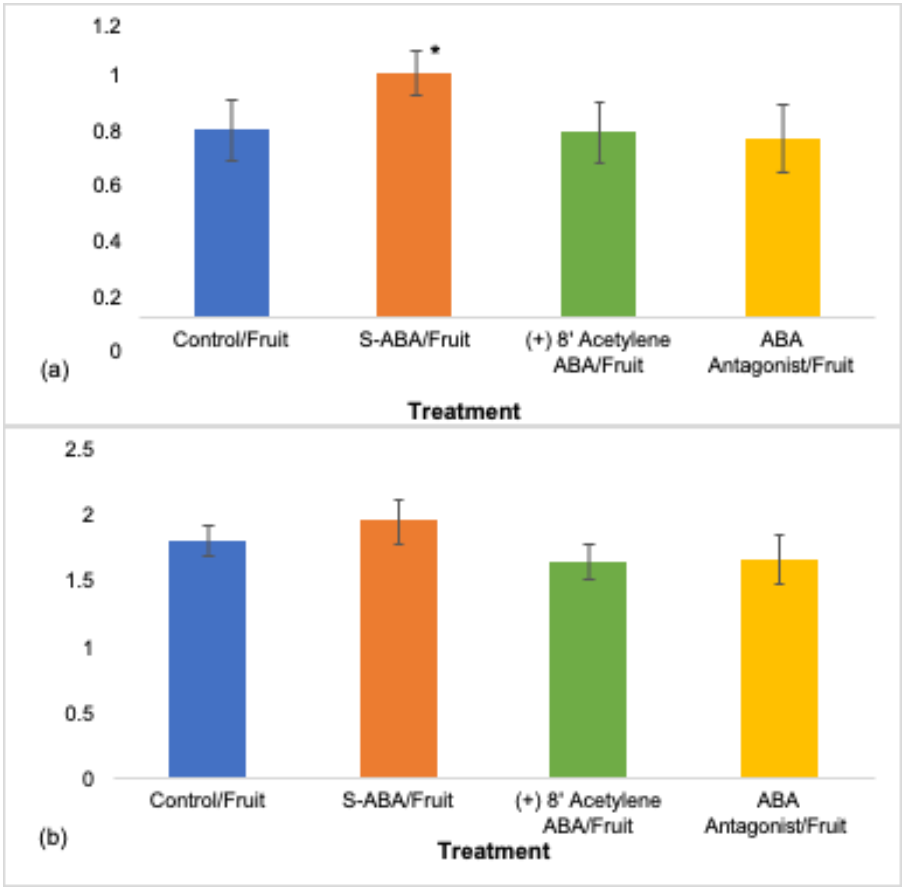
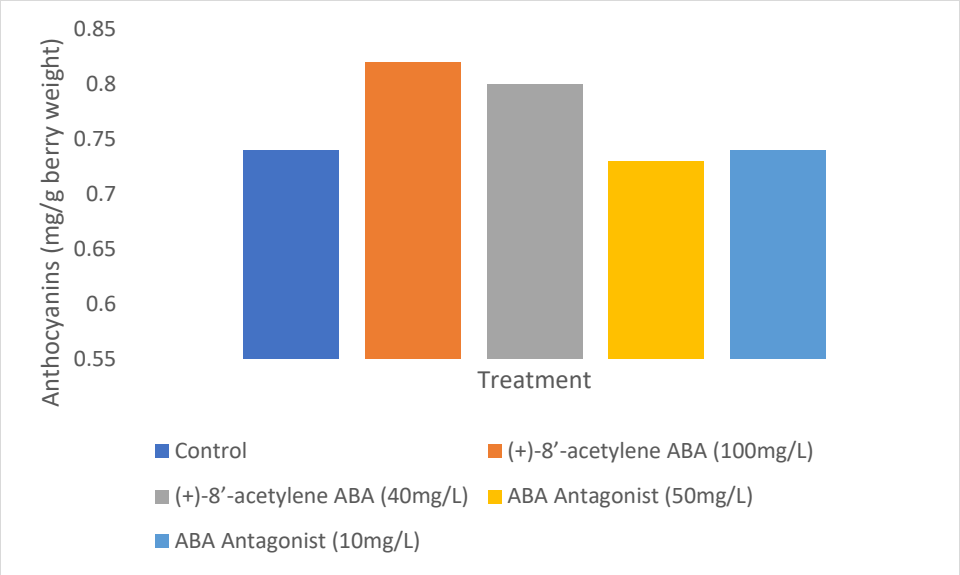


Figure 4: (a) Anthocyanin and (b) total phenolic composition of Cabernet franc treated ABA fruiting zone treatments. * indicates statistical differences within the same application area against the control treatment using Dunnett's Test ($p<0.05$).



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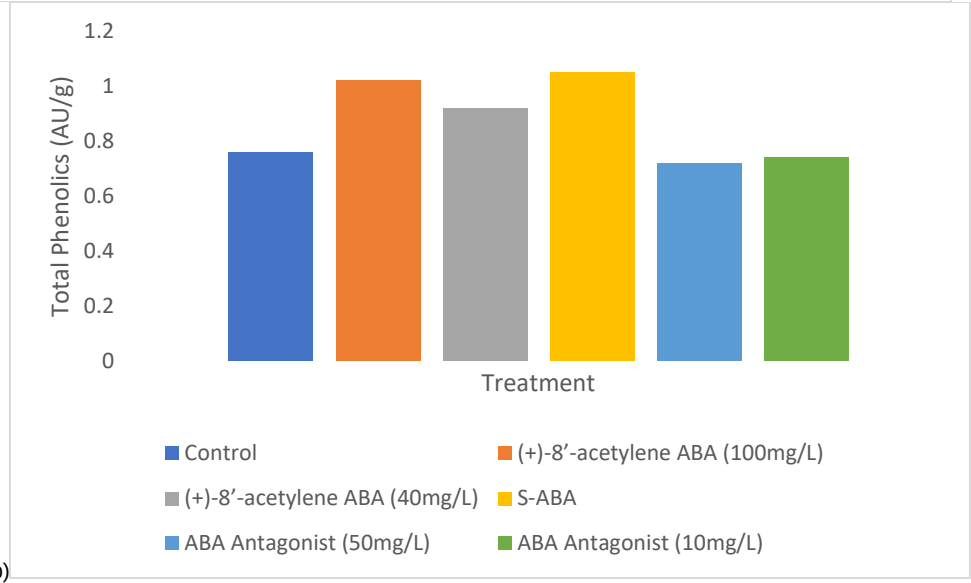
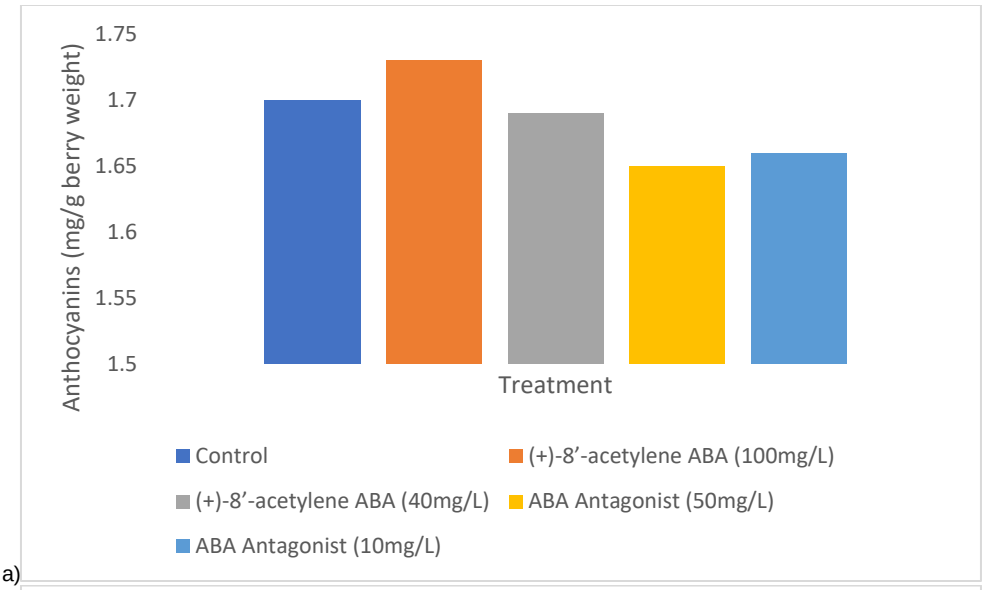
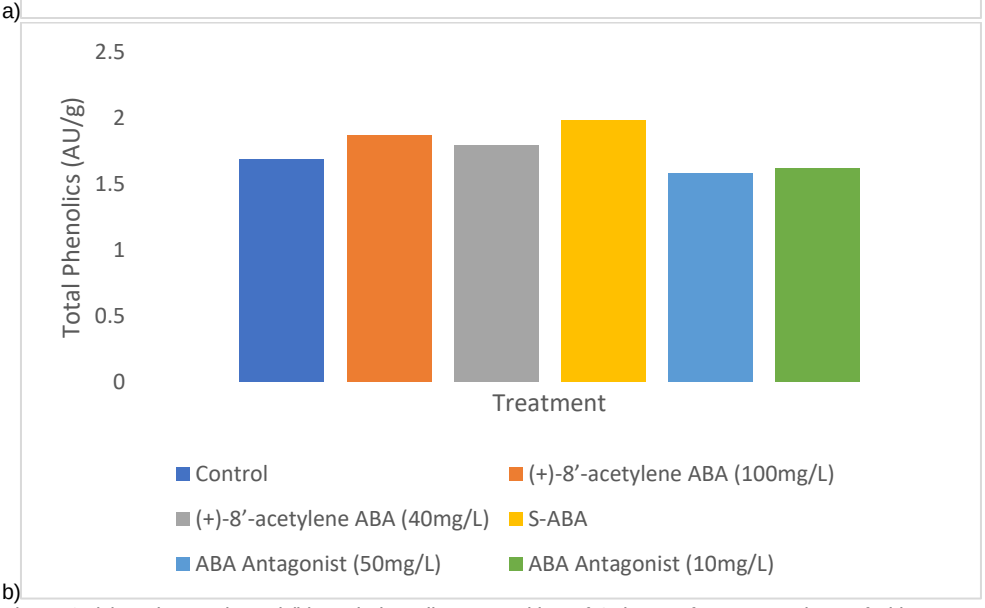


Figure 5: (a) Anthocyanin and (b) total phenolic composition of Cabernet franc treated ABA Canopy treatments. * indicates statistical differences within the same application area against the control treatment using One Way ANOVA $p<0.05$).



a)



b)

Figure 6: (a) Anthocyanin and (b) total phenolic composition of Cabernet franc treated ABA fruiting zone treatments. * indicates statistical differences within the same application area against the control treatment using One Way ANOVA $p<0.05$).

2020.

Anthocyanin and total phenolic compositions from canopy and fruit application treatments are visualized in Figures 5-6. The overall values obtained from the canopy treatments were not significant and did not very far from the control. However, the fruit application anthocyanin values ranged between 0.72-1.05 mg/g berry weight. S-ABA and (+)-8'-acetylene ABA at 100mg/L had a significantly higher colour accumulation compared to the control treatment. Total phenolics from entire berries (skins, pulp, seeds) were further assessed. S-ABA was the only significantly higher total phenolics value at 1.98 AU/g. There were no significant differences from (+)-8'-acetylene ABA and ABA antagonist at either concentration from the control treatment. Total phenolics ranged between 1.54-1.98 absorbance units (au) per berry.

Yield Components

2019. Harvest yields are listed in Table 3. ABA treated infected vines had no significant effect on yield data compared to the controls. Harvest weights were all within a similar range from 2.62 kg to 3.50 kg. Average berry weights were between 1.18 g to 1.35 g in 2019 and 0.80 g to 1.01 g in 2020. Within canopy treatments, ABA antagonist had the greatest yield and (+)-8'-acetylene ABA had the greatest

average berry weight. Within fruiting zone treatments, ABA antagonist had the greatest yield and a significantly greater average berry weight from the control.

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Table 3: Average harvested cluster number, yield per vine weights and berry weights of harvested Cabernet franc ABA treated vines. a) 2019, b) 2020*

a)

Treatment	Application Area	Cluster Number	Yield (kg)	Berry Weight (g)
Control	Canopy	27 ± 7	3.03 ± 0.54	1.39 ± 0.12
(+)-8'-acetylene ABA	Canopy	24 ± 10	2.89 ± 1.25	1.40 ± 0.10
ABA Antagonist	Canopy	25 ± 9	3.50 ± 2.27	1.32 ± 0.12
Control	Fruit	26 ± 6	2.71 ± 0.88	1.18 ± 0.10
S-ABA	Fruit	25 ± 7	2.62 ± 0.92	1.15 ± 0.11
(+)-8'-acetylene ABA	Fruit	24 ± 7	2.70 ± 0.80	1.29 ± 0.10
ABA Antagonist	Fruit	28 ± 7	3.24 ± 1.07	1.35 ± 0.18*****

b)

			October 19th, 2020	
Treatment	Rate	Application Area		
			Number of Clusters	Weight/Berry (g)
Control	0.05%	Canopy	20.4 ± 10	0.95 ± 0.08
(+)-8'-acetylene ABA	100mg/L	Canopy	24.9 ± 9*	0.94 ± .13
(+)-8'-acetylene ABA	40mg/L	Canopy	14.3 ± 5*	1.10 ± 0.11*
ABA antagonist	50mg/L	Canopy	16.0 ± 8	1.02 ± 0.13
ABA antagonist	10mg/L	Canopy	20.9 ± 11	0.95 ± 0.09
Control	0.05%	Fruit	27.4 ± 6	0.78 ± 0.06
(+)-8'-acetylene ABA	100mg/L	Fruit	19.0 ± 8*	1.04 ± 0.15
(+)-8'-acetylene ABA	40mg/L	Fruit	23.1 ± 11	1.01 ± 0.11
S-ABA	400mg/L	Fruit	18.8 ± 7*	0.97 ± 0.10*
ABA Antagonist	50mg/L	Fruit	21.0 ± 10	1.14 ± 0.17*
ABA Antagonist	10mg/L	Fruit	21.4 ± 6	0.95 ± 0.08

*No yield/vine data available in 2020

Application Area

2019-20. Berry composition and yield data in canopy and fruiting zone applications of (+)-8'-acetylene ABA were compared as a way of understanding ABA's optimal uptake in grapevines. Basic berry composition of soluble solids, pH and titratable acidity were analyzed. There was no significant difference between application areas with regard to soluble solids. A significantly higher pH and lower titratable acidity was observed in the fruiting zone applications of (+)-8'-acetylene ABA. Yield results of cluster number, harvest yield and berry weight were analyzed. Cluster number and harvest yield were similar between the two application treatments of (+)-8'-acetylene ABA. The canopy application of (+)-8'-acetylene ABA had a significantly greater single berry weight to the fruiting zone application of (+)-8'-acetylene ABA.

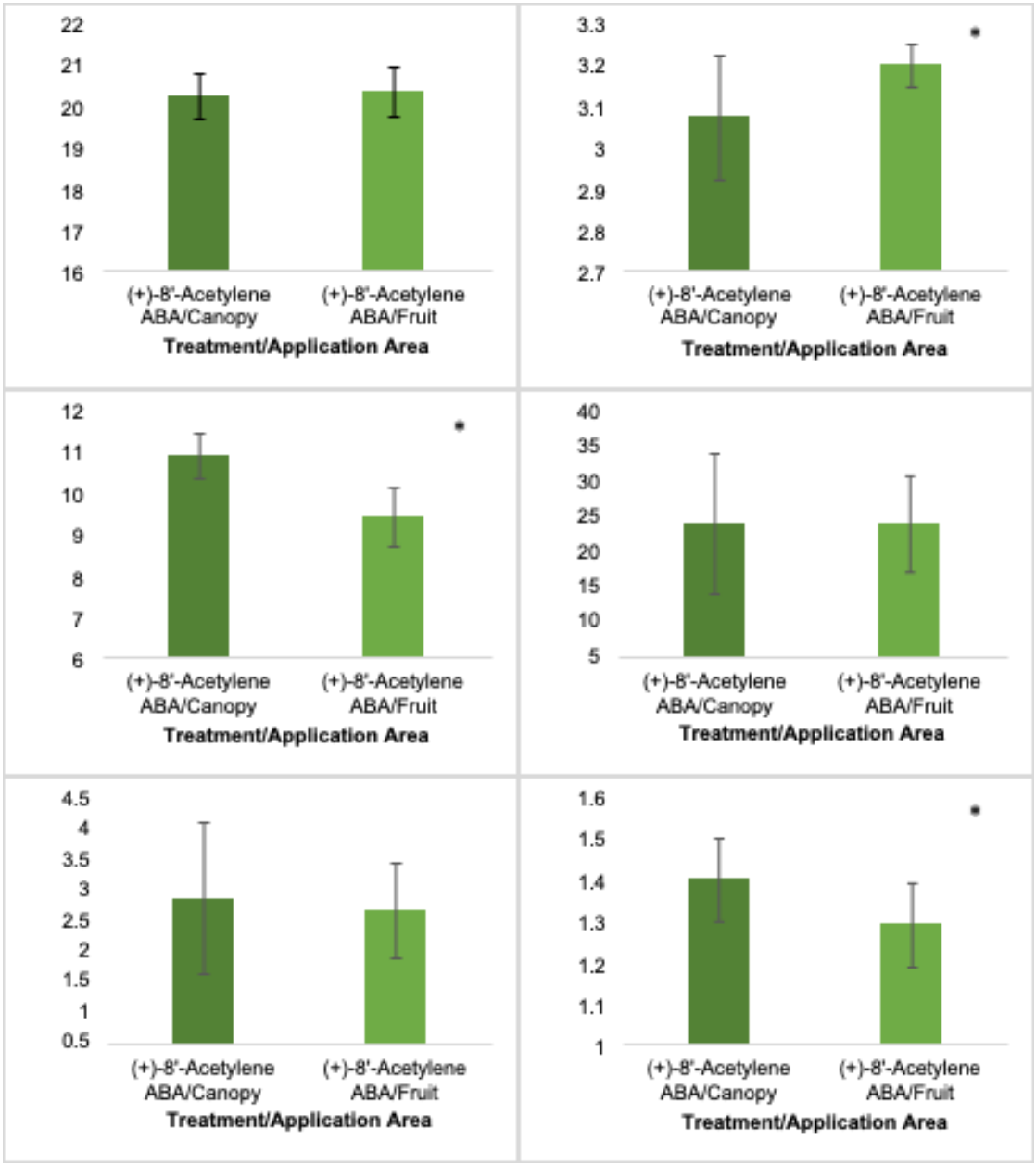


Figure 7: Comparison of application area of exogenous analog ABA treatments in berry composition and harvest yields of Cabernet franc. * indicates statistical difference between application area using Two-Sample T-Test ($p<0.05$).

2020

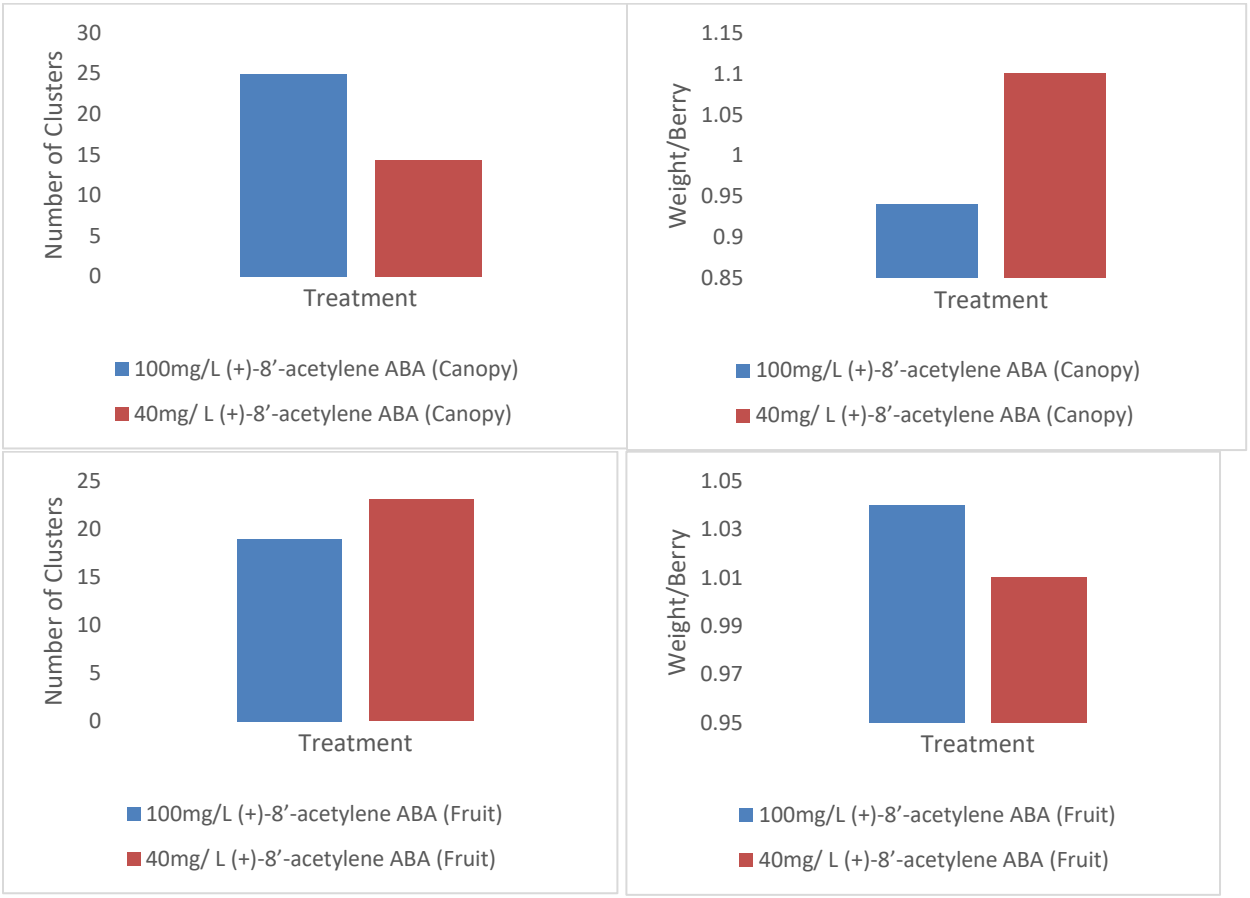
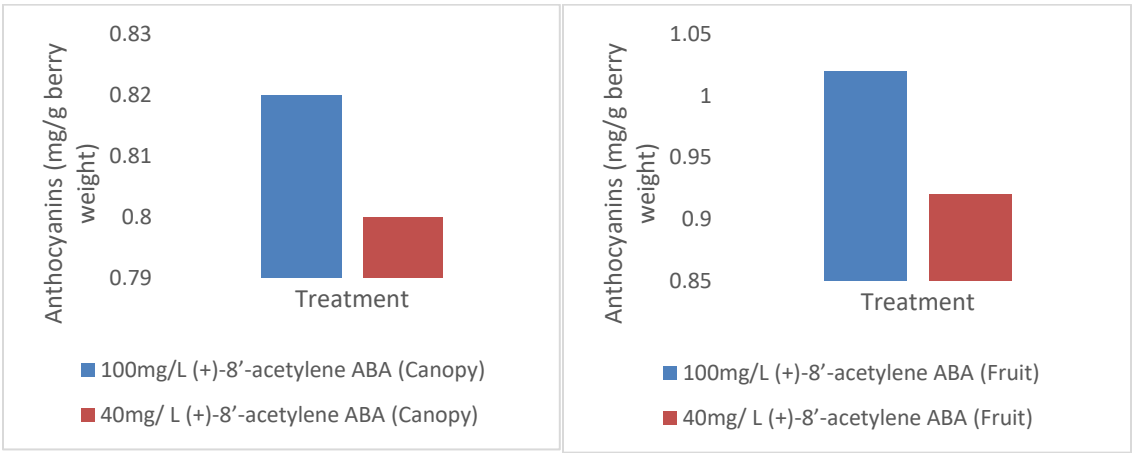


Figure 8. Comparison of rates of exogenous analog ABA treatments on number of clusters per vine and berry weight in canopy and fruit application zones of Cabernet Franc fruit. * Indicates difference between application area using Two sample T-Test ($p<0.05$).



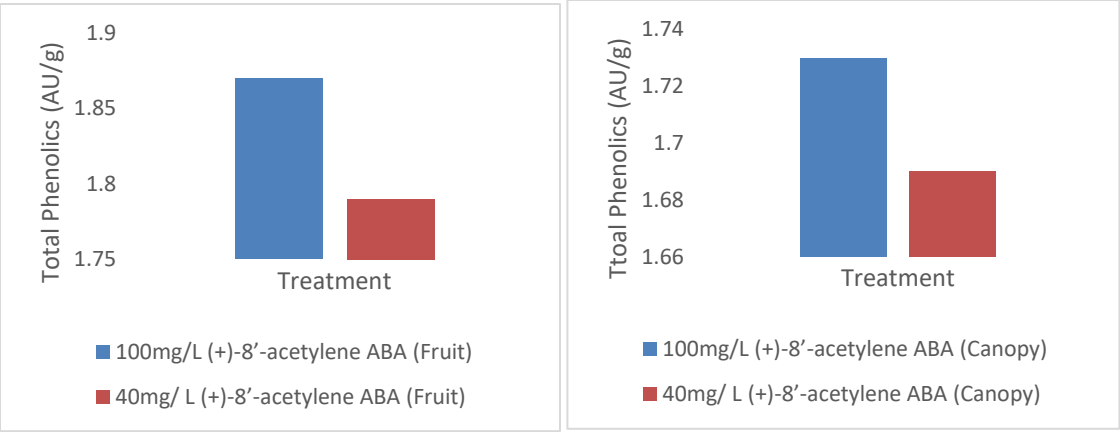


Figure 9. Comparison of rates of exogenous analog ABA treatments on Anthocyanins and Total Phenolics in canopy and fruit application zones of Cabernet Franc fruit. * Indicates difference between application area using Two sample T-Test ($p<0.05$).

2.Cold hardiness experiments

Materials and Methods

Experimental design for field trials

Site selection and description. An established Marquette block was used for this trial in 2019 and 2020. The site was located in Vineland within the Vinemount Ridge sub-appellation (VQA Ontario). The site consisted of clay loam soil. (Kingston and Presant 1989). Each experiment was a randomized block design consisting of four treatments and three replicate blocks. Each block consisted of 1 panel (5-vine) replicates, each representing a specific treatment replicate. Therefore, there were a total of 15 vines per treatment. Buffer panels bordered the plot and buffer panels were assigned between treatment replicate panels.

Treatment applications

Solutions were prepared according to instructions from University of Saskatchewan and Valent BioSciences. All solutions were mixed using deionized water with 0.05% Agral 90 (a surfactant). The treatments were applied once following harvest on October 8 in 2019 and on September 11 in 2020. Whole vines were sprayed with ABA solutions to runoff with a handheld backpack sprayer averaging a spray volume of 0.33 L/vine. Vines were sprayed on both sides of the canopy until leaves were coated thoroughly with material. The treatments were applied during full sun conditions at temperatures between 16.7 and 17°C during the hours of 1100-1200. The formulations and concentrations of the various treatments are described below in Table 1.

Bud cold hardiness determination

Preliminary bud cold hardiness ratings were determined in November and continued throughout the dormant period (3 week intervals) until close to bud break using differential thermal analysis (DTA). Single canes were removed from individual data vines at each sample period (3 canes/treatment replicate; 9 canes total/treatment). The samples were immediately brought to CCOVI for analysis. Additional replicate canes were sampled, and buds and cane sections dissected, frozen under liquid nitrogen and stored at -80°C for future ABA and ABA metabolite analyses. Bud hardiness was determined according to the method described by Mills et al. (2006). The cold hardiness of grapevine buds can be measured by differential thermal analysis, a process that uses a temperature-controlled chamber and energy-sensitive modules to measure bud response to a mimicked cold event. The DTA system consisted of thermoelectric modules (TEM) that detect temperature gradients generated by exotherms and convert the thermal signals to voltage. Nine TEMs were present on a freezer tray with a thermistor located in the centre of the tray. These PVC trays were constructed by Brock University Technical Services out of PVC as described by Mills et al. (2006). Keithley Data Acquisition Systems (DAS) (Model 2700) were used to measure and collect voltage output. The DAS scanned channels of the

TEMs and thermistors every 20 seconds during the course of the freezer run. Two programmable freezer chambers (Tenny T2C) with a capacity of 6 trays each were used for DTA.

Dormant buds from nodes three through were used from single canes of individual data vines at each sampling period. From each cane sampled, these five buds were removed using pruning shears and placed onto one of the nine Peltier plates in the wells on a freezer tray. Once all of the buds were placed in the wells and the trays loaded into the freezer units controlled cooling process began to test the buds' cold tolerance. Customized software (Bud Freezer) created by Brock University Technical Services controlled the temperature run of the freezer. The temperature was decreased steadily (4°C/hr.) over nine hours to -35°C. All data was collected and stored in a database and subsequently processed and analyzed using custom software (Bud Processor and BudLTE) developed by Brock Technical Services. The low temperature exotherm (LTE) and was used to estimate lethal temperatures of the buds. The temperature corresponding to each peak was recorded and compiled. Following data processing and analysis, LTE10, 50 and 90 were calculated. LTE10 was the predicted temperature at which 10% of the buds were killed; the LTE50, the median temperature, and the temperature at which 50% of the buds were predicted to be killed; and the LTE90, the temperature at which 90% of the buds were predicted to killed.

Results

Cold hardiness

2019/2020. Cold hardiness and tissue sampling were performed during the entire dormant period for Marquette but not Merlot as no treatments were applied. As shown in Figure 10, ABA and ABA analogs improved cold tolerance of Marquette grapevines. Natural ABA had some slight improvements but to a lesser extent, especially as deacclimation progressed. Both ABA analog treatments improved and maintained hardiness throughout dormancy and there was not much a concentration effect. Significant improvements of over 5 °C were observed during deacclimation (Figure 10) from February onwards. In general, bud acclimation along the cane was improved for all treatments and varieties for both ABA and analogs but the analog treatments were found to have an overall greater effect compared to natural ABA based on LTE50 calculations. Higher concentrations of ABA analog demonstrated the greatest effects on acclimation, particularly at the rate of 0.5 g/L where was improved by 1.5 °C (Figure 10). Bud break was significantly delayed with the higher concentration of the ABA analog (ABA 1017) by 5 days. No differences in bud break were observed for the other treatments. Some delay in phenology was observed throughout the growing season with the highest rate of ABA 1017. Fruit composition at harvest will provide further insight to determine if these delays impacted fruit maturity significantly.

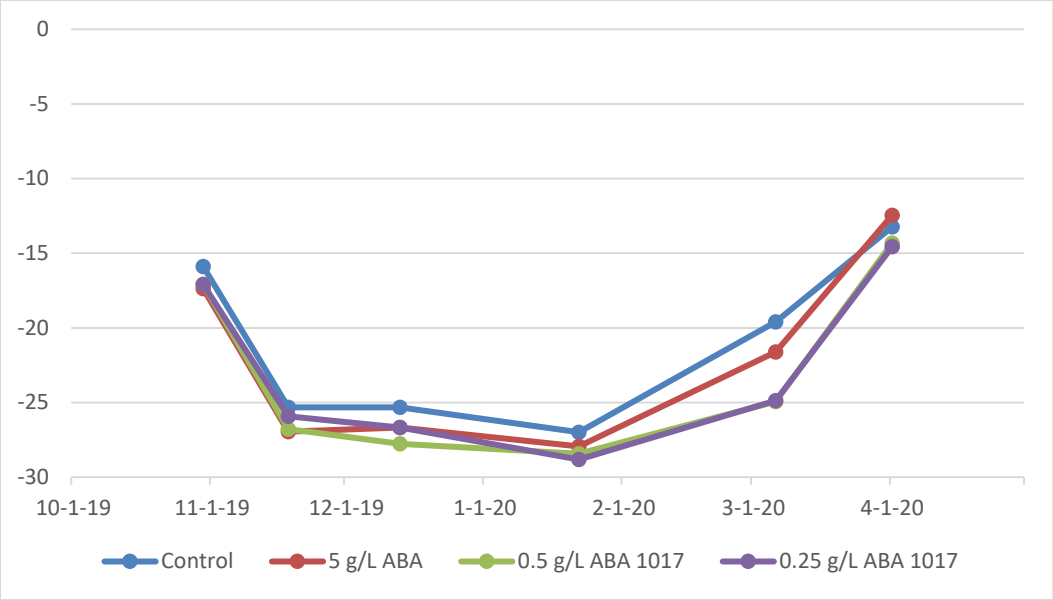


Figure 10. Cold hardiness dynamics (LTE50) of Marquette grapevines based on exogenous ABA treatments. Vinemount Ridge, 2019-20**.**

2020/2021. Cold hardiness and tissue sampling were performed during the entire dormant period for Marquette and Merlot and data to date are shown in Figures 11-12. As demonstrated in previous years and previous studies, ABA analogs improved cold tolerance at certain stages of dormancy particularly later by improving resistant to deacclimation. The 2020/21 data was a bit of anomly compared to previous years' of research. The analogs were not as effective in maintaining or improving hardiness including delaying budbreak, particularly with Marquette. This is difficult to explain and may have to do with climate conditions such as warmer temperatures and/or drier soils during the deacclimation phase. 2020 and 2021 environmental conditions have been shown to be impacted by the COVID-19 pandemic and respective changes in human activity. It could be possible that this massive change could have impacted plants as well, but this is very difficult to know for sure.

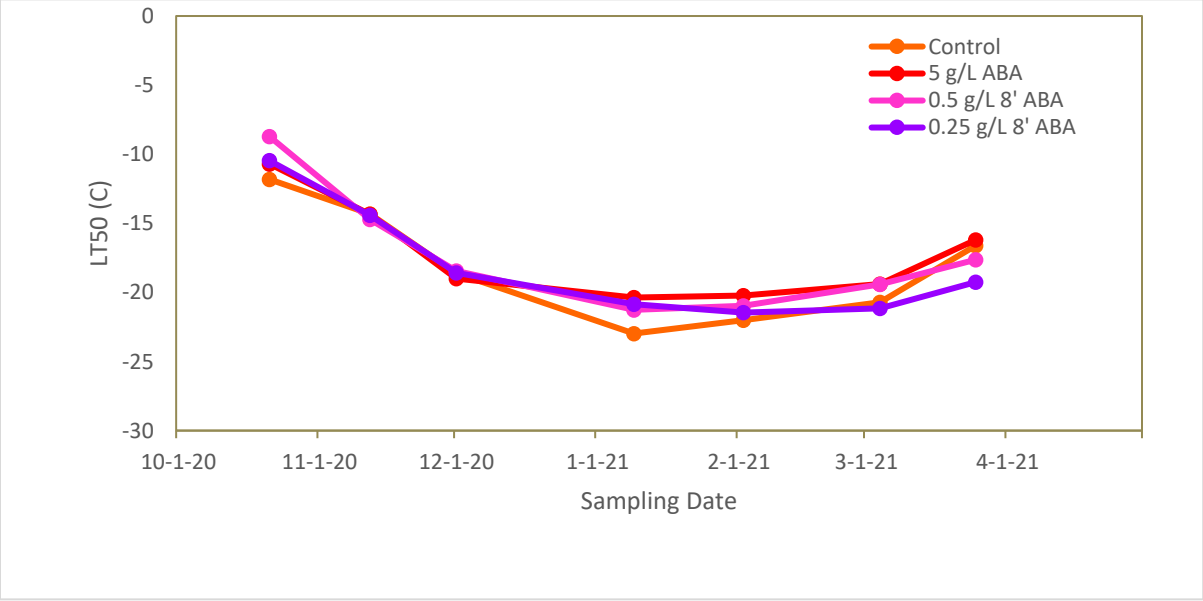


Figure 11. Cold hardiness dynamics (LTE50) of Merlot grapevines based on exogenous ABA treatments. Creek Shores, 2020-21.

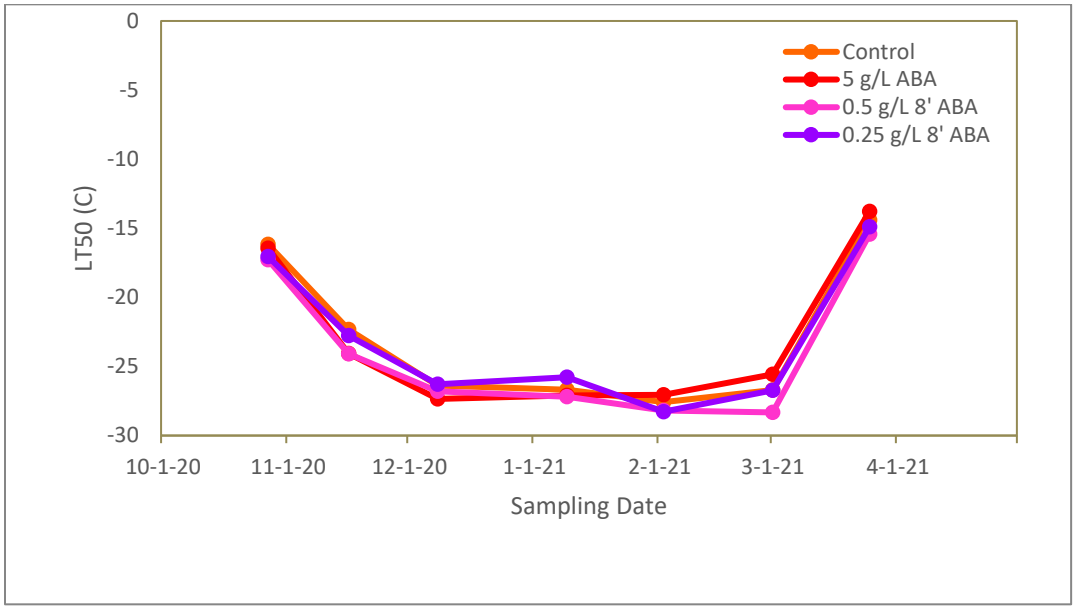


Figure 12. Cold hardiness dynamics (LTE50) of Marquette grapevines based on exogenous ABA treatments. Vinemount Ridge. 2020-21

Results to Date

Short-term

The results to date have been satisfactory and have been expected for the most part. Some improvements and adjustments to the proof of concept testing of ABA analogs to mitigate Red Blotch virus will be made as described below in "Next Steps" but these relate to our objectives with respect to optimizing ABA applications. However, through increasing the rate of application of the 8'-acetylene ABA analog we did observe greater increases in soluble solids for those treatments. Therefore, we do see a dose response that should be greater elucidated.

Public good/benefit of the project. ABA or synthetic ABA analog application may be a novel and practical way to improve cold hardiness in grapevines without negatively impacting fruit composition or quality. This project suggests that abscisic acid analog application may prove to be very beneficial in optimizing cold hardiness in grapevines especially in Ontario's climate. There is potential for ABA analogs to maintain dormancy and improve hardiness particularly in years where winter months may have abnormally warm temperatures. This is important for grapevines to not lose hardiness prematurely or in varieties that lose cold tolerance early (i.e. *V. riparia*-based hybrids). Therefore, ABA analog application may delay deacclimation which could result in less freeze damage associated with sporadic warming and freezing events during dormancy.

Pre-commercialization. ABA analogs do present a potential viable commercialization opportunity and ABAzyne have been activity pursuing ways of getting their Patented technology to the market. These compounds are potent in nature and thus far have outperformed other ABA plant growth regulators currently on the market. Further studies are ongoing and discussions are being had with respect to trials in other regions as well as other tender fruit such as apricots. ABAzyne and OGWRI have had some preliminary discussions concerning business relationships and opportunities for a joint venture or other type of agreement. Letters of Intent have been signed by PI Willwerth, ABAzyne and OGWRI to try to work together to examine research opportunities. This is the first proof of concept study that has examined the effects of ABA analogs on red blotch virus infected vines. Preliminary results indicate that

there may be potential for ABA analogs to be utilized to mitigate the negative effects on sugar levels in infected fruit.

5. Reach and Communications

The primary target of this project is to benefit the grape growers in Ontario, but aspects of this work will also benefit growers across the country and internationally. Our reach was not as significant at this point in time due to COVID restricitons and lack of opportunities to present our findings. However, we have presented the potential benefits of ABA analogs and some preliminary research findings to OGWRI Board members, technical committee members and the Grape Growers of Ontario's grower committee members. Potential commercialization opportunities are being explored with ABAzyne and OGWRI. Researchers from other regions in North America have been briefed on some of this research and there has been some collaborative work in 2019 with Dr. Tony Wolf in Virginia, USA as well as Dr. Michela Centinari in Pennsylvania, USA to further understand the impact of ABA analogs on grapevine cold hardiness physiology and if different climates and varieties may impact the efficacy of these plant growth regulators. We will continue to engage stakeholders and partners as the project continues and present findings to the greater grower community locally, nationally and internationally. OGWRI have been acknowledged at least 4 times formally during the course of this project during presentation of any aspects of this project. Total grower reach has been >100 based on attendance or online views of the various presentations. Overall, this project has gained a lot of traction and interest from growers nationally as well as other researchers both at Brock University and internationally. A current project in collaboration with Dr. Charles Despres at Brock University is examining the impacts of these ABA analogs and red blotch infections on cold hardiness related genes (funded through OGWRI). Hopefully, these research projects will lead to more opportunities for research collaborations and commercialization opportunities for ABAzyne and/or OGWRI through further business development strategies.

Conclusions and Next Steps

This project suggests that abscisic acid analog application may prove to be very beneficial in optimizing cold hardiness in grapevines especially in Ontario's climate. This is the first time that this has been demonstrated using a Riparia-based hybrid grapevine and one that is prone to cold deacclimation. In 2019, ABA analogs reduced the rate of deacclimation in Marquette grapevines which may reduce late dormant season freeze injury. Both natural ABA and ABA analogs improved fruit composition in Red Blotch virus-infected grapevines. However, some results were contradictory and application area had some effects. In general, applications to the fruit had more improvements to fruit maturity compared to canopy applications. There was a dose response observed and in 2020 higher rates of ABA analogs had higher soluble solids and likely higher fruit quality than control vines. Natural ABA had little impacts in 2020.

This is the first proof of study project performed to investigate the effects of exogenous ABA analog applications on mitigating red blotch. One "lesson learned" was increasing the rate of application for the analog. We also were able to see if ABA antagonists would impact fruit maturity. Interestingly, but not surprising the ABA antagonist did not have many impacts of fruit maturation. This demonstrates that other plant hormones (i.e. brassinosteriods) are involved in fruit maturation. This provides more insight that other plant growth regulators (or a cocktail of different ones) may be benefical in improving fruit quality in red blotch infected vines. More research is required to examine more dose responses and some of the mechanisms by which the ABA analogs mitigate these effects as well as understanding how red blotch influences cold hardiness. Our hope is to gain further understanding through current studies funded through MVIP and future studies.