OGWRI Final Report. October 31, 2019

Project number 001700

Project Title: Improving Cold Hardiness and Delaying Deacclimation using Long Lasting Abscisic Acid Analogs

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Executive Summary

Freeze tolerance in grapevines is the most limiting factor for grape production in Ontario and Canada. Cold or freeze injury is the greatest threat to the success and sustainability of the grape and wine sector and the threat will likely worsen as more erratic weather becomes more common with climate change. New innovative approaches can help reduce cold injury and allow growers to continue to enjoy the successes of producing world class *V.vinifera* grapes in Ontario. 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, 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.
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. A plant growth regulator like ABA may prove very beneficial in optimizing cold hardiness in grapevines especially in Ontario's climate where cool and wet fall periods can delay cold hardiness. Furthermore, ABA application may also delay deacclimation which could result in less freeze damage associated with sporadic warming and freezing events during dormancy.

Detailed Description of the Project

a) Objectives and Project input

Objectives

The proposed research will focus on the role of ABA in dormancy and cold hardiness. The project will include examining natural ABA concentrations in grapevine tissue as well as synthesizing and field testing ABA and ABA analogs to accelerate and prolong dormancy and improve cold hardiness acclimation in grapevines and optimize hardiness throughout dormancy.

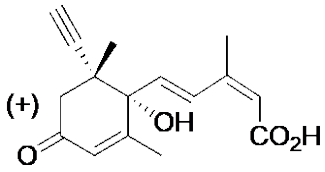
The specific objectives of the project are to:
1. Synthesis of ABA analogs for the specific goal of accelerating and prolonging dormancy and improving cold hardiness in grapevines
2. Evaluate product formulations, concentrations, and timing of application on grapevine cold hardiness and dormancy
3. Examine the role of ABA in dormancy and cold hardiness.

This project involved the PIs, Dr. Jim Willwerth at CCOVI and Dr. Suzanne Abrams at ABAzyne/UofS. Dr. Willwerth coordinated the project along with research technician, Stephanie Bilek and OEVI undergraduate honour's student, Alex Gunn. Dr. Abrams coordinated synthesis of the ABA analogs at U of S and Dr. Randy Purves was contracted to perform hormone profiling of grapevine tissue samples. In-kind contributions were provided by Bill Schenck at Schenck Farms and Greenhouses for vineyard management and fruit donations from the experimental Merlot block. Cash financial support was provided by OGWRI for the project.

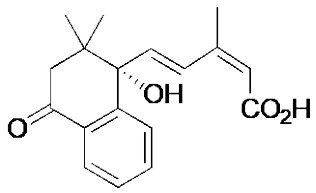
b) Project Activities and Outputs

ABA analog synthesis

Two ABA analogs were synthesized for field trials in 2017 and 2018. In two previous studies, Woolard et al, 2012 and Bowen et al 2016, the racemic form of 8'-acetylene ABA applied at 1.0 g/L was found to be effective in extending dormancy and promoting freezing tolerance in grape vines. The analog 8'-acetylene ABA had previously been shown to have beneficial effects in improving cold hardiness of wine grape vines (Bowen et al 2016). The compound used in the earlier work was a 1:1 mixture of the active (+)-form and the inactive (-)-form. For the present project, we synthesized the (+)-form exclusively, using a method which we have filed a US provisional patent. This method reduces the amount of compound needed by a factor of two. The total amount produced, in several batches, was 8.0 g, coded ABA-1017. The purity of the product was greater than 95%.



In addition, a second biologically active analog, tetralone ABA, was prepared for this year's field trials. This analog had not previously been tested in grape cold hardiness studies. Sixteen grams of this compound, in racemic form coded ABA-1016, was prepared as reported in Nyangulu et al., 2006 in several batches, in purity greater than 95%.



Natural ABA was provided for this study by Valent BioSciences. It had been tested at ten times higher rate than the 8'-acetylene ABA analog (Bowen et al 2016). Sixty grams of ABA was sent to Brock University along with the analogs for the field trials.

Experimental design for field trials

Site selection and description. Established Merlot and Sauvignon blanc blocks were used for this trial in 2017 and Merlot only in 2018. The site was located in West St. Catharines within the Creek Shores sub-appellation (VQA Ontario). The site consisted of sandy loam soil. (Kingston and Presant 1989). Each experiment was a randomized block design consisting of eight 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% Latron B-1956 (a surfactant). The treatments were applied once following harvest on October 26, 2017 and October 22, 2018. 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 formulations and concentrations of the various treatments are described below in Table 1a and 1b.

Table 1a. Description of treatments used for Abscisic Acid (ABA) trials in 2017

Treatment	Treatment
1	Control – surfactant (0.05% Latron B-1956)
2	5 g/L ABA + surfactant
3	1.0g/L ABA-1016 + surfactant
4	0.5 g/L ABA-1016 +surfactant
5	0.1 g/L ABA-1016 + surfactant
6	0.5 g/L ABA-1017 + surfactant
7	0.25 g/L ABA-1017 + surfactant
8	0.05 g/L ABA-1017 + surfactant

Table 1b. Description of treatments used for Abscisic Acid (ABA) trials in 2018

Treatment	Treatment
1	Control – surfactant (0.05% Latron B-1956)
2	5 g/L ABA + surfactant
3	1.0g/L ABA-1016 + surfactant

Bud cold hardiness determination. Preliminary bud cold hardiness ratings were determined on 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.

Vine Performance. Vine phenology was monitored and dates of key stages (bud break, flowering, veraison, harvest, dormancy) were documented throughout the growing season. Vine vigour will be assessed through length of shoot growth and "vine size" through dormant cane pruning weights. Yield components will be determined by cluster weights and berry weights. Digital photography was also used to document vine development.

Fruit composition. All treatments were sampled at harvest and basic fruit composition for Brix, pH, titratable acidity were determined using standard protocols at CCOVI.

Analysis of grape samples for abscisic acid (ABA) and gibberellin (GA) content.
Sample Preparation. The sample preparation and liquid chromatography-multiple reaction monitoring (LC-MRM) analyses were carried out at the University of Saskatchewan. Powdered sample (~50 mg) was accurately weighed into a 2-mL microtube. A volume of 1 mL of an extraction solvent consisting of 80:19:1 methanol: water: formic acid containing deuterated internal standards (namely d_6-ABA, d_3–PA, d_3–DPA, d_4–7-OH-ABA, d_2-GA_7 and d_5-ABA GE) was added to each tube to extract the plant hormones. After vortexing the tube for 5-10 s (Vortex Maxi Mix II, Thermo Scientific), the samples were placed onto a Thermo mixer C (Eppendorf AG, Germany) at room temperature for 30 min at 1400 rpm. Samples were then centrifuged for 5 min at 12,000 rpm on a Thermo Legend Micro 17 (ThermoFisher Scientific, Germany). An 800 µL aliquot of the aqueous supernatant was transferred into a new 2-mL microtube. A second extraction from the remaining pellet was carried out by adding 500 µL of the extraction solvent (80:19:1 methanol: water: formic acid) with no internal standard. After vortexing, mixing and centrifuging as described above, 500 µL from the second extraction was combined with the 800 µL from the first extraction to give 1300 µL of extract. This combined

extract was vortexed and then centrifuged for 5 min at 12,000 rpm. From this combined extract 390 µL were transferred to a new micro tube and evaporated to dryness using a speed vac (Labconco Corp., Kansas City, MO). The dried sample was then reconstituted in 130 µL of 79:20:1 water: methanol: formic acid, vortexed vigorously for 15 s and then mixed on the Thermo mixer at room temperature for 30 min at 1400 rpm. A volume of 110 µL was transferred to an HPLC vial containing a 150 µL insert.

LC-MRM analysis. The instrumentation consisted of a Thermo Vanquish Flex UPLC coupled to TSQ Altis triple quadrupole mass spectrometer (Thermo Scientific, San Jose, CA). A volume of 5 µL was injected from the vial and analytes were separated using an Agilent Zorbax Eclipse Plus C18 column (2.1 x 50 mm, 1.8 µm) and 5 mm guard column prior to analysis using multiple reaction monitoring (MRM) in the negative ion mode. The transitions, collision energies and RF lens for the compounds are shown in Table 2, whereas the binary solvent gradient is shown in Table 3.

Table 2. Transitions, collision energies and RF lens for MRM analysis (5 µL injection)

Compound	Retention Time (min)	Transition	CE (V)	RF Lens (V)
ABA	7.3	263 → 153	10.2	48
t-ABA	6.6	263 → 219	10.2	48
PA	4.7	279 → 139	12.1	54
DPA	2.3	281 → 237	12.9	73
7'-OH-ABA	5.8	279 → 217	10.2	47
ABA-GE	4.5	425 → 263	10.2	77
neo-PA	6.4	279 → 205	11.9	56
GA_1	4.2	347 → 259	18.5	96
GA_3	4.1	345 → 239	14.1	72
GA_4	10.4	331 → 257	22.6	85
GA_7	10.3	329 → 223	17.7	70
1016	9.9	299 → 189	10.2	54
d_6-ABA	7.3	269 → 159	10.2	48
d_3-PA	4.7	282 → 142	10.2	53
d_5-ABA-GE	4.5	430 → 268	10.2	65
d_4-7'-OH-ABA	5.8	283 → 221	10.2	48
d_3-DPA	2.3	284 → 240	13.0	69
d_2-GA7	10.3	331 → 225	18.3	71

Table 3. Binary gradient operated at 400 µL/min for UPLC-MRM analysis*

Time (min)	%A	%B
0	90	10
5	80	20
7	70	30
11	58	42
12	10	90
14	10	90
14.1	90	10
17	90	10

*** A=1% formic acid in water, B=1% formic acid in 90:10 acetonitrile:water.**

Area ratios for each analyte were determined from the peak area of the analyte divided by the peak area of the labelled standard. An eight-point calibration curve was prepared by series dilution of standard solutions for quantification. The concentrations were determined from the area ratios of the samples compared with the calibration curve and based on the weight, the values were reported as µg/g dry weight. For each sample three replicates were analyzed and the average values are reported.

Note: for t-ABA, quantification was based on the calibration curve of its isomer, s-ABA.

Statistical analysis. Data sets was subjected to analysis of variance (ANOVA) and/or multivariate analyses to analyze and interpret the effects of treatments.

4. Project Outcomes

Treatments were applied to grapevines on October 26, 2017 for both Sauvignon blanc and Merlot and repeated the following year on October 22, 2018 for Merlot. This corresponded to a few days following harvest for Merlot and roughly 2 weeks following harvest for Sauvignon blanc in 2017 and 1 day after harvest in Merlot in 2018. Canopies were full and leaves still healthy at the time of application and ambient temperature was 13°C for both 2017 and 2018. Canopies were monitoring following spray applications to observe any leaf senescence as a result of the ABA applications. No leaf senescence was noted, except for the natural ABA applications (5g/L ABA) where some leaf reddening occurred along with some senescence.

Cold hardiness. *2017/18.* Cold hardiness and tissue sampling were performed during the entire dormant period for Merlot but hardiness testing was completed in February for Sauvignon blanc as it was pruned prematurely by the grower. With respect to cold tolerance, indicate that ABA analog applications improved acclimation rates based on the first samplings for cold hardiness determinations following applications of ABA treatments. (see Table 4). ABA-1016 improved hardiness substantially in Merlot, with LTE50 gains of > 2°C for some application rates compared to the control. 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 LTE10 calculations. Higher concentrations of ABA analog demonstrated the greatest effects on acclimation, particularly at the rate of 1.0 g/L for ABA-2016 where LTE10 was improved by 3.9°C (Table 4, Figure 1). Some improvements were found in Sauvignon blanc but to a much lesser extent (Table 4, Figure 2). Post harvest application timing may have impacted the efficacy of the ABA applications as the period of time between harvest and treatment applications were longer in the Sauvignon blanc than Merlot. This trend was evident during all stages of dormancy for Sauvignon blanc.

Table 4. Cold hardiness of Merlot and Sauvignon blanc grapevines during acclimation based on exogenous ABA treatments. Creek Shores, November, 2017.

Variety	Treatment	Date	LTE10	LTE50	LTE90
Merlot	Control	09-Nov-17	-8.39	-11.38	-14.16
Merlot	5 g/L ABA	09-Nov-17	-9.95	-12.52	-14.61
Merlot	1.0g/L ABA 1016	09-Nov-17	-12.25	-13.51	-15.19
Merlot	0.5 g/L ABA 1016	09-Nov-17	-10.01	-12.44	-14.16
Merlot	0.1 g/L ABA 1016	09-Nov-17	-11.74	-13.41	-15.41
Merlot	0.5 g/L ABA 1017	09-Nov-17	-10.82	-12.46	-13.87
Merlot	0.25 g/L ABA 1017	09-Nov-17	-11.06	-12.71	-14.52
Merlot	0.05 g/L ABA 1017	09-Nov-17	-11.29	-12.63	-14.23
Variety	Treatment	Date	LTE10	LTE50	LTE90
Sauvignon blanc	Control	13-Nov-17	-11.82	-15.49	-17.26
Sauvignon blanc	5 g/L ABA	13-Nov-17	-15.51	-16.48	-17.48
Sauvignon blanc	1.0g/L ABA 1016	13-Nov-17	-15.39	-16.32	-17.36
Sauvignon blanc	0.5 g/L ABA 1016	13-Nov-17	-15.24	-16.16	-17
Sauvignon blanc	0.1 g/L ABA 1016	13-Nov-17	-15.17	-16.13	-17.45
Sauvignon blanc	0.5 g/L ABA 1017	13-Nov-17	-14.24	-16.17	-17.69
Sauvignon blanc	0.25 g/L ABA 1017	13-Nov-17	-13.91	-15.88	-18.65
Sauvignon blanc	0.05 g/L ABA 1017	13-Nov-17	-13.08	-15.85	-17.52

Previous work in the Willwerth lab as published in Bowen et al. (2016) have indicated that maximum hardiness is minimally improved with exogenous ABA applications and data from 2017/18 demonstrate similar results. As shown in Table 5 and Figures 1 and 2, little hardiness gains were found with any of the ABA treatments, both natural and analog forms. However, during the deacclimation period both ABA analog treatments had significant impacts (refer to Table 6 and Figure 2). ABA analogs improved (or maintained hardiness) from 4 to 5.4°C for LTE10 values and from 1 to 3.1°C for LTE50 values on April 9. On the April 26th sampling date, similar trends were found but the effects were greater as ABA analogs maintained dormancy by up to 4.1°C based on LTE10 values and as high as 7.3°C for LTE50 values. Natural ABA had little to no effect on maintaining dormancy during deacclimation which corresponded to previous studies (Bowen et al. 2016). The effects of the ABA analogs on cold deacclimation are the most profound results thus far in this research. Through a literature review of research there is no record of any compounds having such an effect on maintain dormancy to this degree in woody plants. There are many potential benefits of delaying deacclimation particularly in warmer winters or dormant periods with large temperature fluctuations, particularly during the period of ecodormancy. Climate change will likely exacerbate the risks of premature deacclimation and therefore, this area of research needs further elucidation beyond the scope of this study.

Table 5. Mid-winter hardiness of Merlot and Sauvignon blanc grapevines based on exogenous ABA treatments. Creek Shores, February, 2018.

Variety	Treatment	Date	LTE10	LTE50	LTE90
Merlot	Control	07-Feb-18	-19.25	-22.05	-23.54
Merlot	5 g/L ABA	07-Feb-18	-20.39	-22.4	-24.84
Merlot	1.0g/L ABA 1016	07-Feb-18	-19.46	-22.21	-24.78
Merlot	0.5 g/L ABA 1016	07-Feb-18	-21.01	-22.67	-25.7
Merlot	0.1 g/L ABA 1016	07-Feb-18	-19.96	-22.04	-24.66
Merlot	0.5 g/L ABA 1017	07-Feb-18	-18.81	-21.98	-24.07
Merlot	0.25 g/L ABA 1017	07-Feb-18	-19.2	-22.13	-25.04
Merlot	0.05 g/L ABA 1017	07-Feb-18	-17.92	-20.69	-23.11
Sauvignon blanc	Control	06-Feb-18	-19.5	-21.58	-22.65
Sauvignon blanc	5 g/L ABA	06-Feb-18	-18.04	-20.3	-23.09
Sauvignon blanc	1.0g/L ABA 1016	06-Feb-18	-20.49	-21.23	-23.58
Sauvignon blanc	0.5 g/L ABA 1016	06-Feb-18	-18.11	-20.58	-22.8
Sauvignon blanc	0.1 g/L ABA 1016	06-Feb-18	-19.27	-21.94	-23.54
Sauvignon blanc	0.5 g/L ABA 1017	06-Feb-18	-17.91	-20.92	-23.33
Sauvignon blanc	0.25 g/L ABA 1017	06-Feb-18	-18.78	-20.88	-22.27
Sauvignon blanc	0.05 g/L ABA 1017	06-Feb-18	-18.89	-22.39	-24.26

Table 6. Cold hardiness of Merlot grapevines during deacclimation based on exogenous ABA treatments. Creek Shores, April, 2018.

Treatment	Date	LTE10	LTE50	LTE90
Control	09-Apr-18	-12.8	-17.31	-19.63
5 g/L ABA	09-Apr-18	-16.06	-18.53	-19.89
1.0g/L ABA 1016	09-Apr-18	-17.41	-19.34	-20.7
0.5 g/L ABA 1016	09-Apr-18	-17.52	-19.61	-21.56
0.1 g/L ABA 1016	09-Apr-18	-16.25	-19.4	-22.38
0.5 g/L ABA 1017	09-Apr-18	-18.16	-20.4	-21.99
0.25 g/L ABA 1017	09-Apr-18	-16.58	-18.85	-20.64
0.05 g/L ABA 1017	09-Apr-18	-16.84	-18.32	-19.58
Treatment	Date	LTE10	LTE50	LTE90
Control	26-Apr-18	-10.24	-12.47	-15.02
5 g/L ABA	26-Apr-18	-9.88	-12.9	-14.97
1.0g/L ABA 1016	26-Apr-18	-13.8	-17.95	-22.39
0.5 g/L ABA 1016	26-Apr-18	-14.38	-19.77	-22.27
0.1 g/L ABA 1016	26-Apr-18	-8.42	-11.09	-13.51
0.5 g/L ABA 1017	26-Apr-18	-13.58	-16.83	-18.39
0.25 g/L ABA 1017	26-Apr-18	-14.8	-17.7	-21.3
0.05 g/L ABA 1017	26-Apr-18	-11.6	-13.25	-16.21

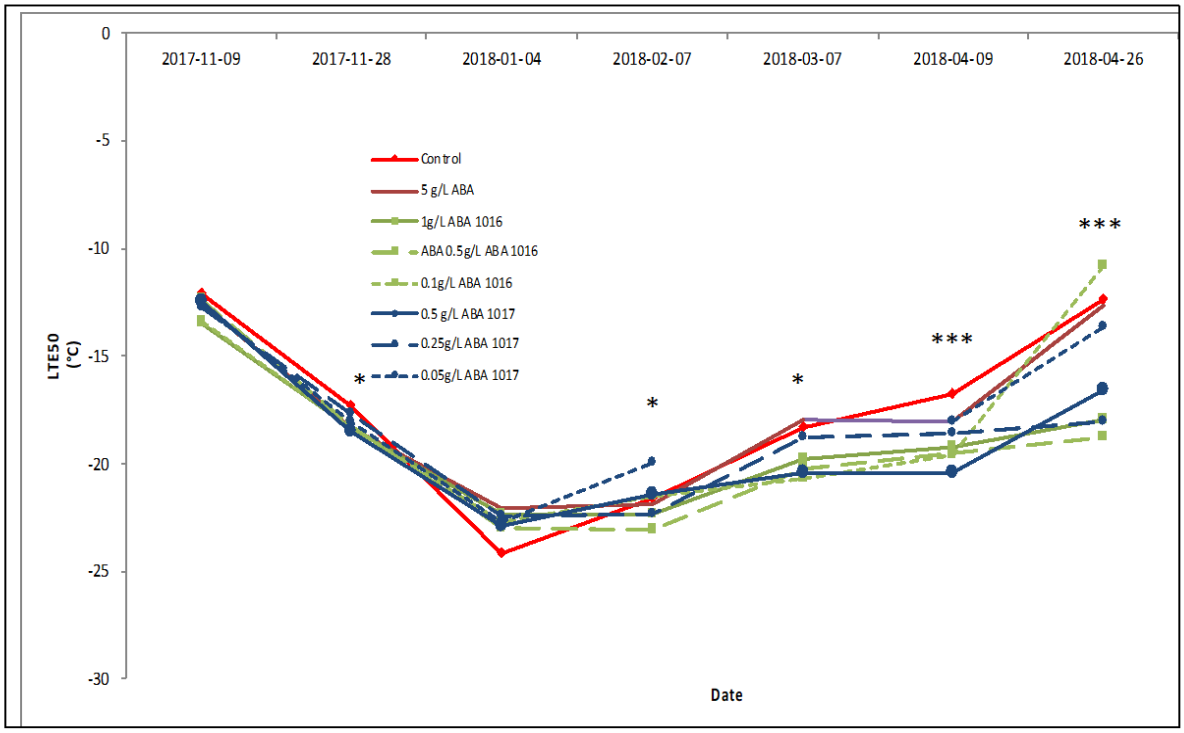


Figure 1. Cold hardiness dynamics of Merlot grapevines based on exogenous ABA treatments. Creek Shores, 2018. (*, ** represent statistical significance @ p<0.05, p<0.001, respectively)

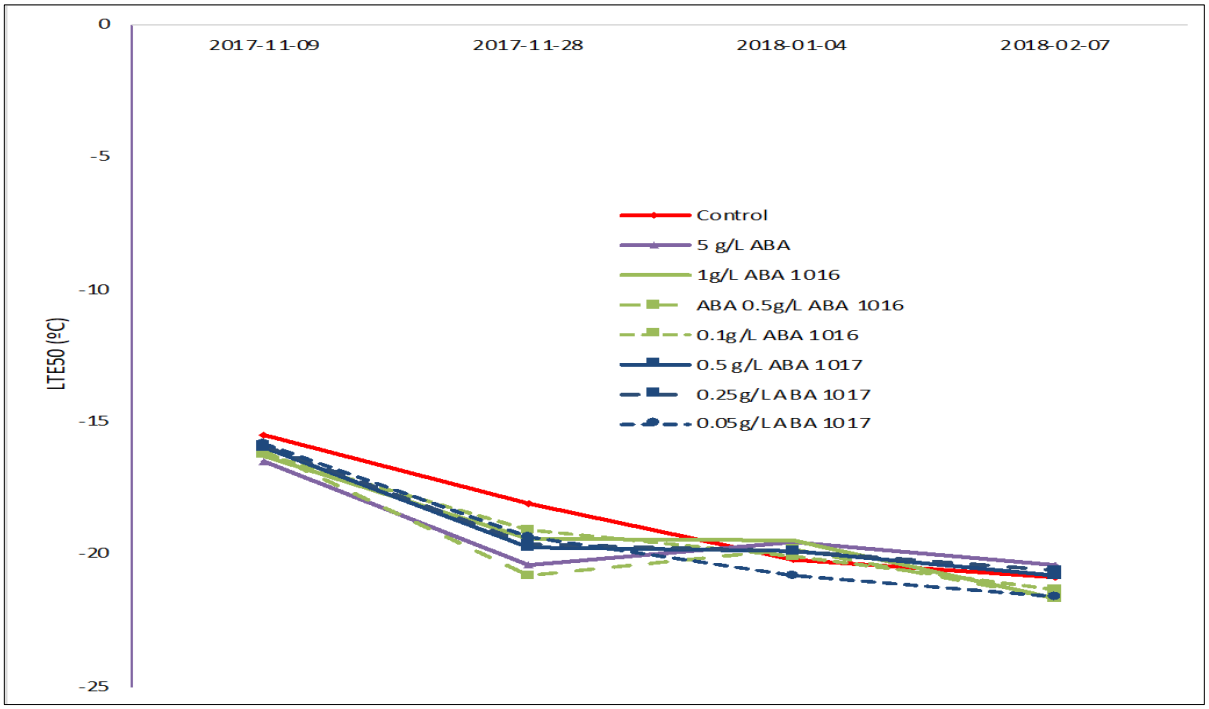


Figure 2. Cold hardiness dynamics of Sauvignon blanc grapevines based on exogenous ABA treatments. Creek Shores, 2018.

2018/19. Cold hardiness and tissue sampling were performed during the entire dormant period for Merlot (November-April). Treatments were limited in 2018/19 and lacking the most proven ABA analog to date, 8'-acetylene ABA, due to synthesis issues from newly contracted services from ABAzyne. This was evident based on the findings. No treatment improved hardiness greatly during acclimation or the mid-winter period but there were some slight improvements with both ABA and ABA analog (see Fig. 3). The ABA analog tetralone did help maintain dormancy that resulted in greater cold tolerance during the deacllimation period. Natural ABA was again found to not improve hardiness during mid-winter months but also did not improve acclimation rates greatly in fall of 2018.

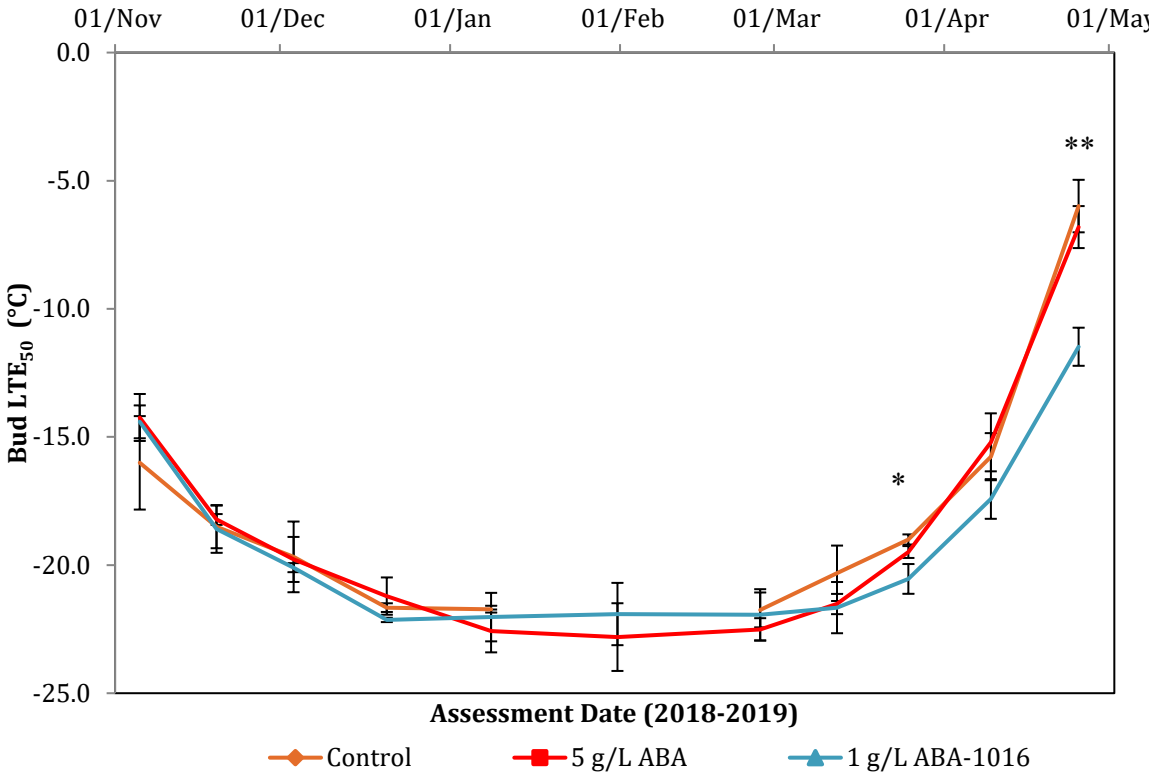


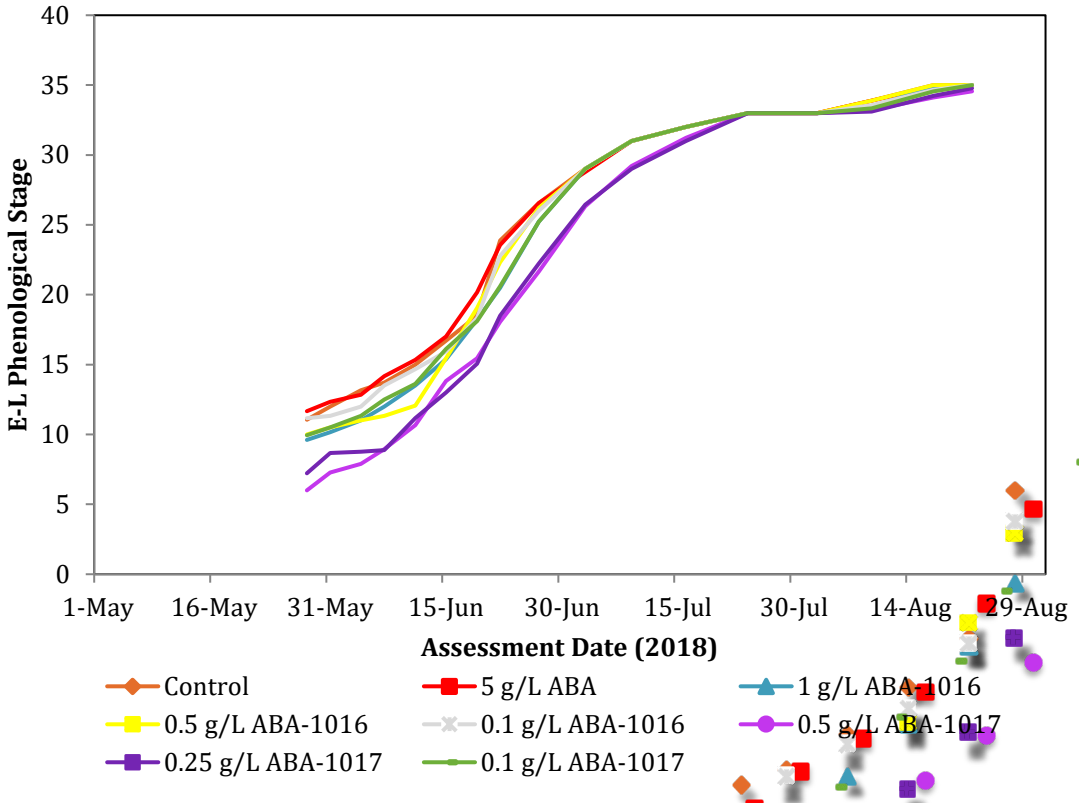
Figure 3. Cold hardiness dynamics of Merlot grapevines based on exogenous ABA treatments. Creek Shores, 2018-19. Significance indicated by asterisks, where *, represent 1 g/L ABA-1016 v. control or 5 g/L ABA at $p < 0.05$ and 0.01, respectively.**

Phenology. *2018.* Phenological stages were monitored during the growing season to determine if delays in deacclimation would further delay key growth stages. Previous research has shown that ABA analogs can delay bud break in Merlot (Bowen et al. 2016) as well as Cabernet franc grapevines (pers. comm. D. Woolard, Valent). Results concerning timing of bud break can be found in Table 7. This study further supports these findings where natural ABA did not have any effect on maintaining dormancy late in winter nor in delaying bud break whereas ABA analogs had significant effects on delaying bud break, particularly at higher concentrations. In

Merlot, ABA-1017 delayed bud break by up to 2 weeks (see Table 7 and Image 1) at the two highest concentrations whereas ABA-1016 delayed bud break up to a week. Results for Sauvignon blanc were similar but to a lesser degree. Again, this seems to indicate that timing of ABA applications following harvest does impact both hardiness and delay of bud break. This is an interesting finding and requires further study as a number of hypotheses are possible. Delays in phenology followed similar trends to bud break based on treatment, however at key stages there was only a slight delay by a few days for the high concentration analog treatments and following veraison it was difficult to visually determine differences for both canopy size as well as fruit maturation based on colour. This is similar to findings in Bowen et al. 2016 where 8'-acetylene ABA treatments were found to delay phenology until berry set but no differences were observed following that stage of growth. Vine vigour will be assessed in 2019 using dormant cane pruning weights to see if overall vine capacity was reduced in any of these delays in timing of phenological stages.

Table 7. Date of budbreak for Merlot and Sauvignon blanc grapevines based on exogenous ABA treatments. Creek Shores, 2018.

	Merlot	Sauvignon blanc
Treatment	Date	Date
Control	17-May	19-May
5 g/L ABA	17-May	19-May
1 g/L ABA 1016	23-May	23-May
0.5 g/L ABA 1016	22-May	22-May
0.1 g/L ABA 1016	17-May	22-May
0.5 g/L ABA 1017	02-Jun	26-May
0.25 g/L ABA 1017	29-May	28-May
0.05 g/L ABA 1017	21-May	22-May



2019. In relation to both the control and natural ABA, tetralone ABA significantly delayed the advancement of treated vines through the modified Eichhorn-Lorenz phenological growth stages from bud break to fruit set (Figure 4). This divergence is most extreme early in the growing season, when at times tetralone ABA repressed the development of vines by 4 E-L phenological stages.

Natural ABA allowed treated vines to emerge from winter dormancy significantly earlier than tetralone ABA, which repressed bud break by 10 days. A near-identical trend is apparent between untreated control vines and those treated with tetralone ABA. As bud break approached 50% for both natural ABA and control treatment vines, only 2% of buds on tetralone ABA treated vines had burst (Table 8).

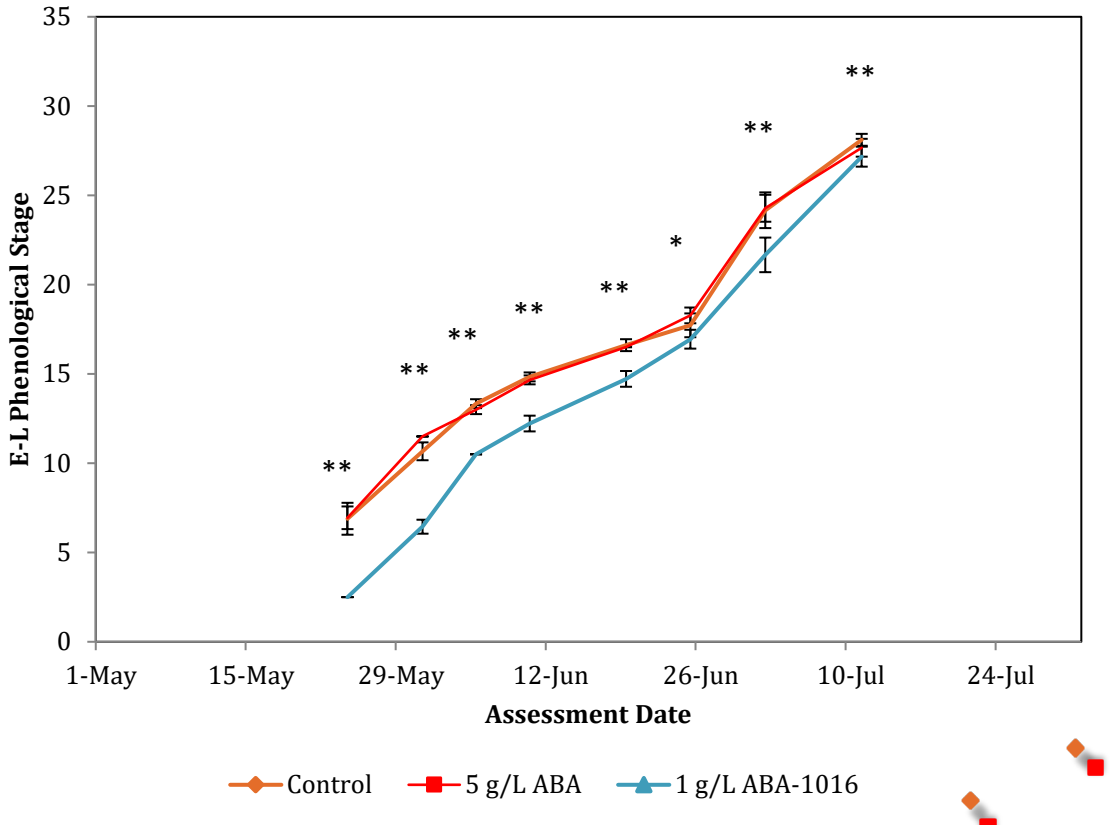


Figure 4. Phenological development of *V. vinifera* L. Merlot grapevines treated with natural ABA and tetralone ABA (ABA-1016). Growth was monitored from bud swell until fruit set on the modified Eichhorn-Lorenz (E-L) system. Significance indicated by asterisks, where *, represent 1 g/L ABA-1016 v. control or 5 g/L ABA at $p < 0.05$ and 0.01, respectively.**

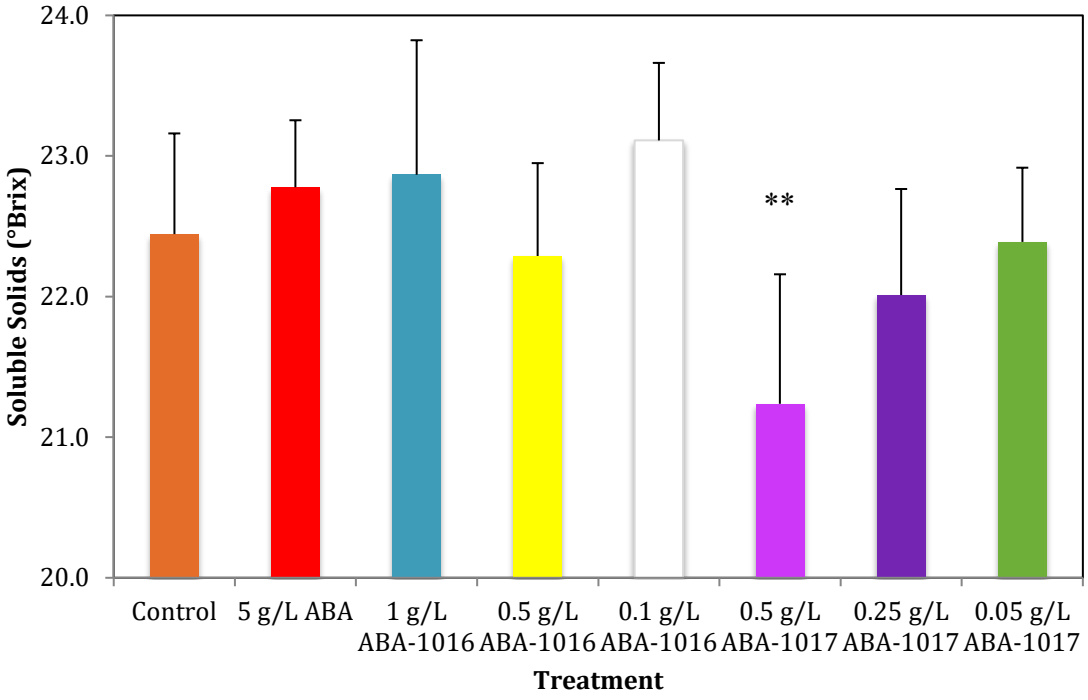
Similar delays were observed between treatments through the flowering period. The onset of bloom was significantly deferred by 2 days with the post-harvest foliar application of tetralone ABA, in comparison to both natural ABA and untreated control vines. Total cap fall was significantly reduced by 32% in vines treated with tetralone ABA, such that bloom was only 36% complete when the control and natural ABA treatments reached 68% flowering (Table 8).

Table 8. Bud break and bloom progression of *V. vinifera* L. Merlot grapevines treated with natural ABA and tetralone ABA (ABA-1016). Bud break was assessed on 20 May 2019 and recorded as the percentage of total buds displaying visible leaf tips; bloom was assessed on 2 Jul. 2019 and recorded as percentage of cap fall on representative clusters. Significance indicated by asterisks, where ** represents 1 g/L ABA-1016 v. control or 5 g/L ABA at $p < 0.01$.

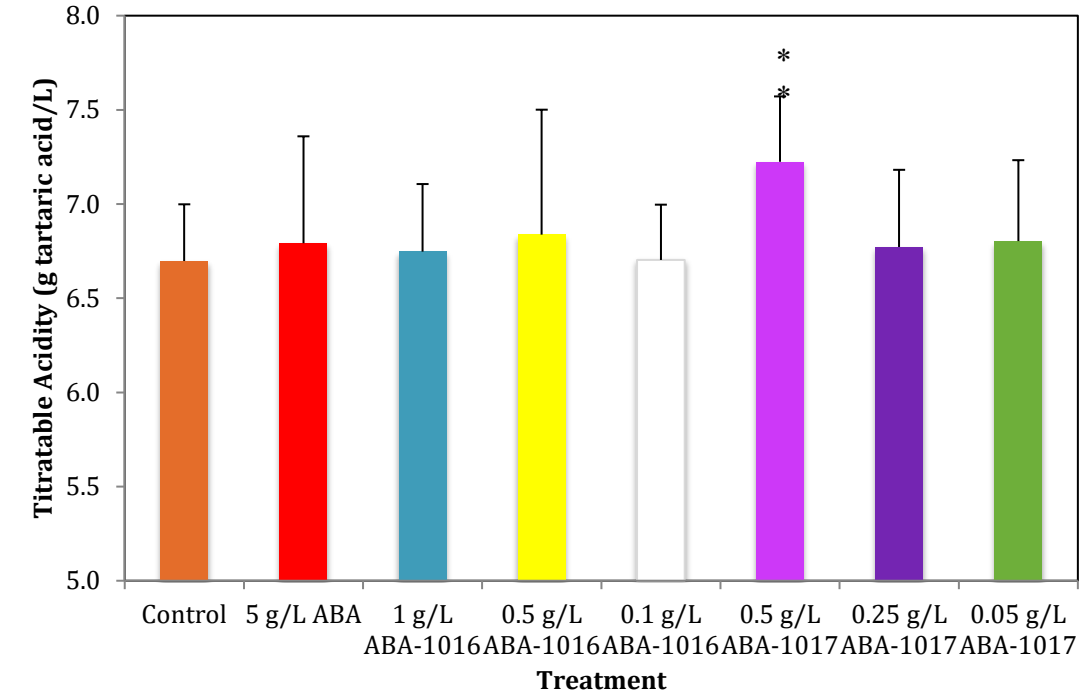
	Bud break (%)	Bloom (%)
	05-20	07-02
Control	48.1	67.2
5 g/L ABA	51.3	68.0
1 g/L ABA-1016	1.7	35.8
1 g/L ABA-1016 v. control or 5 g/L ABA	**	**

Berry Composition

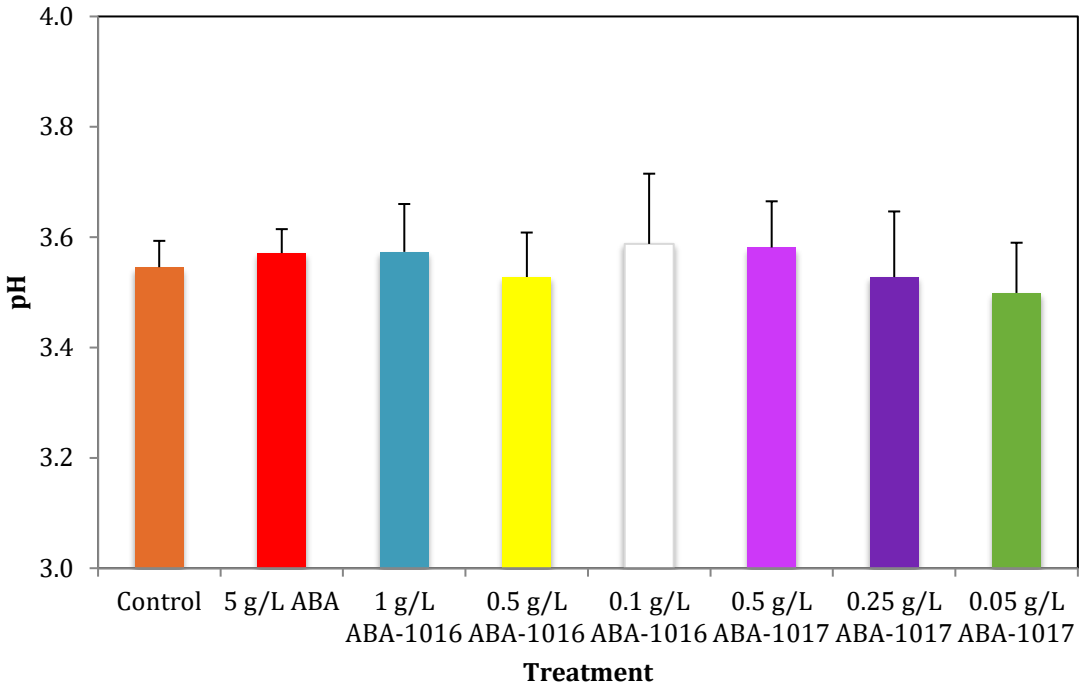
The final objective of this project was to evaluate fruit maturation following the post-harvest foliar application of natural ABA, tetralone ABA (ABA-1016), and 8'-acetylene ABA (ABA-1017) to *V. vinifera* L. Merlot grapevines in 2017. Treatments were compared to determine if either ABA analog yield a discernable impact on the fruit quality parameters of juice soluble solids, titratable acidity, and pH, in relation to natural ABA (Figure 5).



(a)



(b)



(c)

Figure 5. Berry composition of Merlot grapevines treated with natural ABA, tetralone ABA (ABA-1016), and 8'-acetylene ABA (ABA-1017). (a) soluble solids, in °Brix, (b) titratable acidity, in g/L tartaric acid, and (c) pH. Significance indicated by asterisks, where ** represents treatment v. control at $p < 0.01$. 2018.

In relation to untreated control vines, tetralone ABA did not produce a notable effect on berry composition with respect to soluble solids, titratable acidity, or pH
(Figure 10a, b, c). An analogous trend was observed with the application of 0.25 g/L and 0.05 g/L ABA-1017; however, soluble solids were repressed by 1.2°Brix with the 0.5 g/L ABA-1017 treatment (Figure 5a). This 8'-acetylene ABA treatment repressed titratable acidity by 0.5 g/L tartaric acid in harvested fruit (Figure 5b). Conversely, pH was unaffected by the application of 8'-acetylene ABA, regardless of concentration (Figure 5c).

Image 1. Growth in a Merlot grapevine sprayed with 0.5 g/L ABA-1017 (foreground) and control grapevines (background). Creekshores. June 4th 2018.

ABA and metabolite analysis. Mass spectrometric (MS) analyses of Merlot grape bud tissues were performed by Dr. Randy Purves U of Saskatchewan to determine levels of the ABA analog ABA-1016 over winter and before bud break. The levels of abscisic acid (ABA) and its metabolites were also analyzed to see if there was any effect of the analog treatment on levels of the hormone (see Table 9).

Table 9. ABA and metabolite analysis of Merlot grapevine buds during dormancy for control and ABA-1016 treatments. 2017-18.

Sample date	Tetralone (g/L ABA-1016)	ABA	ABA-GE	DPA	7'OH-ABA	t-ABA	PA	neo-PA	Total ABA & metobolites	Tetralone (ABA-1016)	GA4
Nov. 9, 2017	control (surfactant only)	5.8	10.3	10.5	0.04	0.07	0.10	<0.01	26.8	<0.01	0.020
	0.1	6.4	10.8	11.3	0.05	0.06	0.10	<0.01	28.7	0.39	0.026
	0.5	6.9	12.9	7.8	0.07	0.06	0.09	<0.01	27.9	0.76	0.020
	1	4.9	9.0	10.9	0.03	0.06	0.11	<0.01	25.0	3.18	0.034
Jan 4, 2018	control (surfactant only)	3.5	9.2	11.2	0.03	0.06	0.12	<0.01	24.1	<0.01	0.019
	0.1	3.2	9.4	11.2	0.03	0.10	0.12	<0.01	24.0	0.09	0.034
	0.5	3.6	11.1	9.3	0.03	0.09	0.11	<0.01	24.3	0.49	0.023
	1	3.5	9.2	9.9	0.03	0.08	0.14	<0.01	22.9	2.17	0.022
March 7, 2018	control (surfactant only)	1.8	9.3	10.7	0.02	0.08	0.12	<0.01	22.1	<0.01	0.020
	0.1	1.5	9.5	9.4	0.02	0.09	0.13	<0.01	20.7	0.03	0.026
	0.5	1.7	10.0	11.1	0.02	0.08	0.13	<0.01	23.1	0.34	0.021
	1	1.5	10.0	9.4	0.02	0.09	0.13	<0.01	21.1	0.61	0.026
April 9, 2018	control (surfactant only)	1.2	9.5	9.2	0.02	0.08	0.10	<0.01	20.1	<0.01	0.018
	0.1	1.4	9.3	10.0	0.02	0.08	0.12	<0.01	21.0	0.04	0.020
	0.5	1.4	10.6	9.5	0.02	0.09	0.12	<0.01	21.8	0.45	0.017
	1	1.3	8.8	8.1	0.02	0.08	0.10	<0.01	18.5	0.42	0.021
April 26, 2018	control (surfactant only)	1.4	9.5	8.6	0.02	0.06	0.10	<0.01	19.6	<0.01	0.015
	0.1	1.2	10.4	8.4	0.01	0.09	0.10	<0.01	20.2	0.03	0.021
	0.5	1.2	10.8	8.8	0.01	0.08	0.11	<0.01	21.0	0.37	0.020
	1	1.4	10.0	8.2	0.01	0.07	0.12	<0.01	19.7	0.35	0.018
	**Note: all units are ug/g										

Through mass spectrometric analyses of Merlot grape bud tissues ABA levels in the buds decreased over winter from 6 to 1 ug/g dry weight, consistent with what has been found in previous research on dormancy release in grapes and other plant species (Zheng et al. 2015). There was no significant effect of the analog treatments on ABA levels. ABA levels in plants are reduced by conversion to oxidized and glucosylated metabolites (Zaharia et al. 2005). In the merlot grape samples, the principal metabolites were found to be dihydrophaseic acid (DPA) and ABA glucosyl ester (ABAGE) as expected from other plant studies. The levels of these metabolites did not change significantly over time. Again, the analog treatment had little effect on metabolite levels. The levels of the applied ABA analog were analyzed also. The highest levels were observed at the earliest time point, and ranged from 3 ug/g dry weight for the highest concentration applied to (1 g/L solution) to 0.3 ug/g for the lowest concentration. Over the course of the winter, the levels in all samples decreased to one tenth of the initial levels observed. At the highest concentration the residual level of ABA-1016 was found to be 0.3 ug/g of dry plant tissue. This will provide useful information for future studies on fate of the compound in the field and residues in grape plants.

a) Short-term

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 analogues 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 varieites that lose cold tolerance early (i.e. V. riparia-based hybrids). Therefore ABA analogue application may delay deacclimation which could result in less freeze damage associated with sporadic warming and freezing events during dormancy.

Precommercialization. ABA analogs do present a potential viable commercialization opportunity. 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, OGWRI and Brock University 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 to try to work together to examine research and commercialization opportunities.

Value: No product has been sold but further synthesis of ABA analogues has been done to produce product for further studies.

Reach and Communications

The primary target of this project is to benefit the grape growers in Ontario However, aspects of this work will also benefit growers across the country. The project is still ongoing; 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. Willwerth spoke at the OFVGA conference in February as well as the CCOVI lecture series on the ABA research that has been conducted at CCOVI. In August, for the industry grapevine tailgate tour, the experimental vineyard block for ABA analog experiments were shown to a group of over 50 grape and wine industry personnel and results were presented. Growers had the opportunity to see the effects of the applications first hand. Potential commercialization opportunities are being explored with ABAzyne and OGWRI and some preliminary discussions have occurred between parties as well as some industry representatives from companies (i.e., Bartlett). Researchers from other regions in North America have been briefed on some of this research and there is interest in collaborative work in 2019 to further understand the impact of ABA analogs on grapevine physiology across different climates and varieties including other crops susceptible to early freeze injury such as apricots. European producers are also interested in this technology, particularly in regions such as France where spring frost injury has been an issue over the past 5 years. 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 6 times formally during the course of this project during presentation of any aspects of this project. Total grower reach has been >250 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 internationally. Hopefully, this will lead to more

opportunities for research collaborations and commercialization opportunities for ABAzyne and/or OGWRI through further business development strategies.

Conclusions and comments

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. In 2017, both ABA analogs reduced the rate of deacclimation in both Merlot and Sauvignon blanc and also delayed bud break significantly, up to 2 weeks, in Merlot vines at higher concentrations. The highest concentration of 8'-acetylene ABA delayed phenology which ultimately reduced some of the primary fruit chemistry quality parameters such as slight reductions in soluble solids. However, Brix levels were still above VQA Ontario minimum sugar levels. Results using tetralone ABA were not as effective in 2018 during acclimation but some slight improvements were found to exist particularly with delaying deacclimation. Tetralone ABA also delayed bud break so it was found to be effective again at maintain dormancy in Merlot grapevines.

This research is currently being expanded using the most potent ABA analog, 8'-acetelyne, under the guides of a new project with OGWRI in 2019 and also initiative new studies in conjunction with the AAFC AgriScience Cluster to use some of these analogs in field trials to determine if they can mitigate the negative effects of Red Blotch virus on fruit maturation in V. vinifera. We hypothesize that ABA analogs may in fact improve fruit quality in virus effected vines, where other cultural practices have no effect. ABAzyne and Brock would like to explore these possibilities and continue our dialogue with OGWRI with respect to future research and commercialization possibilities.

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