Cool Climate Oenology & Viticulture Institute

Brock University

Effects of Abscisic acid form, concentration and application timing on grapevine cold hardiness

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Acknowledgements

- Funding: OGWRI, OMAFRA (OVIP), Valent Biosciences

<u>Collaborators</u>:

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Valent: Dr. Peter Petracek, Derek Woolard

Overview

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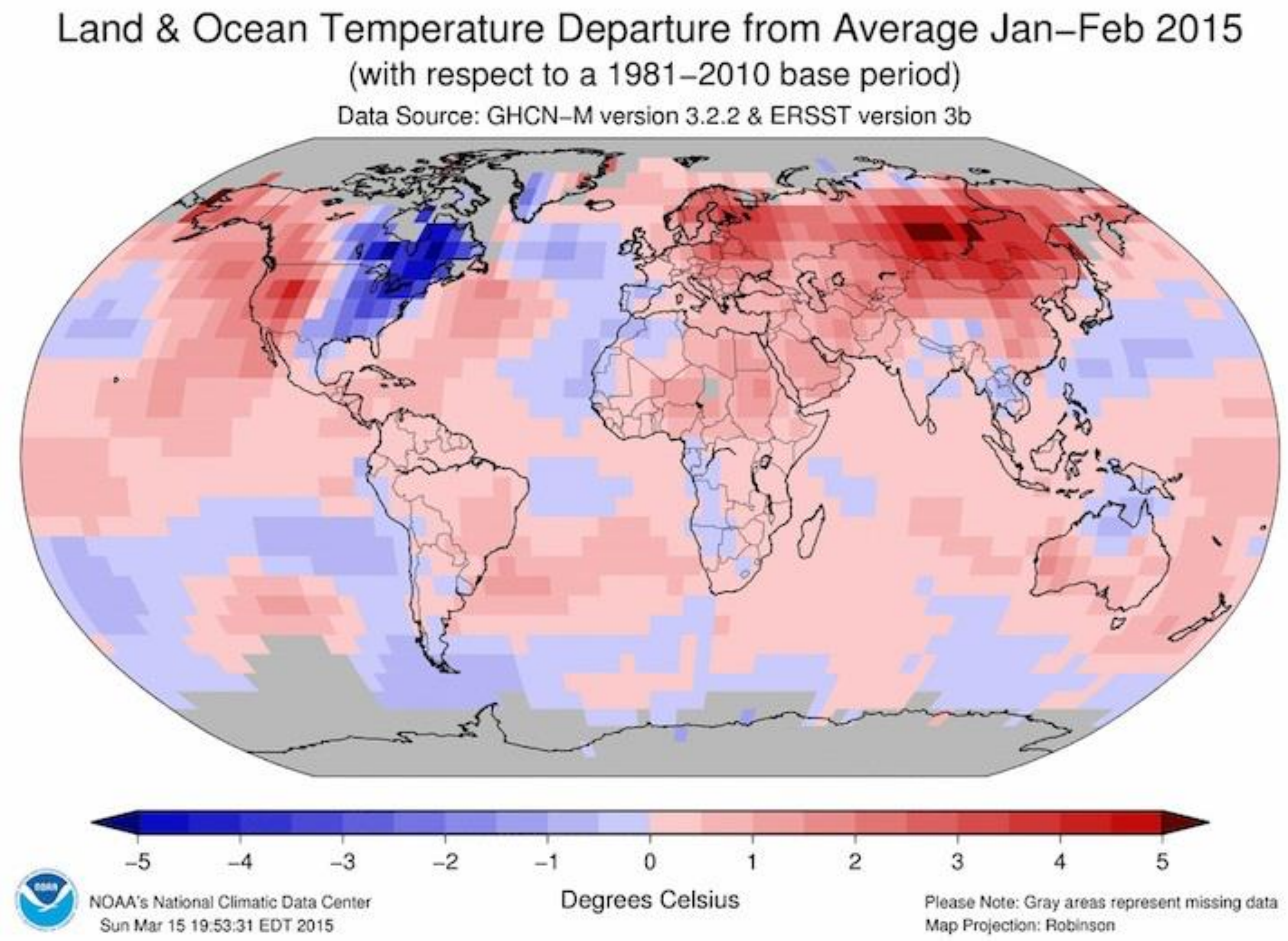
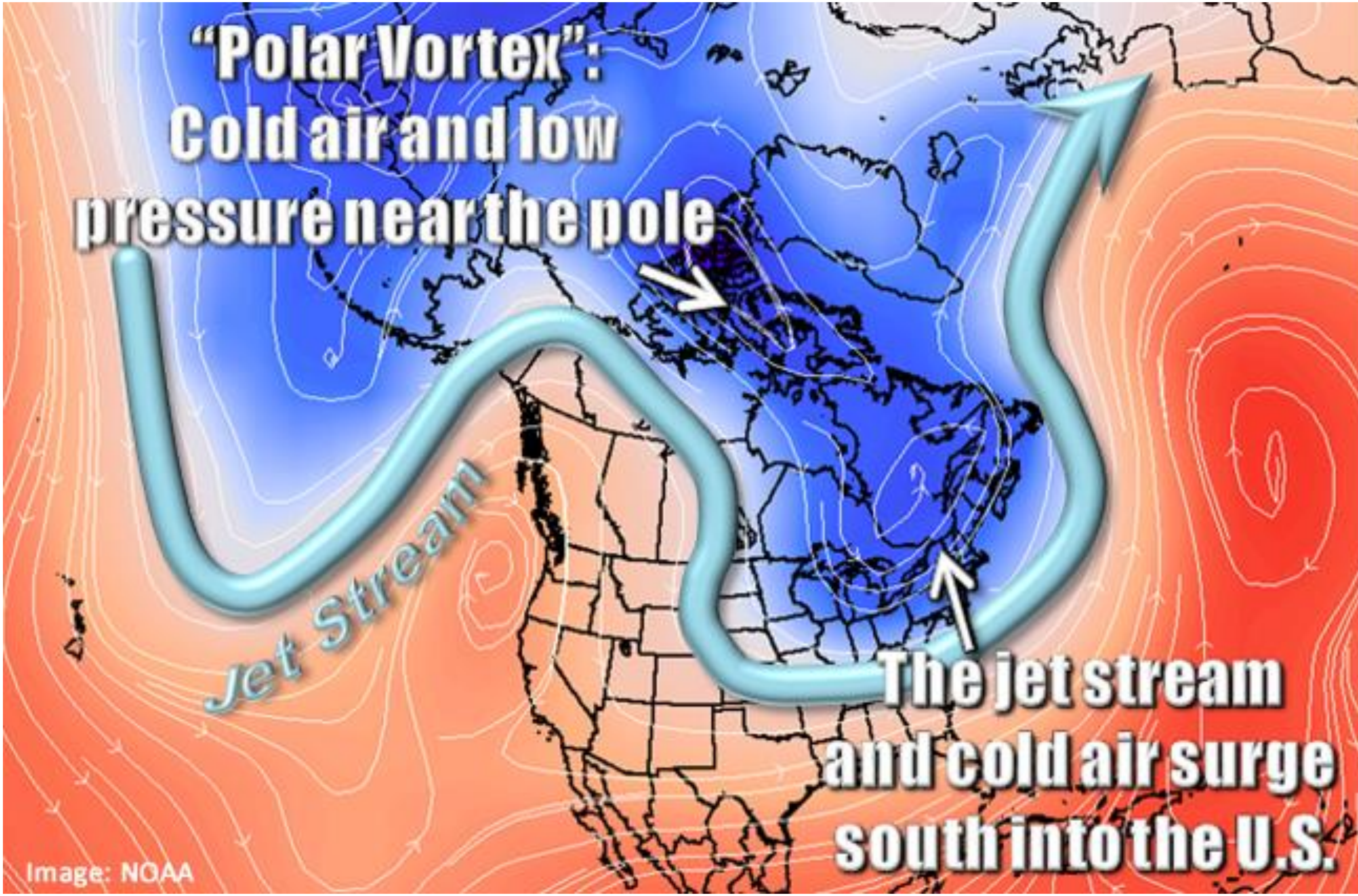
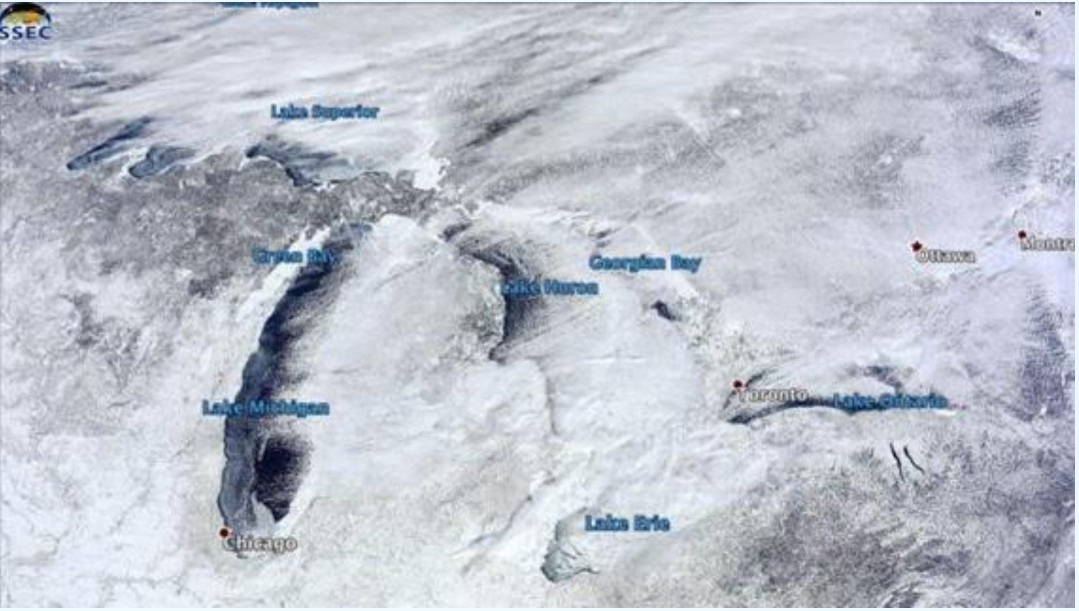
- Freeze injury is one of the greatest threats to grape and tender fruit crops
- Climate change will impact all aspects of agriculture
- Unpredictable future increase the challenges
- Extreme events and volatility prove to be the greatest challenges for agriculture

Changing climate




"Polar Vortex":
Cold air and low
pressure near the pole
Jet Stream
The jet stream
and cold air surge
south into the U.S.
Image: NOAA


SSEC
Lake Superior
Green Bay
Georgian Bay
Lake Huron
Ottawa
Toronto
Lake Ontario
Lake Michigan
Lake Erie
Chicago

Trends for phenological data (Jones et al. 2005)

Table 1 – Location, varieties, and time period of the phenological data for the regions studied in the analysis. Characteristics and trends are given for bud break, bloom, véraison, and harvest (except where noted).

Table 1 – L'emplacement, les variétés, et chronométrez la période des données de phenological pour les régions étudiées dans l'analyse. Les caractéristiques et les tendances sont données pour la coupure de bourgeon, la fleur, véraison, et la moisson (sauf où réputé).

Region/Variety	Time Period	Bud Break		Bloom		Véraison		Harvest	
		X, SD[1]	Trend[2]	X, SD[1]	Trend[2]	X, SD[1]	Trend[2]	X, SD[1]	Trend[2]
Alsace, France **Reisling**	1972-2004	23-Apr (9 d)	-14 days(0.24)	18-Jun (9 d)	-15 days(0.26)	28-Aug (11 d)	-22 days(0.34)	6-Oct (11 d)	-23 days(0.41)
Reims, France **Pinot Noir**	1975-2004	17-Apr (9 d)	NS	21-Jun (8 d)	-14 days(0.29)	15-Aug (12 d)	-22 days(0.28)	25-Sep (9 d)	-18 days(0.24)
Burgundy, France **Pinot Noir**	1952-2004	17-Apr (10 d)	-11 days(0.11)	13-Jun (10 d)	-11 days(0.13)	8-Aug (8 d)	-10 days(0.13)	25-Sep (10 d)	-16 days(0.21)
Bordeaux, France **Merlot/Cab.Sauv.**	1952-2001	No Data	No Data	11-Jun (9 d)	-6 days(0.08)	17-Aug (9 d)	-10 days(0.11)	30-Sep (10 d)	-16 days(0.24)
Conegliano, Italy	1964-2004								
Chardonnay		12-Apr (8 d)	NS	4-Jun (8 d)	-12 days(0.33)	6-Aug (8 d)	-13 days(0.39)	11-Sep (13 d)	-21 days(0.33)
Sangiovese		16-Apr (7 d)	NS	9-Jun (7 d)	-12 days(0.27)	13-Aug (8 d)	NS	28-Sep (10 d)	-14 days(0.19)
Cabernet Sauv.		23-Apr (6 d)	NS	10-Jun (7 d)	-10 days(0.22	15-Aug (8 d)	-12 days(0.26)	27-Sep (8 d)	-8 days(0.10)
Trebbiano		26-Apr (6 d)	+6.5 days(0.11)	13-Jun (6 d)	-6 days(0.10)	20-Aug (7 d)	NS	3-Oct (10 d)	NS
Geisenheim, Germany **Reisling**	1955-2004	27-Apr (7 d)	NS	21-Jun (8 d)	NS	**25° Oechsle** 24-Aug (12 d)	NS	**60°Oechsle** 17-Sep (15 d)	-21 days(0.24)
Dolné Plachtince, Slovakia	1971-2004								
Reisling		23-Apr (6 d)	NS	14-Jun (8 d)	-12 days(0.18)	28-Aug (10 d)	-12 days(0.30)	12-Oct (11 d)	-15 days(0.16)
Müller Thurgau		20-Apr (6 d)	NS	11-Jun (8 d)	-10 days(0.14)	12-Aug (8 d)	NS	23-Sep (11 d)	-14 days(0.14)
Chardonnay		16-Apr (7 d)	NS	8-Jun (8 d)	-12 days(0.19)	20-Aug (10 d)	-19 days(0.30)	4-Oct (10 d)	-17 days(0.23)
Valladolid, Spain	1996-2004								
Verdejo		5-Apr (10 d)	No Trend Analysis	14-Jun (5 d)	No Trend Analysis	25-Aug (4 d)	No Trend Analysis	29-Sep (9 d)	No Trend Analysis
Grenache		4-Apr (11 d)		18-Jun (6 d)		26-Aug (7 d)		5-Oct (10 d)	
Tinta del Páis		10-Apr (10 d)		14-Jun (4 d)		18-Aug (6 d)		3-Oct (9 d)	
Tinta de Toro		9-Apr (10 d)		16-Jun (4 d)		18-Aug (5 d)		30-Sep (9 d)	
Mencia		5-Apr (10 d)		14-Jun (5 d)		18-Aug (4 d)		4-Oct (9 d)	
Albillo Mayor		5-Apr (9 d)		15-Jun (5 d)		18-Aug (4 d)		2-Oct (13 d)	
Albillo Real		28-Mar (10 d)		12-Jun (5 d)		7-Aug (5 d)		17-Sep (16 d)	
Pontevedra, Spain **Albariño**	2001-2004	7-Apr (8 d)	No Trend Analysis	6-Jun (4 d)	No Trend Analysis	21-Aug (13 d)	No Trend Analysis	6-Oct (5 d)	No Trend Analysis

[1] X and SD are the mean (date) and standard deviation (days) of each variety analyzed.
[2] Trend is the trend in days over the time period for each region/variety. The number in parentheses is the R^2 of the trend with all trends shown significant at the 0.05% level.

Examples of post budbreak freeze in Europe

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Photo by: Sophie Nault

Burgundy hit by ‘worst frost since 1981’
http://www.decanter.com

Cold Hardiness: Dynamic condition

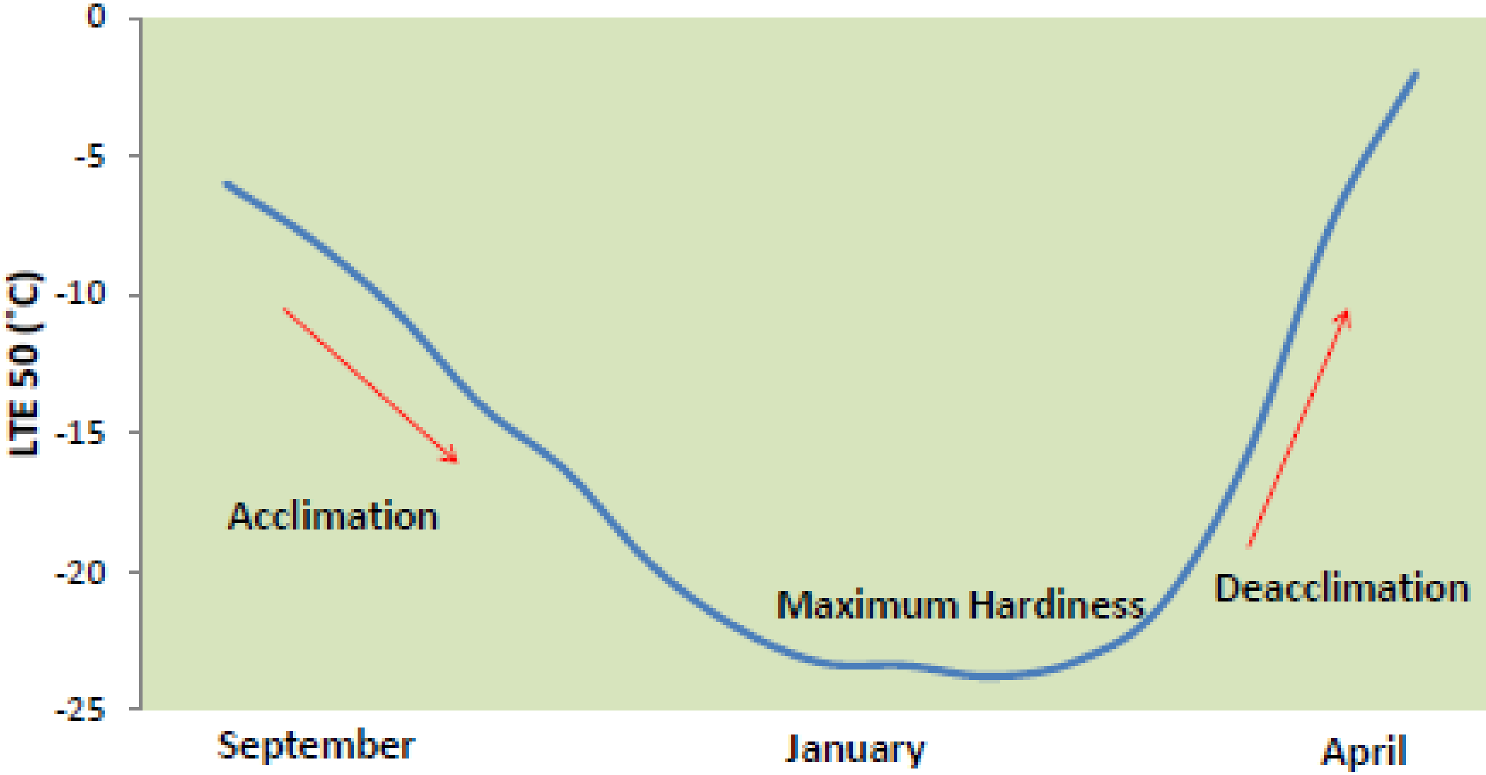

Figure 1. Profile of bud cold hardiness during the dormant season

(CCOVI VineAlert Website)

Weather variation impacts seasonal differences in cold hardiness

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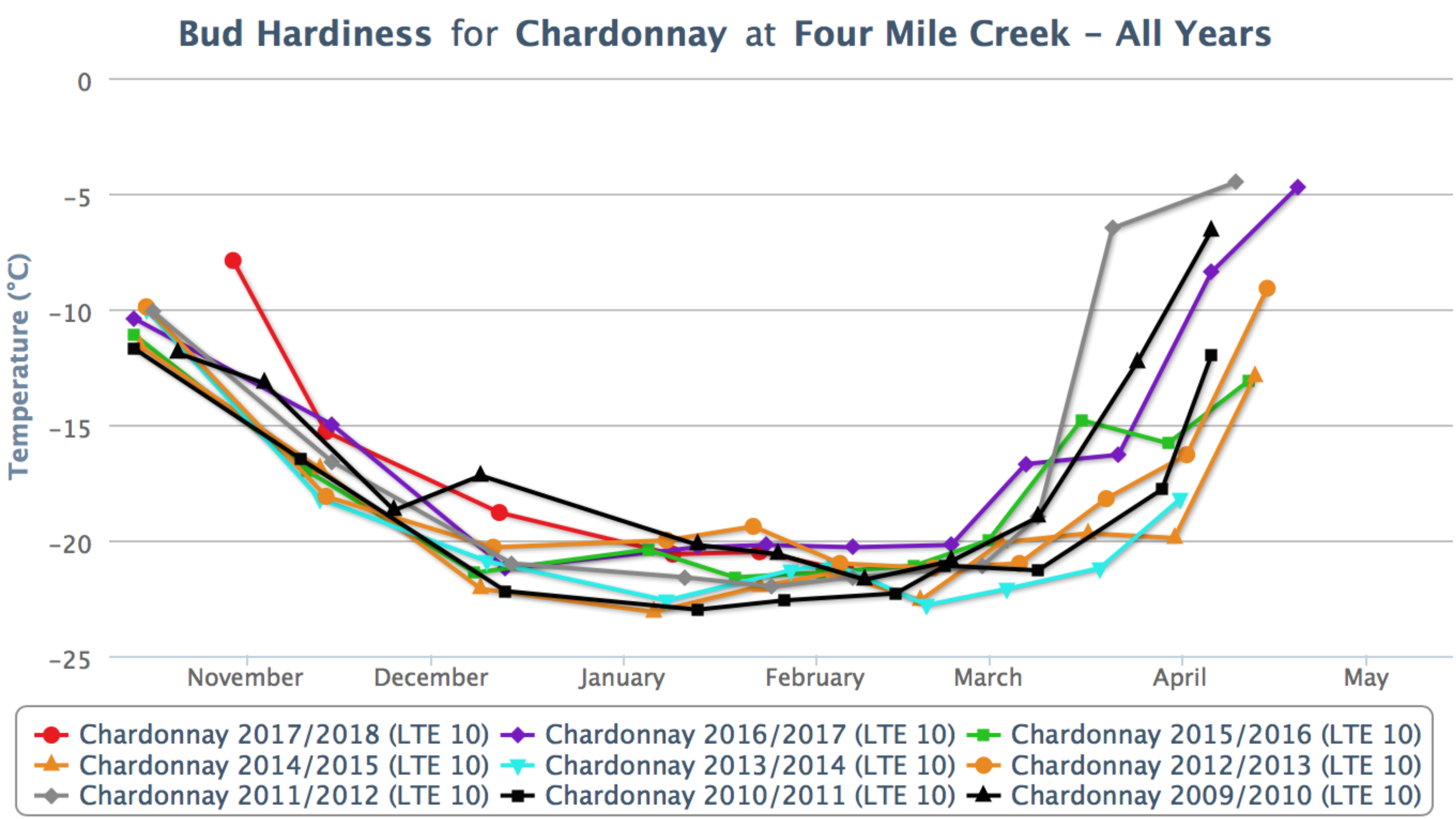

Warming during ecodormancy

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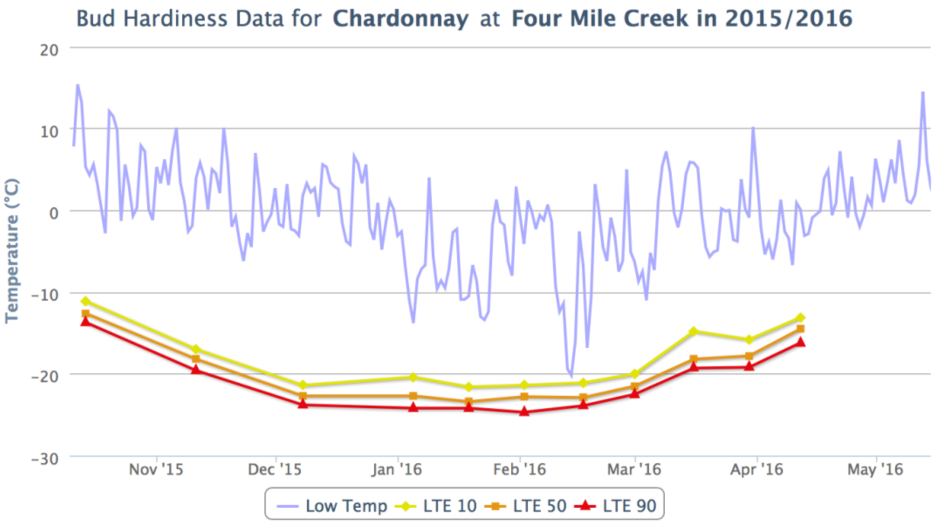
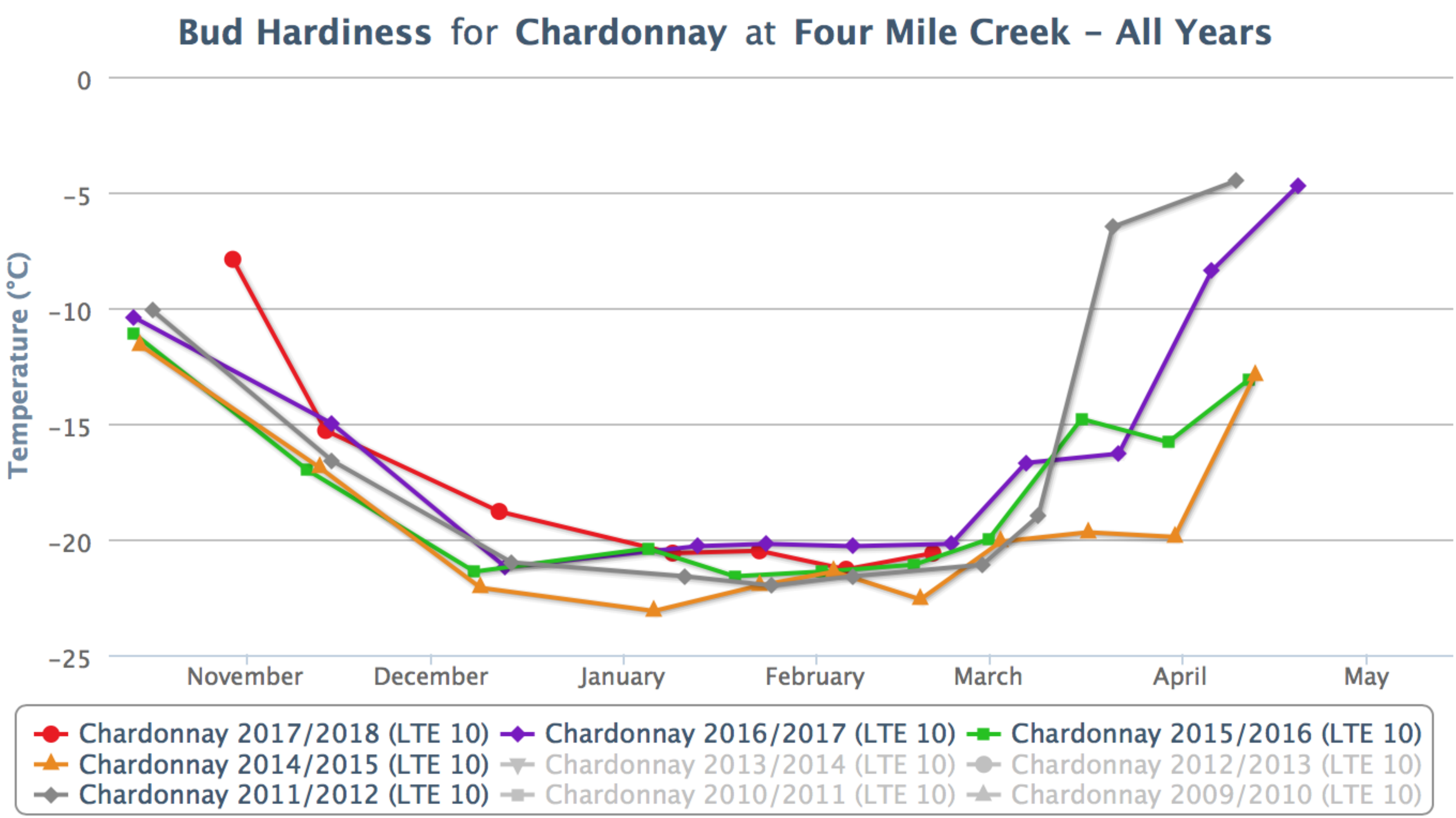
- Probably the greatest risk of freeze injury due to climate change
- More erratic winter temperatures
- Periods of warming followed by ‘extreme’ cold can have devastating consequences



Impacts of warm weather on deacclimation



Potential solution to addressing climate change impacts

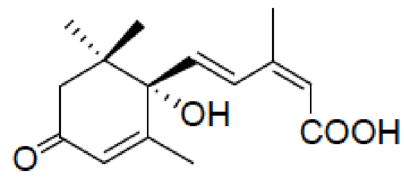
- Plant growth regulators (PGRs) can be a powerful tool
- Commonly used already in hot climate regions
  - Widely used for Table grape production
  - Tropical viticulture
- Various PGRs are used depending on the production or quality issue
- Very limited use in Canada

Use of PGRs in grape cultivation

PGR	Purpose of Use	Stage
Gibberlins (GA)	Cluster Elongation Berry thinning Berry sizing	Pre-bloom Bloom After fruit set
Cytokinins	Berry sizing	After fruit set
Abscisic Acid (ABA)	Berry coloration	Veraison
Ethylene	Berry coloration	Veraison

Background on Abscisic Acid (ABA)

- **Isoprenoid plant hormone found in all plants**
- **Regulates a wide range of processes in plant growth and development**
  - Response to abiotic stress
    - Transpiration
    - Response to heat, drought, salinity and freezing
  - Growth and development
    - Growth inhibition
    - Abscission, senescence
    - Secondary metabolite production
  - Seed Development



S-(+)-Abscisic acid

ABA and cold tolerance

- Enforces dormancy in buds
  - Induction and maintenance of dormancy
- Induces expression of genes encoding proteins to protect cells from dehydration
- Interacts with other signaling molecules (i.e. Ca) to regulate cold tolerance responses
- Impacts expression of CBF genes
- Induce dehydrin gene expression and accumulation of COR proteins
- ABA dependent and independent pathways
- Complex interactions with abiotic conditions

Cold and dehydration responses and ABA

Shi, Yiting & Yang, Shuhua. (2014)

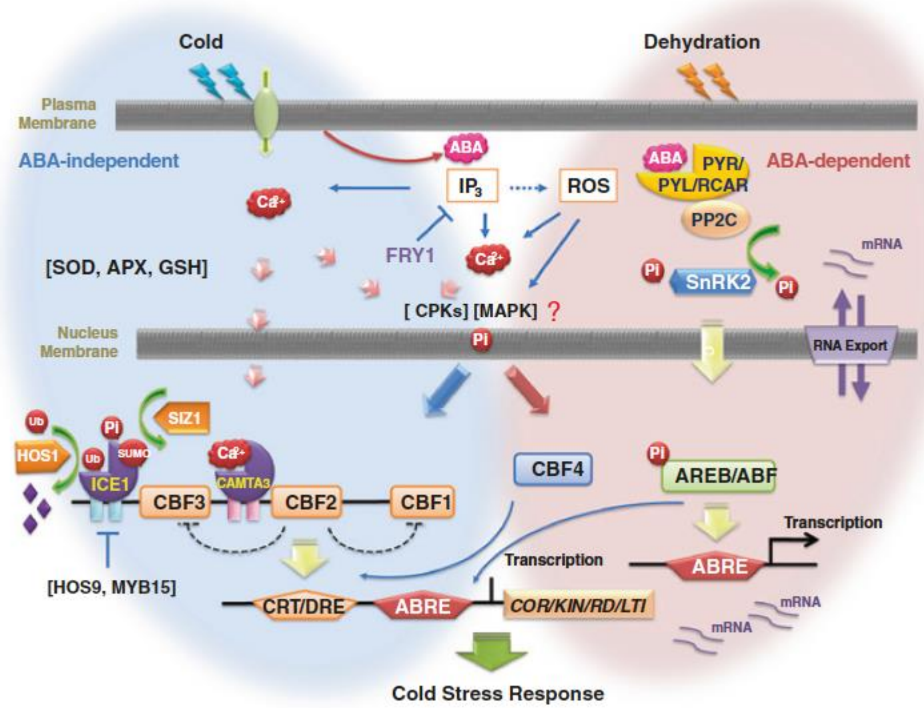


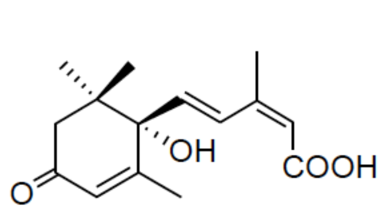
Fig. 17.1 Schematic illustration of the cold and dehydration response regulatory networks in *Arabidopsis*. IP$_3$, Ca^{2+}, and ROS act as second messengers in signalling networks to transduce signals through protein kinases or transcription-factor cascades. CBFs and AREB/ABFs transcription factors are responsible for the regulation of *COR* genes containing CRT/DRE (CCGAC) and ABRE (ACGT) motifs in their promoters, respectively. In *Arabidopsis*, approximately 10 % of the cold-induced transcriptome contains both CRT and ABRE motifs in their promoter regions, which are upregulated by both cold and dehydration stresses. CBFs are activated by ICE1 and CAMTA transcription factors, whereas repressed by MYB15. HOS1 and SIZ1 encode RING E3 ligase and SUMO E3 ligase, respectively, which antagonistically regulate the abundance of ICE1 protein. Cold activates ICE1 protein and AREB/ABFs are induced by ABA-mediated dehydration signalling pathway. *IP$_3$* inositol 1,4,5-triphosphate, *ROS* reactive oxygen species, *CPK* calcium-dependent protein kinase, *MAPK* Ras-mitogen-activated protein kinase, *Ub* ubiquitin moiety, *SUMO* Small ubiquitin-related modifier, *Pi* phosphoryl group

Limitations of ABA as PGR

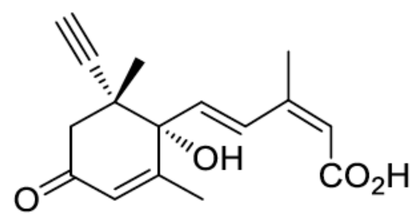
- Few commercial ABA products on the market
- Short half life in plants
- Turned over rapidly in plants to inactive metabolites
  - “Stress” hormone after all
- ABA is also light sensitive and inactive in sunlight

Abscisic Acid (ABA) analogs

- **ABA analogs more effective than natural ABA for improving cold acclimation**
  - 8'-Acetylene ABA
  - Purported to catabolize more slowly in plant tissues
  - Maintain high bioactivity
  - providing enhanced or prolonged effects on dormancy and hardiness



Natural ABA



8′-Acetylene ABA

ABA and ABA analog research

- **Abrams laboratory: Many years of research at NRC and now at Univ of Saskatchewan concerning ABA and analogs**
  - ABA analogs for plant growth regulation
  - ABA metabolism
  - Identification of ABA receptors and binding proteins
  - Method development for hormone and catabolite analyses
- **Various studies have shown that ABA and ABA analogs can be used for:**
  - Improving drought tolerance
  - Thinning of crops
  - Improving colour and hastening maturity in fruits
  - Maintaining dormancy
  - inhibiting germination in seeds

ABA field testing

- Foliar applications may be a practical way to improve hardiness and understand role of ABA better
- Research was conducted on *V. vinifera* grapevines across regions in NA where freeze injury is common
  - ON, BC, Washington, Ohio, Idaho
  - Collaborative effort between Abrams laboratory and Valent Biosciences
    - Examined different formulations and concentrations of ABA as well as timing of application for commercially available formulation from Valent
  - Initial research was funded in part from Valent Biosciences and OVIP

Impacts of exogenous applications of natural ABA

Foliar Applied Abscisic Acid Increases 'Chardonnay' Grapevine Bud Freezing Tolerance during Autumn Cold Acclimatior

Imed E. Dami[1,5], Shouxin Li[1], Patricia A. Bowen[2], Carl P. Bogdanoff[2], Krista C. Shellie[3], and Jim Willwerth[4]

HortTechnology · June 2015 25(3)

Overview of study

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- Collaborative study in 4 distinct grape growing regions
- Exogenous ABA applications at 3 time periods (veraison, post veraison, post harvest)
- Cultivar for study was Chardonnay

Natural ABA impacts on hardiness

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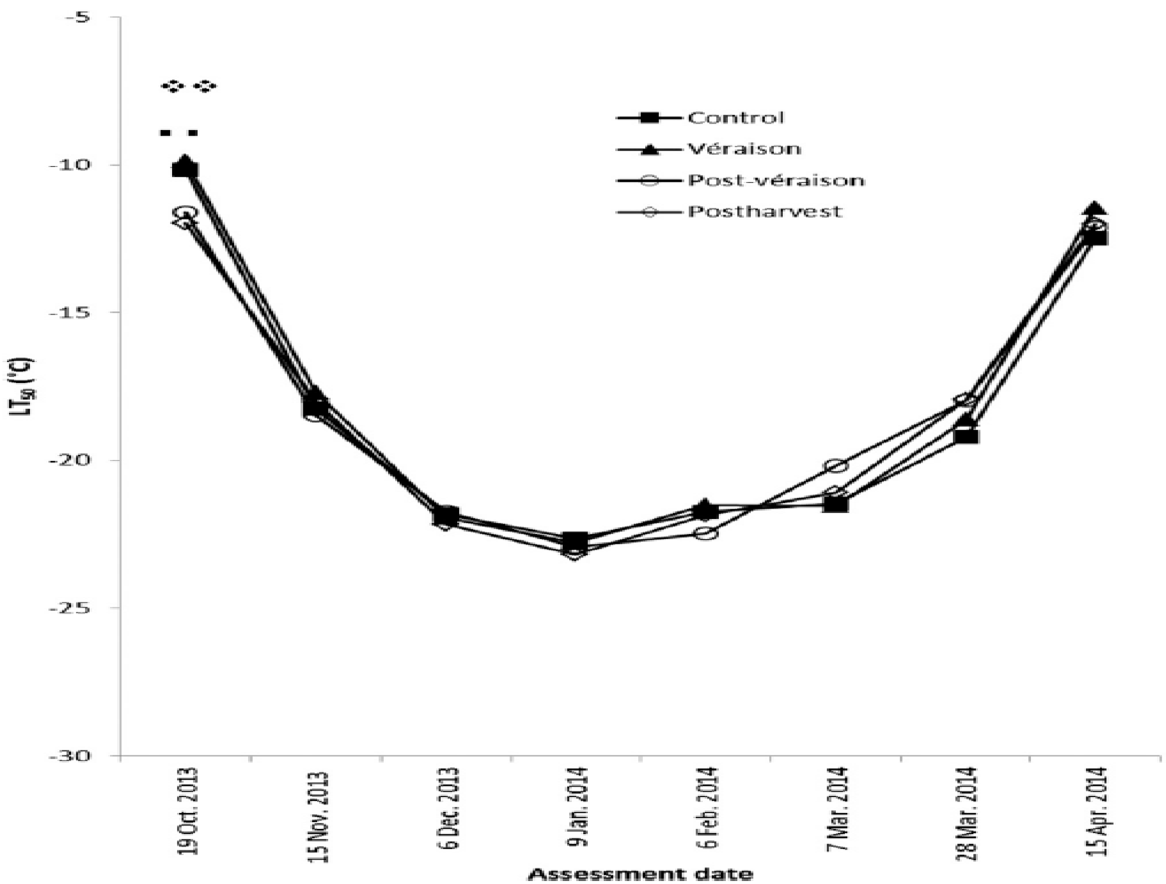

Foliar Applied Abscisic Acid Increases ‘Chardonnay’ Grapevine Bud Freezing Tolerance during Autumn Cold Acclimation

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Fig. 5. Influence of foliar abscisic acid (ABA) applied at véraison (solid triangle), postvéraison (open circle), or postharvest (open diamond) on the lethal temperature for 50% of buds (LT_{50}) of ‘Chardonnay’ grapevine relative to buds from non-ABA control vines (solid square) as determined from low temperature exotherms using differential thermal analysis. Vines were grown in Ontario in 2012–13. Significant difference ($P \leq 0.01$) from control is indicated by ❖❖ (veraison), ■■ (postharvest), respectively; $(1.8 \times °C) + 32 = °F$.

Summary of results

- Some inconsistent effects for timing and regions
- Freezing tolerance improvements during acclimation in 3 of 4 regions
- No real improvements or consistent effects during midwinter and deacclimation periods
- No delay in bud break

Collaborative study examining the use of natural ABA and ABA analogs

Abscisic acid form, concentration, and application timing influence phenology and bud cold hardiness in Merlot grapevines

Pat Bowen, Krista C. Shellie, Lynn Mills, Jim Willwerth, Carl Bogdanoff, and Markus Keller

Can. J. Plant Sci. 96: 347–359 (2016)

Overview of experiment

Cool Climate Oenology & Viticulture Institute

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- Foliar applications of ABA and ABA analogs in 4 distinct regions
- Different forms, concentrations and timing used in Merlot grapevines
- Measured hardiness, monitored bud break/phenology, yield and fruit components

ABA analogs (ABA_A) can delay deacclimation under warm conditions (ON)

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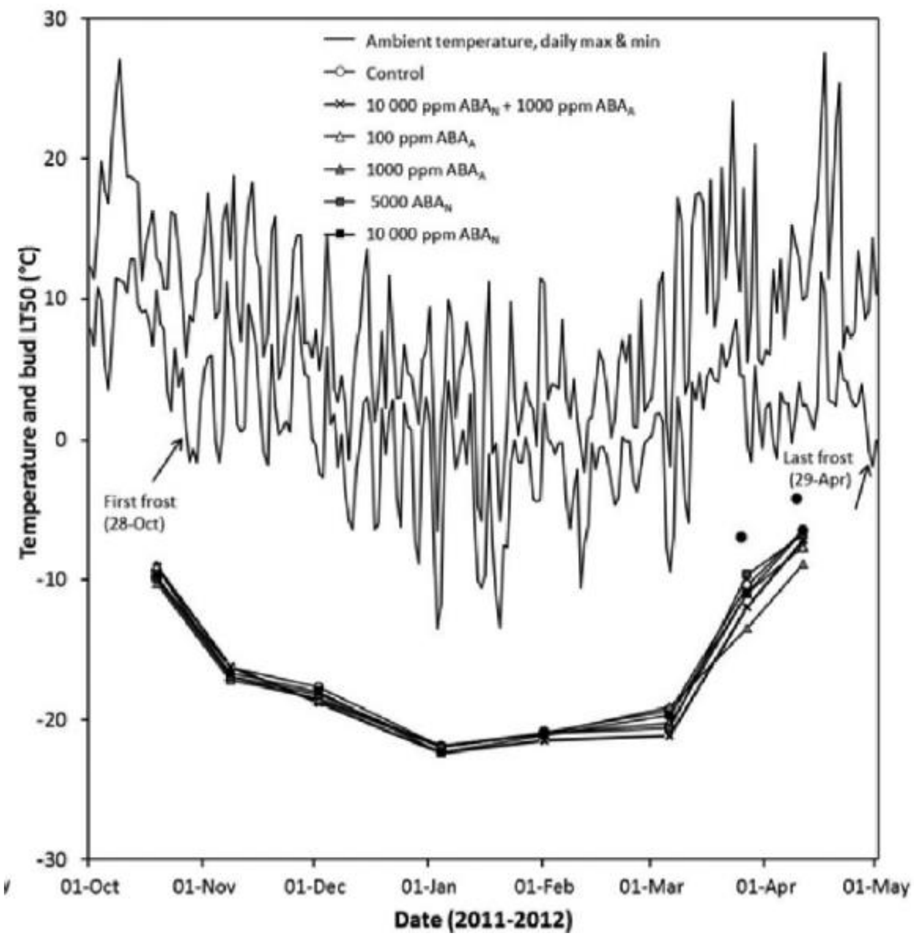

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Fig. 3. Bud cold hardiness of Merlot grapevines in (a) British Columbia, (b) Ontario, (c) Idaho, and (d) Washington during winter 2012–2013 after foliar applications of ABA_A and ABA_N were applied at veraison or postharvest in 2012. Hardiness was identified as the lethal temperature for 50% of sampled buds (LT50). Daily minimum and maximum air temperature and dates of first and last frost at the trial location are presented. Significance of effects: ☼, ABA_A applied at veraison, linear or vs control at $p \le 0.05$; ●, ●●, ABA_A applied postharvest, linear or vs control at $p \le 0.05$ and 0.01, respectively; ◊, ◊◊, ABA_N applied at veraison, linear or vs control at $p \le 0.05$ and 0.01, respectively; ♦, ♦♦, ABA_N applied postharvest, linear or vs control at $p \le 0.05$ and 0.01, respectively; ❖, ABA_N and ABA_A combination applied postharvest vs control at $p \le 0.05$; and ★★, ABA treatments applied at veraison vs postharvest at $p = 0.01$.

ABA analogs (ABA_A) can improve acclimation and delay deacclimation (ON)

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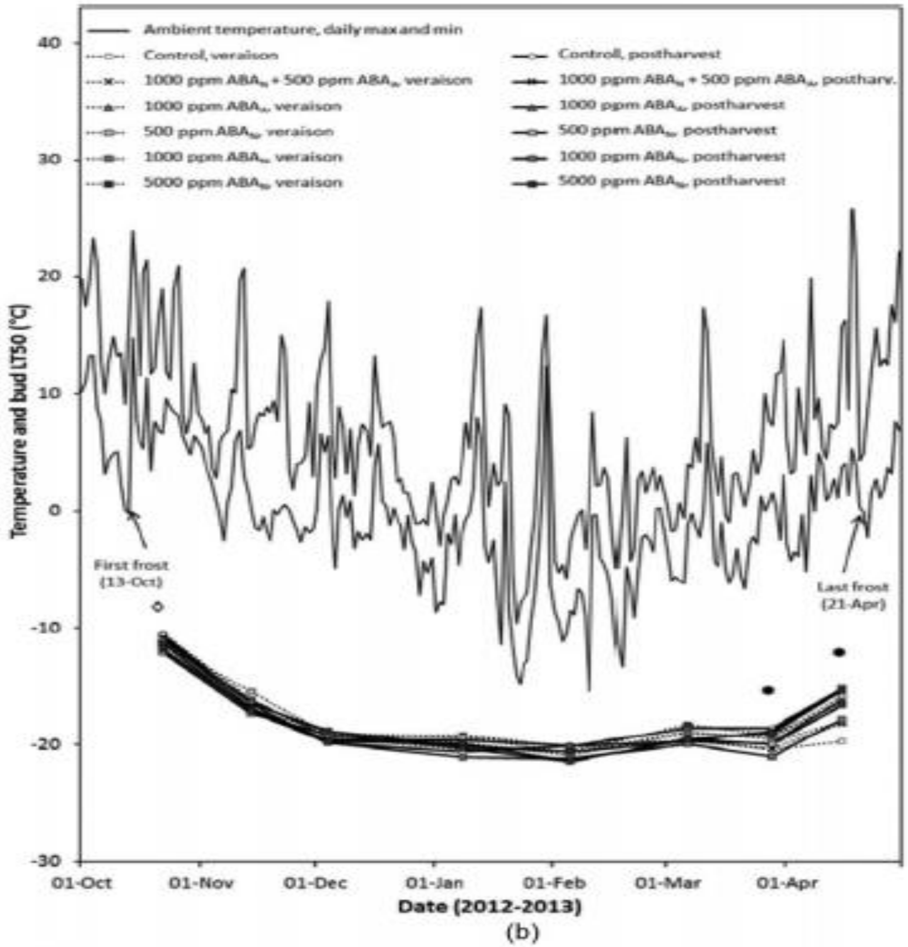

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Abscisic acid form, concentration, and application timing influence phenology and bud cold hardiness in Merlot grapevines

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Delay of bud break in warm winters

Treatment	% Bud break April 27 2012
0.1% Brij 98 (control)	92.7a
100 ppm VBC-30158	11.3c
1000 ppm VBC-30158	10.0c
5,000ppm VBC-30101	74.3b
10,000ppm VBC-30101	69.7b
10,000 ppm VBC-30101 & 1000 ppm VBC-30158	15.0c
Significance of F-value	P<0.0001

ABA$_A$ impact on bud break and early development

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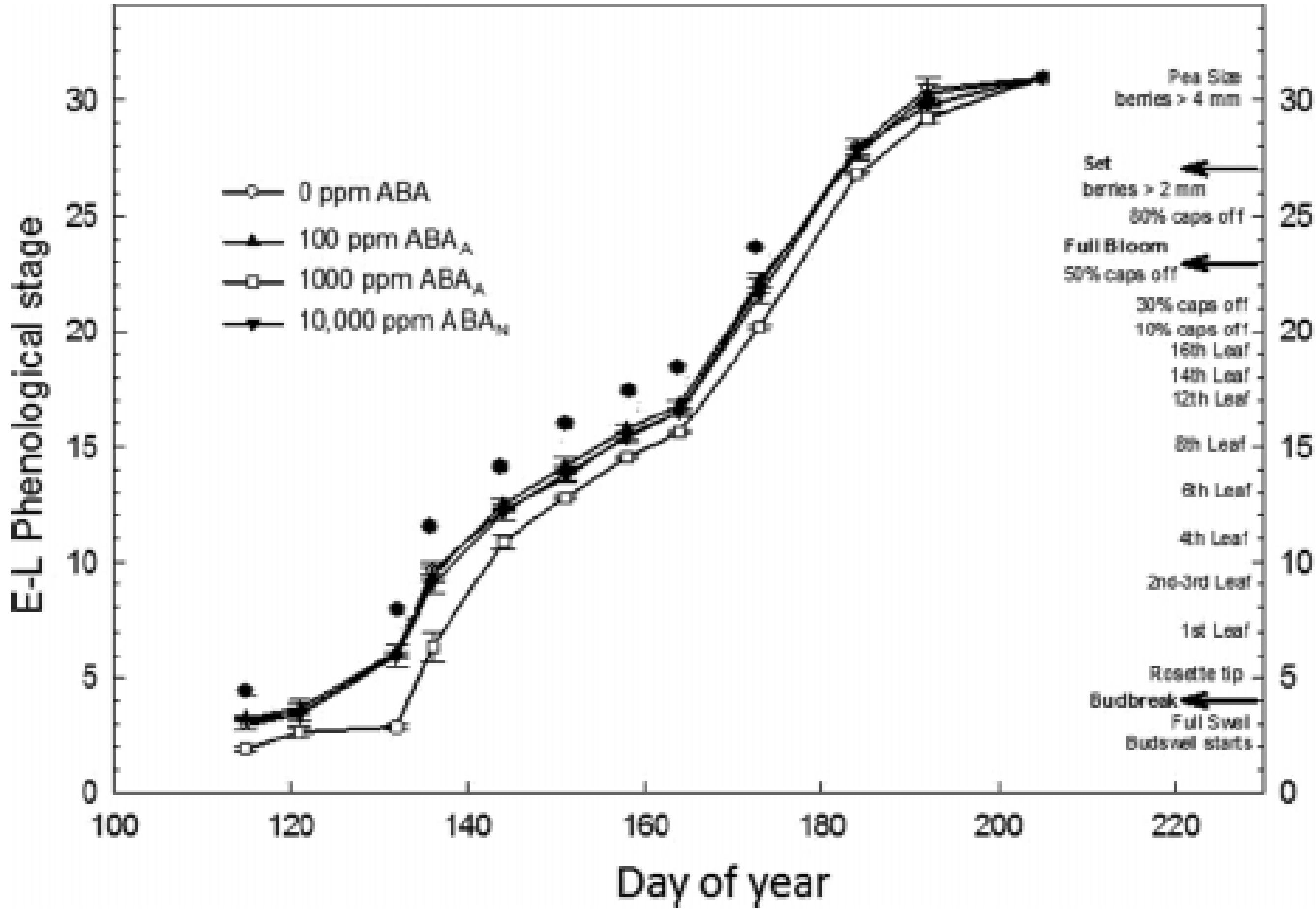

Abscisic acid form, concentration, and application timing influence phenology and bud cold hardiness in Merlot grapevines

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Overall findings

- Natural ABA at higher concentrations advanced leaf abscission and improved hardiness during acclimation
  - Results were more inconsistent and no impacts on delay of bud break even at very high concentrations
- Analogs were more consistent and had greater impacts on improving acclimation (particularly late winter)
- Effects of ABA analogs included delayed deacclimation and bud break.
  - In 2012, delayed bud break over 1 week in Ontario
- Application results and benefits may vary depending on season and growing region.

Current trials

- **Synthesis of long lasting ABA analogs**
  - Abram's laboratory at Univ. of Sask.
  - 2 different compounds compared to natural ABA
- **Field trialing of post harvest applications at different rates for 2 cold sensitive *vinifera* (S. blanc, Merlot)**
- **Measure impact on dormancy, cold tolerance, bud survival, phenology particularly timing of bud break**
- **Biochemical analysis of ABA and catabolites in tissue and how it changes over dormancy**
  - Natural levels vs post-application/analog catabolites?

Preliminary findings
Cold acclimation (fall 2017)

Merlot

Treatment	Date	LTE10	LTE50	LTE90
Control	09-Nov-17	-8.39	-11.38	-14.16
5 g/L ABA	09-Nov-17	-9.95	-12.52	-14.61
1.0g/L ABA 1016	09-Nov-17	-12.25	-13.51	-15.19
0.5 g/L ABA 1016	09-Nov-17	-10.01	-12.44	-14.16
0.1 g/L ABA 1016	09-Nov-17	-11.74	-13.41	-15.41
0.5 g/L ABA 1017	09-Nov-17	-10.82	-12.46	-13.87
0.25 g/L ABA 1017	09-Nov-17	-11.06	-12.71	-14.52
0.05 g/L ABA 1017	09-Nov-17	-11.29	-12.63	-14.23

Treatment	Date	LTE10	LTE50	LTE90
Control	28-Nov-17	-14.38	-17.62	-19.33
5 g/L ABA	28-Nov-17	-16.4	-18.31	-20.95
1.0g/L ABA 1016	28-Nov-17	-16.04	-18.15	-20.36
0.5 g/L ABA 1016	28-Nov-17	-15.73	-18.07	-20.06
0.1 g/L ABA 1016	28-Nov-17	-16.81	-18.05	-20.83
0.5 g/L ABA 1017	28-Nov-17	-15.9	-18.08	-21.79
0.25 g/L ABA 1017	28-Nov-17	-15.61	-17.65	-19.85
0.05 g/L ABA 1017	28-Nov-17	-16.79	-17.91	-19.34

Sauvignon blanc

Treatment	Date	LTE10	LTE50	LTE90
Control	13-Nov-17	-11.82	-15.49	-17.26
5 g/L ABA	13-Nov-17	-15.51	-16.48	-17.48
1.0g/L ABA 1016	13-Nov-17	-15.39	-16.32	-17.36
0.5 g/L ABA 1016	13-Nov-17	-15.24	-16.16	-17
0.1 g/L ABA 1016	13-Nov-17	-15.17	-16.13	-17.45
0.5 g/L ABA 1017	13-Nov-17	-14.24	-16.17	-17.69
0.25 g/L ABA 1017	13-Nov-17	-13.91	-15.88	-18.65
0.05 g/L ABA 1017	13-Nov-17	-13.08	-15.85	-17.52

Treatment	Date	LTE10	LTE50	LTE90
Control	29-Nov-17	-14.24	-18.48	-21.17
5 g/L ABA	29-Nov-17	-18.12	-20.23	-23.37
1.0g/L ABA 1016	29-Nov-17	-17.09	-19.25	-21.67
0.5 g/L ABA 1016	29-Nov-17	-19.35	-20.57	-23.02
0.1 g/L ABA 1016	29-Nov-17	-16.78	-19.14	-21.11
0.5 g/L ABA 1017	29-Nov-17	-18.37	-19.53	-21.24
0.25 g/L ABA 1017	29-Nov-17	-18.09	-19.82	-21.06
0.05 g/L ABA 1017	29-Nov-17	-17.54	-19.35	-20.75

Observed some leaf senescence with ABA but not analogs

Following frost, leaves were healthier on ABA_A vines

Max hardiness (Merlot)

Treatment	Date	LTE10	LTE50	LTE90
Control	06-Feb-18	-19.5	-21.58	-22.65
5 g/L ABA	06-Feb-18	-18.04	-20.3	-23.09
1.0g/L ABA 1016	06-Feb-18	-20.49	-21.23	-23.58
0.5 g/L ABA 1016	06-Feb-18	-18.11	-20.58	-22.8
0.1 g/L ABA 1016	06-Feb-18	-19.27	-21.94	-23.54
0.5 g/L ABA 1017	06-Feb-18	-17.91	-20.92	-23.33
0.25 g/L ABA 1017	06-Feb-18	-18.78	-20.88	-22.27
0.05 g/L ABA 1017	06-Feb-18	-18.89	-22.39	-24.26

Future steps

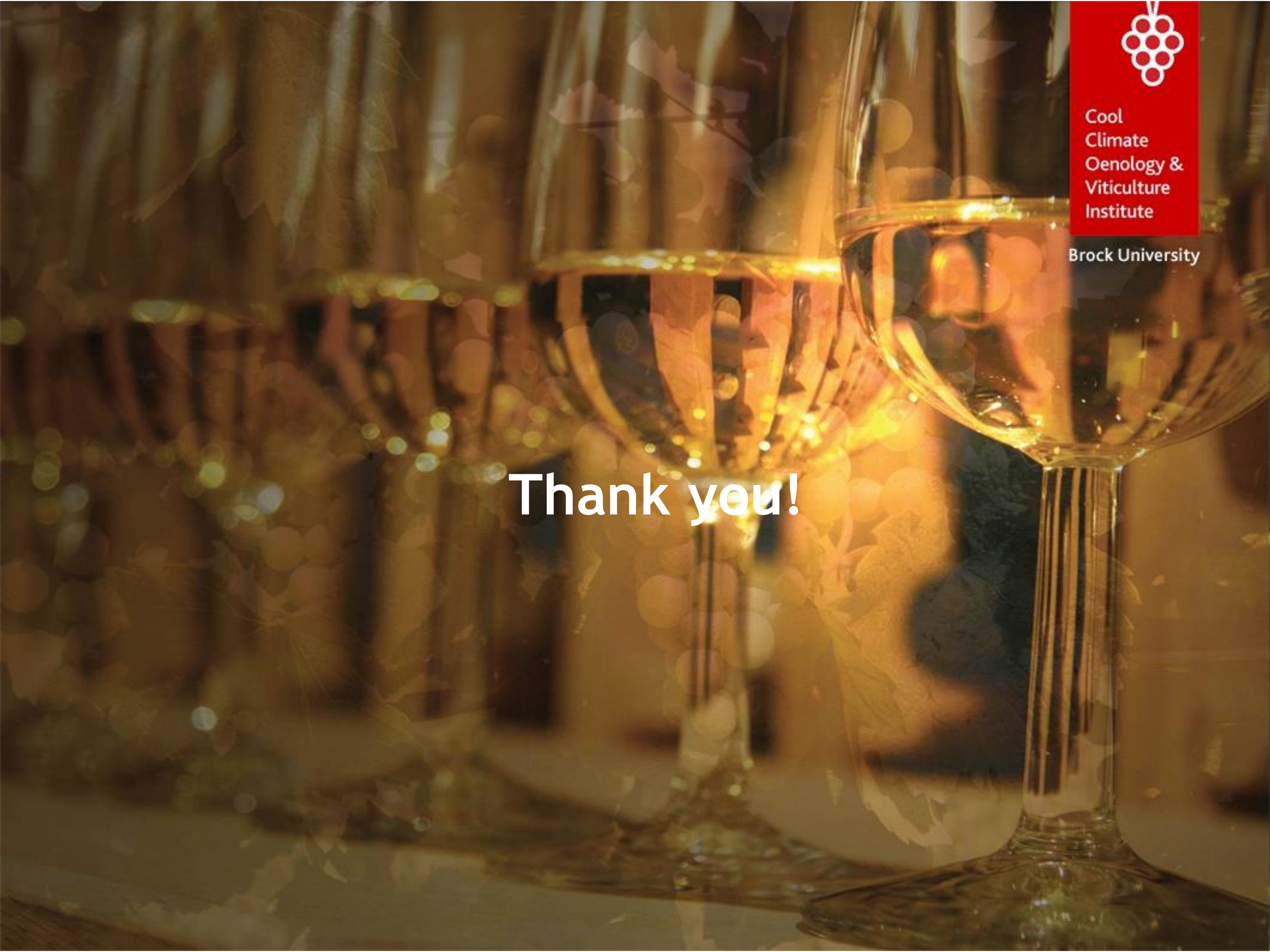
- Continue to track cold tolerance up to bud break
- Determine any impacts on timing of bud break
- Analysis of ABA, ABA_A and their catabolites throughout dormancy from tissue sampling
- Carry over effects?
- Further research and analysis and collaborations based on the findings

Conclusion

- ABA and ABA analogs may be a powerful tool to mitigate climate change risks

Reduce freeze injury due to:

- Enhanced dormancy and acclimation, slow unwanted deacclimation, reduce early bud break
- Form, concentration and timing can matter for desired effects
- Benefits of slowing early development?
- Post bud break applications to address advances in phenology and early maturation in warm climates?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Thank you!