Research in Plain English

X-ray phase contrast imaging of *Vitis spp.* buds shows freezing pattern and correlation between volume and cold hardiness

Research in Plain English provides brief, non-technical summaries of journal articles by Cornell faculty, students, and staff.

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Summary by Rebecca Wiepz.

The takeaway.

- Dormant grapevine buds survive low winter temperatures through supercooling, which allows internal water to remain liquid at temperatures down to a range of -24 to -35° C (-11 to -31 °F).
- During the dormant season, buds pass from endodormancy (non-responsive to warm temperatures) to ecodormancy (where they lose cold hardiness and start development in response to warmer temperatures) following accumulation of sufficient chilling hours (temperatures between 0 and 7°C (32-45°F).
- Bud freezing temperatures are experimentally measured through Differential Thermal Analysis (DTA), which measures heat released from buds, called the “low temperature exotherm" (LTE) during controlled freezing runs. DTAs measure the result of bud freezing.
- Xray phase contrast imaging is a nondestructive technique to observe the process of bud freezing in real time. It allows visualization of the internal structure of the buds without dissecting them (microtomography), and creation of time-lapsed video imagery during the process of freezing.
- The authors used Xray microtomography to image buds of V. *vinifera* (cv “Riesling”), V. *riparia* (wild North American species) and V. *amurensis* (wild Asian species) at different stages of deacclimation and during the process of freezing.
- Different species of buds differed in size, amount of green tissue, and amount of wool material (small hair-like structures) occupying the empty space.
- Increase in bud volume upon freezing was positively correlated with deacclimation possibly indicating that increases in volume and water content are reducing the buds ability to supercool.

- Freezing started in the center of the bud and propagated outwards, and the freezing event took several minutes.

Background.

Cold hardiness is a critical component to the success of agricultural industries in many regions of the world, including New York. Grapevines, in particular, are vulnerable to cold temperatures as *Vitis vinifera*, one of the most common parents of wine grapes, is sensitive to cold temperature. Grapevine buds survive largely by supercooling water in their tissues.

Throughout the winter the accumulation of chilling hours causes the grapevine buds to transition from endodormancy to ecodormancy and break bud. Bud break is typically the standard to assess differences in dormancy, but this relies on similarities across genotypes that may not exist, particularly with respect to the rate at which grapevines deacclimate. Grapevines protect buds from cold temperatures by supercooling water in tissues, and despite the prevalence of cold damage in many agricultural systems, the process of this supercooling is still largely unknown.

Understanding bud morphology and the mechanisms of supercooling in bud tissues is critical to understanding cold hardiness and bud survival. Current techniques for examining bud morphology are generally destructive, while time-resolved x-ray microtomography allows us to observe ice development to determine what causes failure of supercooling and therefore what controls it.

Using x-ray microtomography, this study evaluated bud development of different Vitis species leading to bud break and imaged the freezing of buds to identify where in the bud freezing starts.

Methods.

Buds of three different *Vitis* species, V. *amurensis*, V. *riparia*, and V. *vinifera* were collected at the end of January and placed in water in a cold room. Buds were then placed into forcing conditions and back into cold storage at varying times so buds were at varying states of deacclimation.

Differential Thermal Analysis (DTA) was used to determine cold hardiness as represented by low temperature exotherm (LTE) and then compared with the number of days to bud break. DTA involves laying cut buds onto a thermocouple and freezing them at a slow and constant rate. The thermocouple measures the temperature of the bud, and the moment of freezing and subsequent bud death that creates a low temperature exotherm (heat that is recorded by the

thermocouple), and allows researchers to determine the temperature at which the bud died.

X-ray microtomography involves the use of high energy electromagnetic radiation, which passes through materials at various rates, to create images of the inside of various plant tissues nondestructively.

Bud morphology

The three species of buds were imaged at different points in deacclimation to observe morphological differences within and between species using X-ray technology at the Cornell high energy synchrotron source. By comparing bud tissue volume at different time points to the initial day 0 volume, researchers were able to describe morphological changes in the buds as they approached bud burst.

Clear morphological differences were seen between buds, with reference to size, amount of green tissue, and amount of wool material occupying space, even before deacclimation began. The images below show vinifera, riparia and amurensis buds before being placed in forcing conditions. These difference correlate with differences in areas of origin and also with differences in cold hardiness.

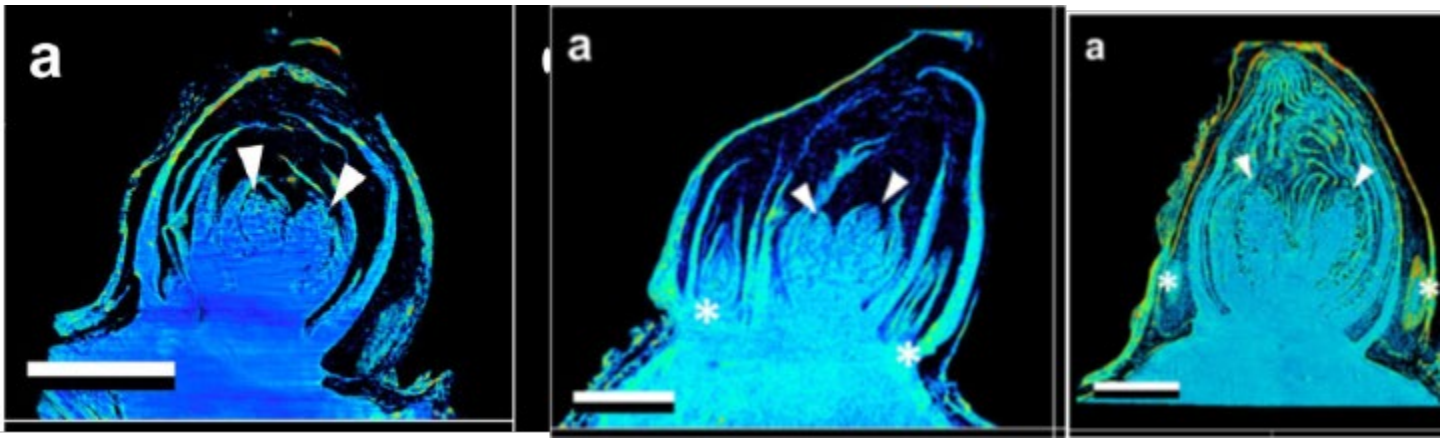


Figure 1. V. *riparia (left)*, V. *vinifera (center) and* V. *amurensis (right)* buds on Day 0, prior to exposure to de-acclimating temperatures. Full arrow heads indicate inflorescences, asterisks indicate secondary and tertiary bud. Scale bar = 1mm.

Both the vegetative structures and cluster primordia were visible despite varying stages of development between species. V. riparia showed very little change in the bud until day 8 but then expanded noticeably. V. vinifera buds only showed noticeable expansion in the base of the primary bud. For V. amurensis, there was no visible difference in bud development between days 0 and 5. The image below compares riparia and vinifera buds at 13 days under forcing conditions, and the expansion of the primary buds is very noticeable.

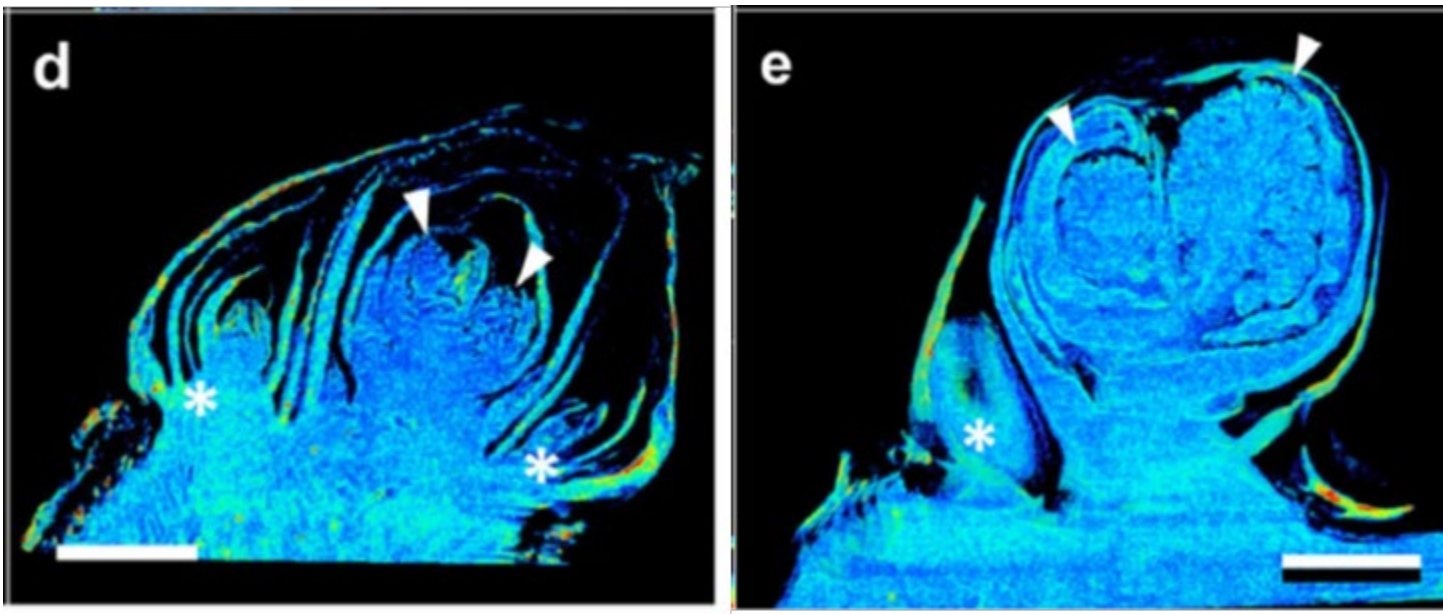


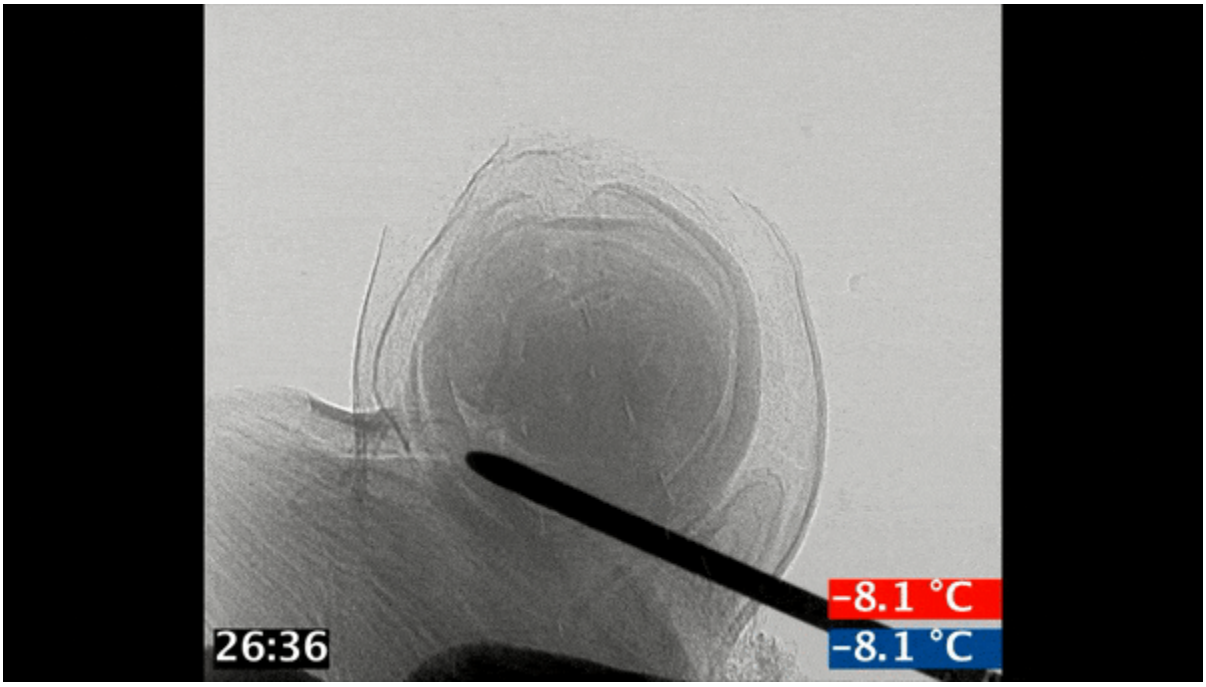
Figure 2. V. *vinifera* (d-at left) buds showed very little growth after 13 d of deacclimation, while V. *riparia* (e-at right) showed expanded stem and cluster development, indicating more rapid response to deacclimation temperatures. Full arrow heads indicate inflorescences, asterisks indicate secondary and tertiary bud. Scale bar =1mm.

Cold hardiness and X-ray microtomography imaging.

Bud freezing temperatures (LTEs) from standard DTA measurements were compared to X-ray microtomography imaging. To freeze the buds while imaging, a stream of cold air (cryostream) was passed over the top of the bud and a thermocouple probe inserted into the bud's base. Despite more rapid chilling using the cryostream, bud freezing temperatures (LTEs) were comparable to those collected by standard DTA methods.

Buds were frozen and imaged using 2D time-lapse imaging. The images at 'time 0' were compared to subsequent images to analyze changes in different regions of the bud. By comparing the expansion of different areas of the buds over time, the images collected clearly show that tissue freezing starts at the center of the bud and propagates outward. The freezing event is not instantaneous, as previously thought from DTA, but rather lasted several minutes. Secondary and tertiary buds freezing were completely separate events from primary bud freezing.

The supplementary video below shows the expansion of the buds during their freezing event, although the differences are rather subtle. To see the expansion, watch the right side of the bud right after 28 seconds. Elapsed time in the bottom left. The temperature of the needle probe is in the red box, the blue box is external temperature.



Increase in volume upon freezing was positively correlated with deacclimation, possibly indicating that increases in volume are reducing the buds' ability to supercool. As can be seen in figure 3, V. *riparia* deacclimated much more quickly, and increased in volume at a much higher rate than V. *vinifera*, particularly after day 5.

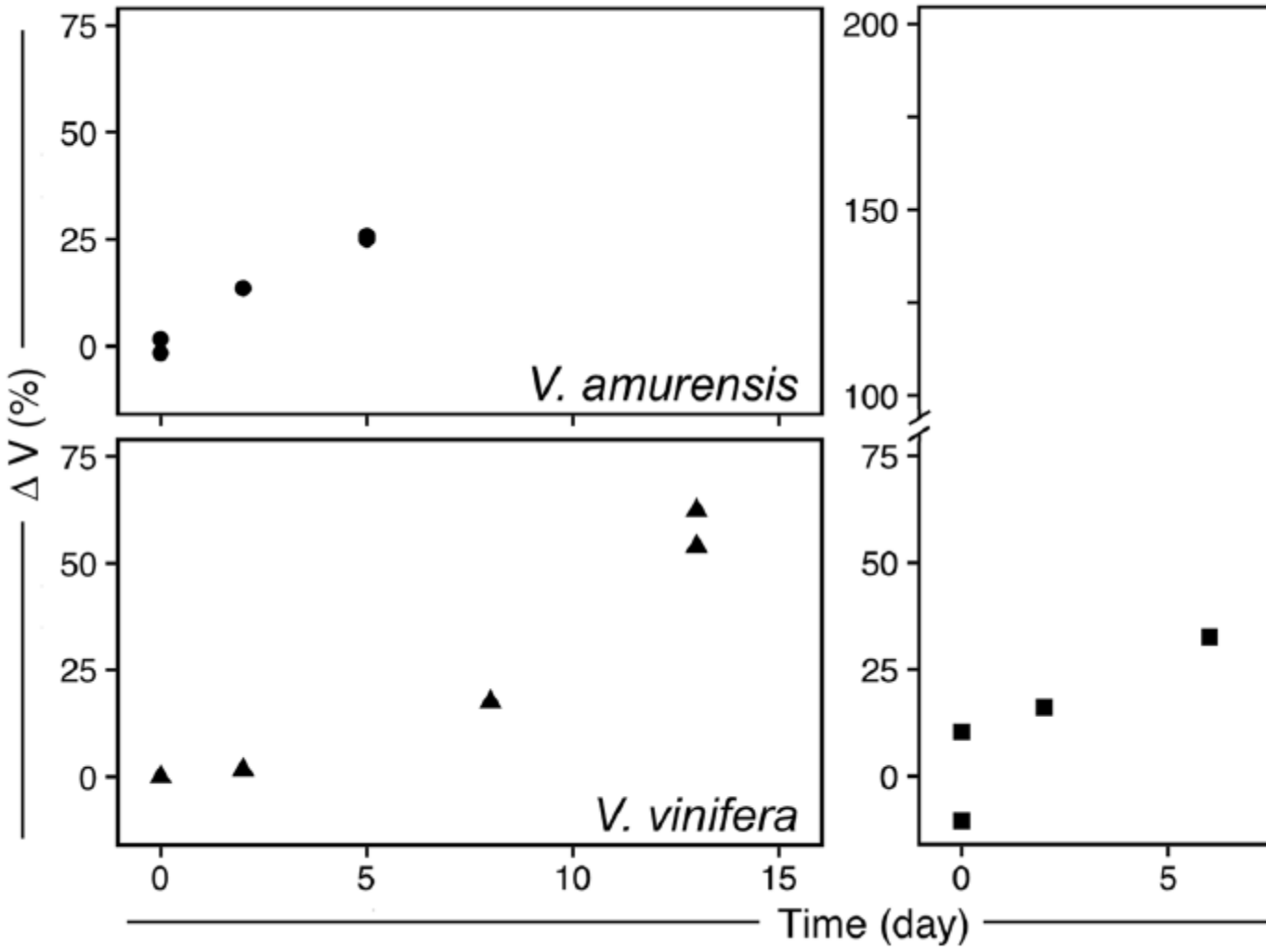


Figure 3. Increase in volume (D V) of V*itis amurensis*, V. *riparia*, and V. *vinifera* during deacclimation. Volume was determined by counting the number of 'voxels' (3d pixels) in xray tomography reconstructed buds, therefore not including air space. D v was calculated as the percent increase in volume from sample (or average of samples) at day 0. Each point represents an individual sample.

Conclusions and looking forward.

X-ray microtomography proved to be a useful approach to investigating bud development and cold hardiness. Although there was concern for the new method of potential cell death due to x-ray exposure and placement of the thermocouple probe, neither affected the bud readings.

This study demonstrated that differences in bud morphology are directly correlated with differences in their cold hardiness, and also showed that freezing propagates from the inside of the bud, potentially due to an increase in size causing

a failure of the supercooling mechanism. Finally, the freezing event lasts several minutes, unlike what was previously thought based on the brief peak in standard DTA.

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