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# Tissue Moisture Loss during Sample Preparation Lowers Exotherm Temperatures in Dormant Grape Buds

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***Abstract.*** **Grapevine cold hardiness is often assessed with differential thermal analysis (DTA) of excised dormant buds. Such small tissues are prone to rapid dehydration when exposed to air during sample preparation. We show that excised buds of grape cultivars 'Vignoles' and 'Norton' lose as much as 6.3% and 2.9% of their total water content, respectively, during a two-minute exposure to air at 24 °C. In order to assess the impact of moisture loss on cold hardiness measurements, we prepared dormant bud samples with reduced water content and subjected them to DTA. The results demonstrate a positive correlation between average gross bud water content and median low temperature exotherm ($LTE_{mean}$). In 'Vignoles' and 'Norton' buds, a 6.5% and a 4.3% reduction in gross water content, respectively, were sufficient to result in lower LTE temperatures ($P < 0.001$). The data suggest that even moderate dehydration of excised grape buds may influence the results of cold hardiness assessment by DTA. It is important that investigators be vigilant to the potential artifacts that can arise during sample preparation in order to ensure that the LTE temperatures of samples reliably characterize the cold hardiness of field populations.**

Cold injury to dormant grapevine buds causes serious losses to the viticulture industry in cool-climate production regions. Cold hardiness of grapevine buds is determined by the ability of cytoplasmic water in shoot primordia to supercool. At a certain subfreezing temperature, however, the cytoplasmic water loses its ability to remain in liquid phase and crystallizes into ice. Ice formation within plant cells is lethal, and therefore the temperature at which ice forms represents the limit of cold hardiness. When water crystallizes into ice, heat is released. This heat, termed the heat of fusion, can be detected using differential thermal analysis (DTA). DTA typically involves placing excised buds or other tissue sections in contact with thermoelectric modules, cooling them in a controlled environment, and electronically recording the temperatures at which freezing occurs (Wample et al., 1990). Freezing events that occur in dormant shoot primordia are referred to as low temperature exotherms (LTEs). Median LTE ($LTE_{mean}$) temperatures approximate field temperatures that are lethal to 50% of buds ($LT_{50}$), and thus DTA has become a preferred technique for the characterization of grapevine cold hardiness (Clark et al., 1996; Gu, 1999; Wolf and Cook, 1994).

Water content of dormant buds was shown to be inversely related with cold hardiness in several woody species (Bittenbender and Howell, 1975; Johnston, 1923; Quamme, 1983; Wiegand, 1906). It is not well understood, however, how tissue water content affects cold hardiness in dormant grapevine buds. Wolpert and Howell (1984) observed that a gradual decrease in gross bud water content correlated with an increase in cold hardiness in primary buds of the *Vitis labrusca* hybrid cultivar 'Concord' during a 14-week acclimation period. They found in another study, however, that the inverse relationship between these parameters did not exist through the entire dormant season (Wolpert and Howell, 1985). Similarly, Hamman and coworkers (1990) reported that changes in bud water content were not consistently paralleled by changes in primary bud cold hardiness in *Vitis vinifera* cv. 'Merlot' during a 5-week deacclimation period. All these observations were made on buds whose moisture level changed naturally under field conditions, and therefore the effect of moisture on cold hardiness was confounded by the effect of physiological events and environmental factors such as air temperature and day length.

We observed that even a short extension of the sample preparation time lowers the temperatures at which LTEs occur (Du and Kovacs, unpublished results). A potential explanation for this is that evaporative water loss during sample preparation would lead to a decline in tissue moisture, which, in turn, may depress the temperatures at which LTEs occur. The purpose of this present work was to determine the impact of dehydration on DTA assessment of cold hardiness. The working hypothesis was that a reduction in average gross water content in dormant bud samples is associated with lower $LTE_{mean}$ temperatures.

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## Materials and Methods

*Plant material*. The experiments were performed on dormant buds from twelve year old 'Norton' (*Vitis aestivalis*) and 'Vignoles' (*Vitis vinifera* x *Vitis rupestris* x *Vitis aestivalis*) vines grown at the Missouri State Fruit Experiment Station in south-central Missouri. 'Norton' and 'Vignoles' vines were trained to Geneva Double Curtain and Scott Henry systems, respectively. Buds were taken from well-exposed canes that ranged from 0.6 to 0.9 mm in diameter. Cane segments containing nodes at positions 2 through 7 (position 1 being nearest to the base) were collected from unpruned vines on 18 Jan. and on 16 Feb. 2000 for the first and second experiments, respectively. The canes were tightly wrapped in plastic and incubated at 0 °C for 10 d prior to processing.

*Bud tissue moisture evaporation.* In preliminary experiments, we observed that water evaporation from excised buds was so rapid that it resulted in an excessively high sample variation. In order to slow and thereby better control the evaporation process, bud water was allowed to evaporate through the vascular tissues of attached stems. This was achieved by the excision of 2-cm-long nodal segments and the exposure of segments to air under controlled environmental conditions. The node on the excised segment was located at 1 cm from either end. Only segments of node positions 3 to 6 were used, those of positions 2 and 7 were discarded. A sample of 36 segments was placed on open Petri dishes, and another sample of 36 segments was tightly wrapped in plastic. While enclosure in plastic does not completely prevent tissue water loss, it impedes dehydration. The exposed and plastic-wrapped samples were incubated simultaneously at 0 °C in the same incubator. Incubation periods were 180, 270, 360, 450, and 540 min. Bud samples from exposed and plastic wrapped segments will be referred to as treated and control samples, respectively.

*Differential thermal analysis*. Following the evaporation treatment, buds were excised from cane segments and immediately mounted on the surface of thermoelectric modules (Melcor, Trenton, N.J.). The cut surface of the buds directly contacted the surface of the modules, and the buds were held in place by parafilm. Each module held four buds, which represented one replicate. In the first experiment, most samples were tested in nine replicates, but several could be tested in only six to eight replicates because of module error. In the second experiment, each sample was successfully tested in nine replicates. The modules with the buds were placed in a chamber inside a programmable freezer (Tenney Environmental, Williamsport, Pa.). The chamber was cooled from a starting temperature of 5 °C to a final temperature of –36 °C at a rate of 4 °C /h. Chamber temperature and thermoelectric module voltage were recorded on a personal computer every 10 s. High-temperature exotherm (HTE) module voltage peaks occurred only above –8 °C for both 'Vignoles' and 'Norton' buds, and were sepa-

rate and distinguishable from LTE voltage peaks. For each module, the four highest LTE voltage peaks were considered the result of freezing events in primary shoot primordia. Because the primary shoot primordium is fruitful and is of economic importance, its LTE temperature is considered to be the practical limit of the buds cold hardiness, even though that temperature may not be lethal to the secondary and tertiary shoot primordia. Therefore, the terms "bud LTE" and "bud cold hardiness" refer to the LTE and cold hardiness of the primary shoot primordia.

*Statistical analyses*. $LTE_{mean}$ temperatures from treated bud samples were compared to those from corresponding control samples with a two-sample *t* test. The strength of the relationship between average gross water content and $LTE_{mean}$ temperatures of the bud samples was determined with least-squares regression analysis. The *t* test and least-squares regression analysis were performed with Axum 6 data analysis software (MathSoft, Seattle).

*Gross bud water content.* Gross bud water content is defined here as the amount of water collectively contained in the bud scales and in the primary, secondary, and tertiary shoot primordia, and is expressed as the percentage of total bud fresh weight. At the completion of the evaporation treatment, each cane segment was weighed, its bud was excised and immediately attached to a thermoelectric module. The remaining cane tissue was then weighed again. This method allowed the precise measurement of bud weight with minimal exposure of the excised buds to ambient air. Following DTA, the buds were oven-dried and their dry weight was determined. Water evaporation from excised buds exposed to ambient air was monitored by placing samples of 10 freshly excised buds on an analytical scale and recording their total mass every minute for a 10 min period. Water evaporation was monitored under two different conditions: in ambient air of 24 °C and 53% relative humidity (RH) and in ambient air of 2 °C and 78% RH. Monitoring was performed three times, each time with a separate freshly excised 10-bud sample. Subsequently, the samples were oven-dried and their dry weight was determined. Gross bud water content was calculated as the difference between fresh and dry weight and was expressed as a percentage of fresh weight.

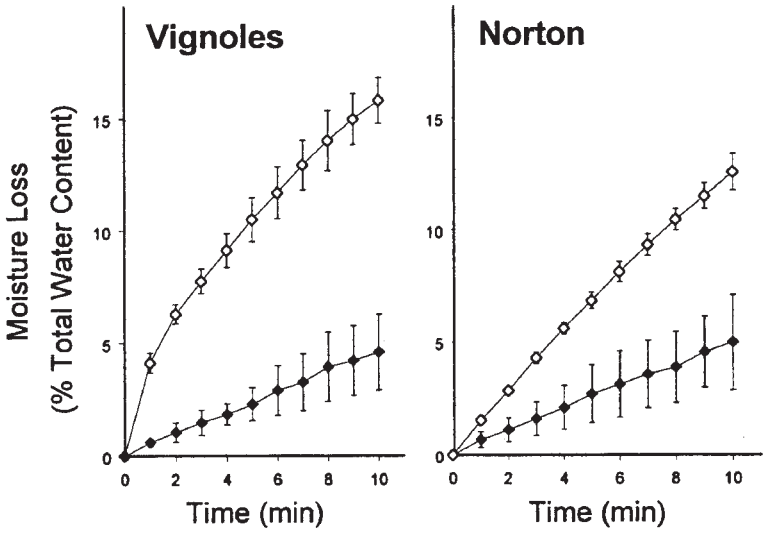


Fig. 1. Loss of tissue water from excised dormant 'Vignoles' and 'Norton' grape buds exposed to air. Open and filled symbols indicate moisture loss at 24 °C and 2 °C, respectively; error bars represent standard deviation in the data derived from three measurements.

## Results and Discussion

*Evaporation of tissue water from buds exposed to air.* Cold hardiness of grape cultivars is commonly assessed by DTA on excised buds. For 'Norton' and 'Vignoles', the mass of such excised buds ranges from 12.5 to 32.5 mg and from 10.0 to 32.5 mg, respectively. Explants of such small size can lose tissue water rapidly through cut surfaces. Results of our measurements showed that excised dormant 'Vignoles' buds lost 6.3% of their gross water content during a 2-min exposure to air at room temperature (24 °C), and lost as much as 10.5% of their water content during a 5-min exposure (Fig. 1). 'Norton' buds lost 2.9% and 6.9% of their water content during a 2- and 5-min exposure, respectively, at room temperature. The rate of dehydration was considerably slower at 2 °C: 'Vignoles' and 'Norton' buds lost 2.3% and 2.7% of their water content, respectively, during the first 5 min of air-exposure (Fig. 1).

*Gross water content and $LTE_{mean}$ temperature.* To test whether the loss of moderate amounts of tissue water influences the temperatures at which LTEs occur, we prepared bud samples of reduced water content and subjected them to DTA. We conducted two independent experiments and obtained similar results in both. Table 1 summarizes the results of the second experiment. 'Vignoles' bud samples that freely evaporated water for 180 to 540 min ranged in average gross water content from 29.2% to 38.6 %, and in mean LTE temperatures from –21.2 °C to –24.4 °C. The corresponding control buds whose water evaporation was impeded ranged in water content from 41.5% to 47.4%, and in mean LTE temperatures from –13.7 °C to –17.1 °C. The lowest and highest LTE temperatures ($LTE_{max}$ and $LTE_{min}$, respectively) also were consistently

Table 1. Cold hardiness parameters of dormant grape bud samples with various gross water content.

| Grape | Treatment | Water content[y] (%) | | $LTE_{mean}$[x] (°C) | | $LTE_{max}$[w] (°C) | | $LTE_{min}$[w] (°C) | | $LTE_{20}$[v] (°C) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| cultivar | time[z] (min) | Control | Treated | Control | Treated | Control | Treated | Control | Treated | Control | Treated |
| Vignoles | 180 | 45.5 | 38.6 | –15.3 ± 2.5 | –22.3 ± 2.9*** | –10.8 | –15.9 | –20.7 | –30.0 | –13.4 | –19.7 |
| | 270 | 47.4 | 36.9 | –16.9 ± 3.4 | –21.3 ± 4.4*** | –10.0 | –14.3 | –26.5 | –30.8 | –14.0 | –16.9 |
| | 360 | 41.5 | 35.0 | –17.1 ± 2.6 | –21.2 ± 4.1*** | –13.1 | –14.6 | –22.0 | –30.5 | –14.4 | –17.3 |
| | 450 | 45.1 | 33.0 | –13.7 ± 2.9 | –24.4 ± 4.0*** | –8.7 | –18.5 | –19.1 | –33.3 | –10.8 | –20.5 |
| | 540 | 46.0 | 29.2 | –15.6 ± 3.8 | –23.2 ± 3.6*** | –8.8 | –16.8 | –24.1 | –28.9 | –12.7 | –20.0 |
| Norton | 180 | 43.1 | 40.3 | –16.1 ± 3.7 | –18.6 ± 4.8* | –11.6 | –11.2 | –22.6 | –26.6 | –12.6 | –13.7 |
| | 270 | 40.8 | 36.5 | –13.9 ± 3.8 | –20.2 ± 5.2*** | –9.6 | –12.7 | –23.1 | –30.7 | –10.6 | –16.1 |
| | 360 | 41.9 | 36.1 | –16.3 ± 4.4 | –22.6 ± 6.1*** | –8.2 | –13.5 | –25.8 | –31.5 | –12.0 | –15.3 |
| | 450 | 41.1 | 34.6 | –17.9 ± 5.8 | –24.5 ± 6.2*** | –9.1 | –15.0 | –29.9 | –33.6 | –12.8 | –17.4 |
| | 540 | 41.0 | 30.8 | –16.4 ± 5.0 | –26.3 ± 5.9*** | –10.0 | –17.2 | –29.2 | –34.0 | –12.1 | –19.0 |

[z]Length of time for which buds were allowed to lose moisture through attached stem segments at 0 °C in order to adjust their water content.
[y]Average gross water content of a sample of 36 buds expressed as percentage of fresh weight. In treated samples, water content was adjusted by unrestrained evaporation; in control samples, evaporation was impeded by plastic wrapping.
[x]$LTE_{mean}$, mean of temperatures (±SD) at which low temperature exotherms (LTEs) occurred in a sample of 36 buds.
[w]$LTE_{max}$ and $LTE_{min}$, temperature at which the highest and the lowest LTE, respectively, occurred in a sample.
[v]$LTE_{20}$, temperature above which 20% of the buds in a sample produced an LTE.
*, *** LTE temperatures differ from the corresponding control at the 95% to 99.9% probability levels, respectively.

lower for samples of lower average gross water content, and higher for buds of higher water content. The $LTE_{20}$ temperature, the parameter indicating the highest lethal temperature at which bud injury has an economic impact, also was consistently lower for buds of lower water content. The same relationships can be observed between the average gross water content and the $LTE_{mean}$, $LTE_{max}$, $LTE_{min}$, and $LTE_{20}$ temperatures in the bud samples of 'Norton' (Table 1). The *t* test analyses revealed that the $LTE_{mean}$ temperatures for the treated buds were lower than those of the corresponding controls at the 95% to 99.9% probability level (Table 1).

Fig. 2 shows the effect of tissue water loss on the percent of buds injured by lethal temperatures in treated and in control samples. This figure presents the original LTE temperatures data for buds whose moisture evaporation was facilitated or impeded for 540 min and that were the source of the cold hardiness parameters presented in lines 5 and 10 of Table 1. The graphs of Fig. 2 demonstrate that the same lethal temperatures result in lower injury levels in samples of reduced water content than in the corresponding control samples.

In order to further examine the association between tissue moisture and LTE temperature, we performed a regression analysis, where average gross water content was the explanatory variable and the $LTE_{mean}$ temperature was the response variable. We found that the relationship between these two variables could be adequately described by a linear response in the range of 29% to 45% water content. The square of the correlation ($r^2$) was 0.74 and 0.87 for 'Vignoles' and 'Norton', respectively, and the linear response was statistically significant at the 99.9% probability level in both cultivars (regression lines not shown).

We conclude from these results that lower average gross water content is associated with lower $LTE_{mean}$ temperatures in dormant 'Vignoles' and 'Norton' grape bud samples. The experimental conditions applied in this study allow us to infer that the decrease in $LTE_{mean}$ temperatures was a consequence of the reduction in water content and was not significantly influenced by other factors. Consequently, evaporative water loss that occurs during sample preparation is likely to depress LTE temperatures in 'Vignoles' and 'Norton' buds, and may lead to overestimation of cold hardiness in buds of other grape cultivars also.

*Implications of the results in DTA studies.* The data presented here indicate that a decline in tissue moisture during sample preparation is a likely source of experimental error in DTA. Tissue water can evaporate not only during sample preparation, but also during extensive periods of treatment or storage prior to DTA. In experiments where tissues are stored or pretreated, the samples are usually wrapped in plastic (Pierquet et al., 1977; Wolf and Pool, 1987). While enclosure in plastic impedes evaporation, it does not completely prevent water loss, and a decline in tissue moisture can occur over an extended period of time. We propose that tissue preparation procedures be carefully standardized and that preparation time be minimized in order to reduce experimental error in DTA studies. Furthermore, in experiments where extended periods of storage and pretreatment are applied, the water content of the tissues at the time of DTA should also be determined and reported.

Interestingly, it has been noted in various plant species that small tissue sections tend to test hardier than larger sections of the same tissue (Ashworth, 1990; Gross et al., 1984; Scarascia-Mugnozza and Valentini, 1989). This recurrent observation led several authors to question the accuracy of cold hardiness assessment by DTA (Barney, 1989; Ceccardi et al., 1995; Flinn and Ashworth, 1994). In the light of our results, it is tempting to speculate that the relationship between tissue size and cold hardiness may reflect the effect of water evaporation: sections of smaller size lose relatively more of their moisture because their surface to mass ratio is higher than that of larger sections. An adequate discussion of the tissue size–cold hardiness relationship, however, requires further investigation.

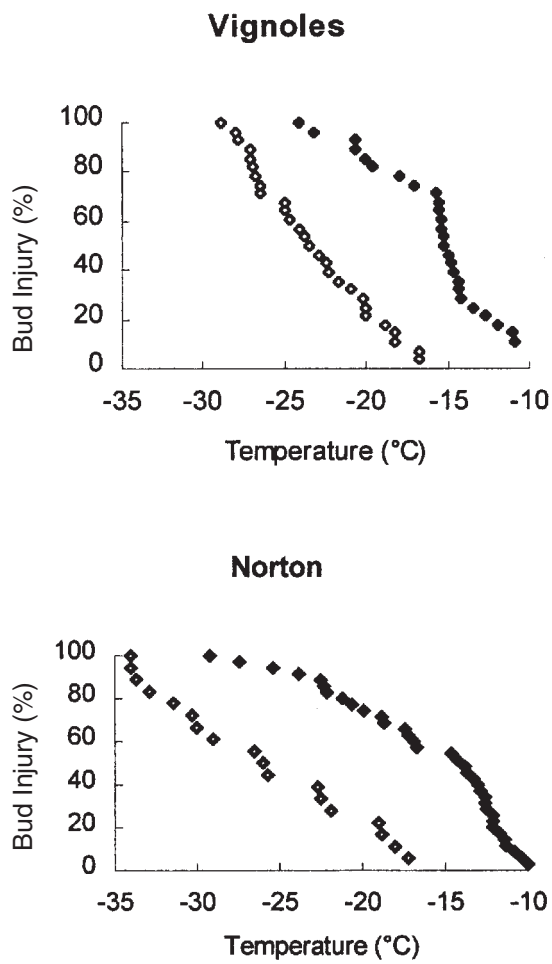


Fig. 2. Lethal temperature-bud injury response curves of grapevine bud samples of differing water content. LTE temperatures of primary buds were determined by DTA and plotted against the percentage of injured buds. Open symbols corespond to buds in samples of reduced water content 29.2% and 30.8% for 'Vignoles' and 'Norton', respectively); filled symbols correspond to buds in control samples (46.0% and 41.0% water content for 'Vignoles' and 'Norton', respectively).

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