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Factors Affecting Exotherm Detection in the Differential Thermal Analysis of Grapevine Dormant Buds

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Abstract. **Differential thermal analysis was evaluated as a means of determining the cold hardiness of excised dormant buds of *Vitis vinifera* L. cv. Chardonnay grapevines. The manner in which buds were excised and cooled affected the freezing characteristics of bud primordia. Buds excised with 1 to 2 mm of subjacent nodal tissue exhibited both high temperature exotherms (HTEs) and low temperature exotherms (LTEs). HTEs apparently resulted from freezing of supercooled moisture in bud scales and/or in the subjacent nodal tissue and occurred at inconsistent temperatures. Cooling similarly excised buds on a water-saturated substrate caused HTEs to occur at −4° to −8°C and did not affect the occurrence of LTEs, which were consistently associated with primordia death. Median LTEs associated with primary bud death were 1.5° to 2.0° warmer than LT_{50}s derived from temperature/survival freezing tests of similar buds. Buds killed by freezing did not supercool appreciably when refrozen. Bud cold hardiness increased when single-node cuttings were exposed to a step-wise cooling cycle; however, the ability to detect exotherms decreased under these conditions. The decreased detection of exotherms was due to increased bud death and, presumably, a decrease of freezable (and thus detectable) moisture in the supercooled primordia of viable buds. DTA provides a useful and reliable means of determining grapevine bud cold hardiness when conducted in a standardized fashion.**

Dormant grapevine buds escape freezing by supercooling (2, 6, 15). This mechanism has two important viticultural ramifications. First, the survival of grapevine buds and their potential crop appears from previous reports (2, 15) to be limited by the tendency of individual shoot primordia (typically three) of the compound bud to supercool. Ice nucleation of supercooled tissues results in intracellular freezing, which is invariably lethal (13). A second and related implication is that the detection of bud freezing provides information on bud cold hardiness. A reliable index of plant cold tolerance is central to the conduct of cold hardiness experiments. Although bud survival is not necessarily indicative of plant survival, it is one index by which viticulturists can gauge vine response to cultural practices or other treatments affecting vine cold hardiness.

The freezing of supercooled tissues can be monitored using thermal or differential thermal analysis (DTA), which detects heat evolved (exothermic) by the freezing of supercooled fluids (6). DTA of the reproductive buds of a number of species typically reveals both high temperature exotherms (HTEs) as well as low temperature exotherms (LTEs) (2, 3, 8, 9, 12). LTEs have been associated with floral primordia death, while the HTEs are considered to result from the freezing of moisture in the bud scales and/or subjacent nodal tissue.

Pierquet and Stushnoff (14) first described a relationship between LTEs and cold injury in dormant grapevine buds. Primary bud death was observed consistently when excised buds of *V. riparia* were cooled at 110° to 120°C/hr and removed immediately after the occurrence of an LTE. When other buds were cooled to within several degrees of the average LTE temperature and promptly removed, bud injury was absent, provided LTEs had not occurred. The relationship between LTEs and grapevine bud injury was reexamined by Andrews et al. (2) using 'White Riesling' (*V. vinifera*) buds cooled at 2°/hr. Exotherms were detected with noninvasive thermoelectric modules (1). Invariably, when buds were removed from the freezer immediately after the occurrence of LTEs, injury to primary buds was observed. Multiple LTEs from a single bud reportedly resulted from independent freezing of the individual shoot primordia of the compound bud.

Evidence exists suggesting that floral and shoot primordia barriers to external (e.g., frozen bud scales) ice nucleation are organized at the tissue level rather than exclusively at the cellular level (3, 8, 11, 17, 19). Thus, the manner in which dormant grapevine buds are excised from canes might affect bud freezing characteristics. The following experiments were conducted to characterize the freezing events occurring in excised 'Chardonnay' compound buds and to determine how sample preparation affected their supercooling.

Materials and Methods

Experiments were conducted on dormant 'Chardonnay' buds obtained from vines grown at the New York State Agricultural Experiment Station, Geneva, or from a commercial vineyard near Geneva. Buds were collected from visually mature (dark brown periderm) nodes, excluding those with physical defects, those from node positions less than three or greater than 18 (from base), and those associated with a persistent summer lateral shoot.

Differential thermal analysis. Thermoelectric (TE) modules (1) were used to detect exotherms. Unless otherwise indicated, buds and 2 to 3 mm of subjacent nodal tissue were excised from canes and mounted singly or multiply on TE modules bearing strips of filter paper moistened with water. Buds were positioned such that their cut surfaces were in contact with the moistened filter paper. Modules then were wrapped with Parafilm and aluminum foil and inserted in 2.5 × 15 cm stoppered test tubes.

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Test tubes were inserted in 4-kg cylindrical aluminum heat sinks bored to close tolerances to accept eight tubes. The aluminum heat sinks were placed in a programmable freezer, and samples were cooled at 3°C/hr.

The millivolt output of the TE modules was recorded either graphically on a Linseis LS65 six-channel strip chart potentiometric recorder (0.5 mV full scale) or digitally on Campbell Scientific CR21 microloggers. The millivolt output of the TE modules is a function of the temperature differential between the two faces of the module (1, 22). The temperature of one sample in each heat sink was monitored with thermocouples or thermistors and recorded as with the millivolt data. Samples within a given heat sink had been measured to cool uniformly. The temperature at which endotherms occurred when frozen buds were thawed was evaluated to assess tissue freezing point.

LT_{50} determination. The temperature lethal to 50% of the primary buds (LT_{50}) was evaluated by dividing 120 nodes into six equal groups. One group (control) was stored at 4°C. The remaining groups were placed in separate styrofoam chests and cooled at 3°/hr. The ambient temperature within each chest was recorded with several 24-gauge copper–constantan thermocouples. Samples of buds were removed from the freezer in 2° decrements bracketing a predicted LT_{50} and placed at 4°. Buds were sectioned after 48 hr and examined for primordia viability on the basis of tissue browning (21). Primary bud survival (%) of each group was plotted against the minimum temperature to which those buds were exposed, and an LT_{50} was derived graphically by interpolation. The percentage of dead control (exposed only to field conditions) buds (5–10%) was subtracted from the percentage dead at each programmed temperature decrement to obtain an LT_{50} based only on those buds living prior to controlled freezing.

Relationship between exotherms and bud injury. Buds were excised either to include or exclude a 2- to 3-mm portion of subjacent nodal tissue to determine how bud excision affected bud freezing characteristics. The freezing characteristics of buds as affected by the presence or absence of a water-moistened filter paper substrate placed on the TE modules also was measured.

The relationship between exotherm occurrence and tissue injury was evaluated by monitoring the strip chart recorder and removing samples from the freezer immediately after or before exotherms were observed. Buds were sectioned after 48 hr and primordia were evaluated for viability.

Median LTE vs. LT_{50} as measures of bud hardiness. DTA and a temperature/survival curve method were compared as measures of primary bud hardiness. DTA methods in these comparisons involved freezing single buds in the absence of moistened filter paper.

Cooling rate effects. Freezing patterns of buds cooled at 2° or 5.6°C/hr were determined on two occasions in Nov. 1984. Low temperature exotherms generated in these comparisons were subjected to analysis of variance using the GLM procedure of the Statistical Analysis Service (SAS Institute, Cary, N.C.). The impact of a slow, step-wise cooling rate on bud hardiness and exotherm appearance also was evaluated. Visibly well-matured canes (nodes 3–15) were collected 9 Dec. 1984. Canes were randomized and one-half were cut to single node sections, rerandomized, and partitioned into four groups of 125 nodes each. The intact canes were partitioned into four groups of 10 canes each. Samples were bagged in plastic, then cooled to −10°C over a 24-hr period. Twenty additional buds were subjected to DTA on 9 Dec. After 6 days at −10°, buds from one group of node sections and one group of canes were evaluated for hardiness using both DTA and temperature/survival techniques. Minimal bud warming was allowed between preparation and controlled freezing. Following hardiness assays, the freezer was cooled to −15° over a 24-hr period. After 7 days at −15°, buds of both categories were again subjected to hardiness determinations. The freezer then was cooled to −20° and held there for 10 days. Bud hardiness again was evaluated, and the freezer was cooled to −25°. A final assessment of bud hardiness was made after 5 days at −25°. Freezer temperature varied no more than ±1.5° about the desired temperature.

Bud refreezing. Two separate experiments were conducted to evaluate the freezing characteristics of buds that had been previously frozen then thawed. The first experiment (14–15 Mar.) involved buds excised from canes collected 15 Nov. 1984 that had been wrapped in plastic and maintained at 2°C. Primary bud injury of this material was absent. Buds were cooled either to the point where exotherms no longer appeared on the strip-chart recorder (−16°) or to −34°, a temperature lethal to all tissues of the bud. Buds of both categories were subsequently warmed to 20°, held for 12 hr, then refrozen to −34°. The second experiment used buds collected from the field on 18 Mar. 1985. Primary bud injury was 6%. Initial freezing was either to −21° (LTEs no longer occurring) or −34°. Again, buds subsequently were warmed for 12 hr and refrozen to −34°.

Results

Relationship between exotherms and bud injury. Freezing single buds on dry TE modules produced a series of thermally distinct exotherms (Fig. 1A). Initial (warmest) exotherms occurred between −9° and −16°C. The occurrence of these ex-

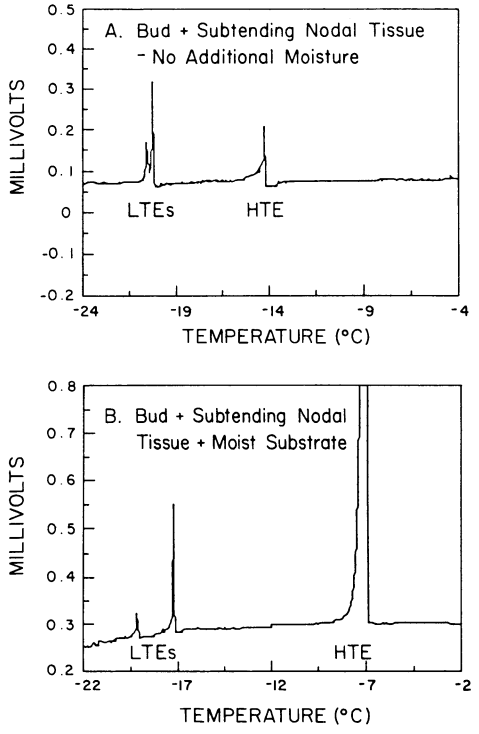

Fig. 1. Representative DTA profiles generated by single 'Chardonnay' dormant buds excised with 2 mm of subtending nodal tissue and frozen at 3°C/hr on dry thermoelectric modules (**A**) or thermoelectric modules bearing water-moistened filter paper (**B**). HTE = high temperature exotherm; LTE = low temperature exotherm.

otherms did not relate consistently to primary bud injury (Table 1). The occurrence of two or more exotherms was, however, associated consistently with injury to at least one shoot primordium (Table 1).

Buds excised with subjacent nodal tissue and cooled in contact with moistened filter paper exhibited two predominant exotherms: 1) a single large (2–3 mV) HTE at −4° to −8°C; and 2) a smaller (0.05–0.20 mV) LTE at temperatures comparable to the LT_{50} of primary buds (Table 2 and Fig. 1B). LTEs frequently consisted of a single, relatively large exotherm followed some degrees later by a second, smaller, and, sometimes smaller still, third exotherm (Fig. 1B).

Buds excised with subjacent nodal tissue and cooled in the absence of moistened filter paper generally exhibited multiple exotherms, the warmest of which (HTE) was inconsistent with bud hardiness predicted from temperature/survival evaluations of LT_{50}s (Table 2). When cooled without moistened filter paper, buds lacking nodal tissue produced LTEs at temperatures comparable to the primary bud LT_{50}, as well as occasional HTEs (Table 2). Buds excised so as to exclude subjacent tissue failed to exhibit LTEs when frozen on moistened filter paper (Table 2 and Fig. 2). A single large endotherm was observed at 0° to −2°C when frozen buds were monitored during a slow thaw (data not shown).

The relationship between exotherms and specific tissue injury was evaluated on several occasions using buds with subjacent nodal tissue and mounted on moistened filter paper (data not shown). Primordia were uninjured when buds were removed from a cooling cycle after the HTE and prior to the occurrence of an LTE. The occurrence of a single LTE was associated with injury to the primary bud. Injury to secondary and tertiary primordia was observed following additional LTEs; however, a single large LTE was often associated with injury to two or more primordia.

Table 1. Occurrence of exotherms and injury to individual 'Chardonnay' bud primordia with and without nodal tissue subtending the excised bud. Data are the composite of three evaluations conducted between 29 Oct. and 16 Nov. 1984 and are ranked in order of decreasing temperature only for clarity of presentation. Single buds were mounted on dry TE modules.

Nodal tissue[y]	Exotherm temp (°C)	Sample removal (°C)	Bud viability[z] 1°	2°	3°
+	−9	−9	+	+	+
+	−9	−9	+	+	+
+	−11	−11	+	+	+
+	−11	−11	+	+	+
+	−11	−11	+	+	+
+	−13	−13	+	−	+
+	−14.5	−14.5	+	+	+
+	−16	−16	+	+	+
+	−14.5, −19	−19	−	+	+
+	−14, −15, −15.5, −16	−16	−	−	−
+	−19.5	−20.5	−	+	+
−	−9, −11	−11	−	+	+
−	−14	−14	−	−	−
−	−14	−14	−	+	+
−	−16	−16	−	+	+
−	−16	−16	−	+	+

[z]1°, 2°, and 3° represent primary, secondary, and tertiary buds, respectively. Buds were rated as dead (−) or alive (+) on the basis of tissue browning (21) after holding 48 hr at 4°C.
[y]Bud excised with (+) or without (−) a 2- to 3-mm disk of subtending nodal tissue.

Table 2. Effects of nodal tissue subtending bud primordia and presence or absence of moistened filter paper on occurrence of 'Chardonnay' bud exotherms. Data are the composite of three evaluations conducted between 10 Nov. and 2 Dec. 1984 and are ranked in order of decreasing temperature only for clarity of presentation. Data are based on freezing of single buds.

Nodal tissue present[z]			Nodal tissue absent[z]		
Moistened filter paper[y]	Temp observed exotherms (°C)[x]		Moistened filter paper[y]	Temp observed exotherms (°C)[x]	
+	−7.0	−14.0	+	−5.0	---
+	−4.0	−15.0	+	−5.5	---
+	−6.0	−17.0	+	−7.0	---
+	−7.0	−17.0	+	−7.5	---
+	−7.5	−17.0	+	−8.0	---
+	−7.0	−19.0	+	−8.0	---
+	−6.0	−20.0	−	---	−13.5
+	−8.0	−20.5	−	---	−14.5
+	−7.0	−21.0	−	---	−15.0
+	−6.5	−21.0	−	---	−15.0
+	−7.0	−22.0	−	---	−16.5
+	−8.0	−22.0	−	---	−16.5
+	−7.0	−23.0	−	---	−17.0
−	−13.0	---	−	---	−18.0
−	−14.0	---	−	−16.0	−18.0
−	−14.0	---	−	−13.0	−18.5
−	---	−17.0	−	−14.0	−19.0
−	−11.0	−18.5	−	---	−19.0
−	−11.0	−18.5	−	---	−19.0
−	−11.0	−19.0	−	---	−21.0
			−	---	−20.0
			−	−15.0	−24.0

[z]Buds excised from canes to include (present) or exclude (absent) a 2- to 3-mm disk of subtending nodal tissue.
[y]Buds mounted on TE modules in presence (+) or absence (−) of water-moistened filter paper.
[x]Cooler exotherms represent either a single LTE or, where multiple LTEs were observed, the largest.

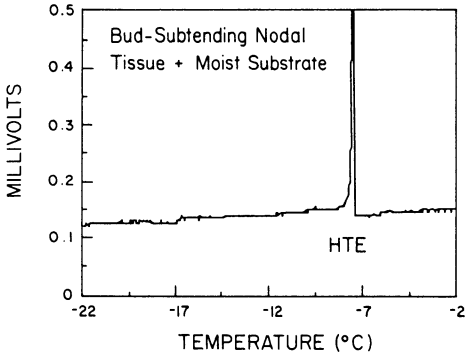

Fig. 2. Representative DTA profile generated by a single 'Chardonnay' dormant bud excised without subtending nodal tissue and frozen at 3°C/hr on a thermoelectric module bearing water-moistened filter paper. HTE = high temperature exotherm.

Median LTE vs. LT_{50} as measures of bud hardiness. The median LTE temperature was ≈2°C warmer than the graphically derived LT_{50} on 27 Oct. and 3 Nov. 1984 (Table 3).

Cooling rate dependency. Buds cooled at 5.6°C/hr exhibited median LTEs 1° cooler than buds cooled at 2°/hr in two separate experiments in Nov. 1984 (Table 4). Differences in LTE tem-

Table 3. Comparison of 'Chardonnay' primary bud hardiness as determined by differential thermal analysis and by a temperature-survival curve method on two dates.

Date	Differential thermal analysis			Temp/survival	
	N[z]	Median LTE (°C)[y]	1° LTE range (°C)	N[x]	LT_{50} (°C)[w]
27 Oct. 1984	22	−13.5 ± 0.3	−10.0 to −16.5	120	−15.0
3 Nov. 1984	24	−15.0 ± 0.3	−13.0 to −19.5	120	−17.0

[z]Number of exotherms upon which median LTE is based.
[y]Median low temperature exotherm temperature and mean SE.
[x]Number of buds used to derive LT_{50}.
[w]Temperature lethal to 50% of primary buds.

Table 4. Effect of two cooling rates on occurrence of low temperature exotherms (LTE) of 'Chardonnay' primary buds at two dates in 1984.

Date	Cooling rate (°C/hr)	Median LTE (°C)[z]	N[y]	1° LTE range (°C)
23 Nov.	2.0	−20.5 ± 0.2	10	−18.5 to −21.5
	5.6	−21.5 ± 0.3	11	−19.5 to −23.0
25 Nov.	2.0	−20.0 ± 0.2	18	−17.0 to −22.0
	5.6	−21.0 ± 0.2	17	−20.0 to −23.5
Significance				
Date		NS		
Cooling rate		***		
Date × rate		NS		

[z]Median low temperature exotherm temperature and mean SE.
[y]Number of exotherms upon which median LTE is based.
NS,***Nonsignificant and significant ($P = 0.001$) effects, respectively.

peratures due to cooling rate were small but significant at $P \leq 0.001$.

Step-wise cooling experiment. The median LTE of buds collected on 9 Dec. was −21°C with no primary bud injury due to field exposure at this time (Table 5). Six days at −10° decreased the median LTE of buds excised from frozen canes to −21.2° and of buds excised from single nodes to −22° while having little impact on primary bud survival. The LT_{50} values at this time for the two bud categories were both about −24° (Table 5). The similarity in bud hardiness between single nodes and whole canes persisted at subsequent sampling dates. Therefore, the LTE and LT_{50} values in Table 5 are based on the combined single node and whole-cane data.

The median LTE and LT_{50} decreased to −25° and −28°C, respectively, following 7 days at −15° (Table 5). Many buds exhibited a reduction in the size of exotherms or failed to produce detectable exotherms at this date. The median LTE therefore was derived from only those samples exhibiting discernible peaks. Exposure to −15° for 7 days killed 20% of the primary buds of the control group. Subsequent exposure of buds to −20° for 10 days resulted in a complete absence of LTEs, resulted in the death of an additional 30% of the control primary buds, and did not increase hardiness of surviving buds (Table 5). Cooling remaining buds to −25° and holding for 5 days killed an additional 35% of primary buds (Table 5). An LT_{50} was not derived from those data due to the number of killed control buds.

Bud refreezing. The freezing pattern of previously frozen and thawed buds was affected to some degree by the prior freezing temperature. Previously unfrozen buds cooled to −16°C on 14 Mar. 1985 had a median LTE of −13°, which was based on 22 LTEs judged to arise from primary buds (Table 6). Refreezing these same buds after thawing resulted in the occurrence of only 13 LTEs, which had a median value of −6°, which was significantly warmer than the initial median LTE temperature. The median LTE of buds frozen to −21° on 18 Mar. was −18° (Table 6). Refreezing these buds resulted in the appearance of only four LTEs that had a median LTE temperature of −7°. Buds initially cooled to −34° on either date did not exhibit any LTEs when refrozen.

Discussion

Excised 'Chardonnay' buds frozen on dry TE modules produced HTEs over a wide range of subfreezing temperatures. These HTEs were not associated with primordia injury and, presumably, resulted from the freezing of supercooled moisture

Table 5. Effect of slow, step-wise cooling on 'Chardonnay' primary bud hardiness as assessed by differential thermal analysis and by a temperature/survival method.

Temp preceding evaluation	Evaluation date	Differential thermal analysis			Temp/survival	
		N[z]	Median LTE (°C)[y]	1° LTE range (°C)	% dead controls[x]	LT_{50} (°C)[w]
Ambient	9 Dec. 1984	19	−21 ± 0.4 b	−17 to −24	0	---
−10	15 Dec. 1984	37	−22 ± 0.3 b	−19 to −25	5	−24
−15	22 Dec. 1984	15	−25 ± 0.4 a	−24 to −29	20	−28
−20	1 Jan. 1985	---	---	---	50	−28
−25	6 Jan. 1985	---	---	---	85	---

[z]Number of exotherms upon which median LTE is based.
[y]Median low temperature exotherm temperature and mean SE. Mean separation within column by Duncan's multiple range test, 5% level.
[x]Based on 40 primary buds at each sample date.
[w]Temperature lethal to 50% of primary buds.

Table 6. Relationship between 'Chardonnay' bud freezing and the recurrence of exotherms in the same buds following thawing and refreezing on two occasions in 1985.

	Initial freeze			Refreeze			
Date[z]	Final temp (°C)	No. 1° LTEs[y]	MLTE (°C)[x]	Final temp (°C)	No. 1° LTE[y]	MLTE (°C)[x]	Sig.[v]
14 Mar.[w]	−16	22	−13 ± 0.2	−34	13	−6 + 0.2	***
	−34	23	−13 ± 0.2	−34	0	---	
18 Mar.	−21	23	−18 ± 0.2	−34	4	−7 + 0.5	***
	−34	21	−18 ± 0.3	−34	0	---	

[z]Twenty-five buds were evaluated for each final temperature at each date.
[y]Number of discernible primary bud low temperature exotherms upon which MLTE temperature was derived.
[x]Median low temperature exotherm temperature and mean SE.
[w]Buds were collected in Nov. 1984 and maintained at 2°C.
[v]*t* tests were conducted between initial and refreeze LTEs. ***Indicates that MLTE temperatures differed significantly ($P = 0.001$) between initial and refrozen condition.

in the bud scales and/or subjacent nodal tissue. That this moisture supercooled to −16°C in the absence of externally applied water may be an artifact of the small mass of tissue being cooled; excised bud fresh weight was on the order of 75–100 mg. Ashworth and Davis (4) noted a logarithmic relationship between the fresh weight of peach stem samples and tissue nucleation temperature. Peach stem samples weighing <5 g supercooled several degrees cooler than 20–g samples, which froze at about the same temperature as intact trees (−2°).

The inconsistent appearance of HTEs made it difficult to distinguish LTEs that were associated with bud death. This problem was most pronounced in early fall, when LTEs occurred at relatively warm, subfreezing temperatures. Freezing buds on a hydrated substrate, however, ensured the consistent occurrence of HTEs at temperatures distinct from the injurious LTEs. Conceivably, the external water provided an aqueous continuum with moisture in the nodal tissue and/or bud scales that facilitated the propagation of ice to these tissues when the free water froze.

Failure of moistened bud primordia to supercool when excised to exclude subjacent nodal tissue suggests that the integrity of the primordia–nodal interface is a critical component of the barrier to ice nucleation of supercooled primordia. These results are consistent with similar experiments involving excised peach buds (3, 17, 19) and emphasize that bud excision must not negate structural barriers contributing to primordia supercooling.

The slightly lower LT_{50}s compared to median LTEs observed in the current study might have resulted from measuring air temperature immediately adjacent to buds rather than the bud tissue itself, as in DTA. Although not measured here, it is conceivable that a lag in heat removal from whole-node sections can occur even when samples are cooled at 3°C/hr. Thus, air temperature could be some degrees cooler than tissue temperature. DTA might therefore offer a more accurate estimate of a population's hardiness than a temperature/survival technique in that the exact temperature of the freezing of individual buds is known with DTA.

Data presented here on cooling rate dependence of LTEs suggest the possibility that rapid cooling rates may lower the temperature slightly at which bud primordia freeze. This suggestion contrasts with a number of reports with other species in which median and average LTEs are consistently warmer with faster cooling rates (3, 5, 8, 15, 20). A standard cooling rate of 3°C/hr is rapid enough to expedite hardiness evaluations, and yet slow enough to simulate conditions that might be encountered in the field.

Reports of injury to grapevine buds due to the duration of cold are lacking, as most emphasis has focused on effects of temperature minima. Pogosyan and Sarkisova (16) reported that step-wise artificial cooling of several grape cultivars to −30°C over an ≈26-day period elicited an increase in bud hardiness from −20° to −28°. Proebsting et al. (18) presented data from the state of Washington that indicated that prolonged (about 2 weeks) subfreezing ambient temperatures depressed the LT_{50}s of 'Concord,' 'White Riesling', and 'Cabernet Sauvignon' (*V. vinifera*) to an estimated −40°, −37°, and −31°, respectively. 'White Riesling' and 'Cabernet Sauvignon' reportedly sustained injury although the extent of injury and whether it was due to prolonged exposure or minimum temperatures (about −20°) were not reported. On the basis of 7 years of grape bud hardiness data and comparisons with *Prunus* hardiness data, Proebsting et al. (18) suggested that: " . . . it is likely that cold resistance of dormant grape buds increases slowly while they are frozen, regardless of how cold they are." Results of step-wise cooling of 'Chardonnay' buds observed here would suggest that prolonged periods of subfreezing temperature (−10° to −15°) do increase bud hardiness. Additional exposure to these relatively warm temperatures might have increased bud hardiness further. Exposure of buds to −20°, which was warmer than the range of LTEs observed following exposure to −15°, did not appreciably increase bud hardiness, but did kill up to 50% of buds. The diminution and eventual nondetection of exotherms, coupled with the death of control primary buds held for prolonged periods at temperatures well above the median LTE, suggests that water migrated from bud primordia, and that this dehydration ultimately was associated with bud injury. This form of injury might correspond to that consequence of extraorgan freezing described by Ishikawa and Sakai (10, 11), in which primordia dehydrate during freezing but do not survive severe desiccation. From a practical standpoint, this consequence would imply that buds in situ under field conditions would become susceptible to dehydration injury following prolonged exposure to stable, subfreezing temperatures. Thus, the possibility exists that cold injury to grapevine buds might consist of additional forms of injury other than intracellular ice formation alone. Transient thawing of buds, as by radiant heating, might mitigate the effects of prolonged freezing in the field by allowing primordia to reabsorb water; however, Damborská (7) reported that buds of 'White Riesling,' 'Muller-Thurgau,' and 'Blue Portuguese', all *V. vinifera* cultivars, lost very little hardiness when warmed to 10° to 12° for 24 hr following hardening under natural and artificial conditions. Warming periods of 72 and 120

hr were, however, associated with decreased cold resistance. In *Rhododendron* flower buds, water withdrawn from florets during freezing took 3 days to return to florets when buds were thawed (11). The impact of transient thawing on grapevine bud hardiness, therefore, may not be significant.

Whether the viability of the tissue subtending primordia is critical to supercooling of grapevine shoot primordia, as appears to be the case with peach floral primordia (3), is unresolved by the experiments involving bud refreezing reported here. 'Chardonnay' buds that produced LTEs when cooled to relatively warm temperatures retained a slight degree of supercooling capacity when refrozen. The hardiness of tissue subtending bud primordia cooled to relatively warm temperatures was not specifically assessed. It would, therefore, be presumptuous to conclude that viability of tissue subtending primordia affects primordia supercooling. The reduced capacity of freeze-killed buds to supercool does, however, reduce the risk of ascribing unwarranted hardiness to dead buds.

Pierquet and Stushnoff (14) reported that *V. riparia* buds cooled to −50°C and thawed exhibited LTEs when refrozen either 24 or 48 hr later at essentially the same temperature as in the initial freezing. Two possible reasons are offered to explain differences between results presented here and those of Pierquet and Stushnoff (14). First, bud tissues of *V. riparia*, regardless of viability, might have a greater structural capacity than *V. vinifera* to facilitate supercooling. Primary buds in that study were reported to exhibit LTEs at −35° to −42°, considerably cooler than any observed here with 'Chardonnay'. Viability of bud tissues other than primordia were not reported by Pierquet and Stushnoff (14). Second, buds used in our study to assess recurrences of exotherms were collected during acclimation (15 Nov. 1984) or deacclimation (18 Mar. 1985). Tissues of buds collected at these times might not exhibit the same refreezing characteristics as buds evaluated by Pierquet and Stushnoff, which were collected 7 Feb.

Freezing buds on a hydrated substrate ensured the consistent occurrence of HTEs at temperatures distinct from those of LTEs. Buds excised without subjacent nodal tissue did not demonstrate primordia supercooling if they were frozen on a hydrated substrate. Killed buds did not retain the capacity to supercool to temperatures that would create confusion with exotherms of previously uninjured buds. Differential thermal analysis conducted in a standardized fashion offers a convenient and reliable means of assessing grapevine bud cold hardiness. The utility of DTA of grapevine buds might be reduced following prolonged periods of subfreezing temperature when moisture in bud primordia is insufficient to detect its freezing and/or when primordia injury results from causes other than intracellular ice formation.

Note added in proof. H.A. Quamme recently reported on similar aspects of grape bud thermal analysis: Quamme, H.A. 1986. Use of thermal analysis to measure freezing resistance of grape buds. Can. J. Plant Sci. 66:945–952.

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