Grapevine Cold Hardiness: Mechanisms of Cold Acclimation, Mid-Winter Hardiness Maintenance, and Spring Deacclimation

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Freeze injury and damage is a reality in temperate zone viticulture. The past 35 years have been a period of considerable activity on the topic of perennial woody plant cold hardiness. The opening presentation of the Symposium will provide information relating to woody plants in general and grapevines specifically. Aspects to be covered include, physiology of cold hardiness with information at the molecular, organelle, cellular, tissue, organ, and vine level; genotypic and cultural influences on vine cold hardiness, the changes in hardiness over the dormant cycle; differences among cultivars and rootstock influences; and a brief projection about the potential for improving hardiness of cultivars and the factors that are exciting and those that may be limiting.

KEY WORDS: cold hardiness, vine physiology, genotype, deacclimation

A small portion of Earth's land surface is cultivated and Parker [52] suggests that drought and cold are the most limiting of natural plant distribution, and by extension, most limiting to the range of cultivated plants such as grapevines.

Tissue temperatures below -2°C can kill actively growing grapevine tissues. Yet, fully cold-hardy tissues of *Vitis riparia* can survive to -40°C [56]. The mechanisms by which freezing stresses kill cells and tissues, and the chemical, physical and physiological changes that vines undergo which allow cells and tissues to resist damage when subjected to such freezing stresses, are complex. They include: *(1)* influence of genotype; *(2)* the environment of the vine's culture; and *(3)* the influence of vine culture and management on the expression of a vine's genetic potential for cold hardiness.

These are of economic significance in every viticultural region where temperatures fall below -2°C. It is for grape growers and grapevine researchers in such regions that this symposium has been organized.

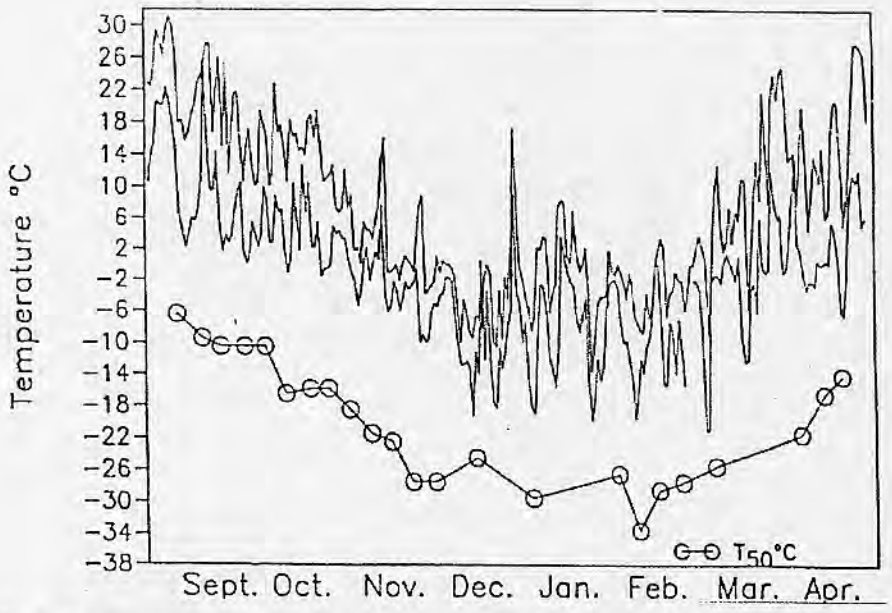

Fig. 1. Plot of temperature and grapevine bud hardiness from September to May.

Injury and Damage

Levitt [37] and Shaulis [70] have provided some terms that are useful. Levitt draws a distinction between *injury* and *damage*. The distinction is one of magnitude. The freeze kill of a primary bud on a grape cane is damage to that bud, but only injury to the cane or grapevine. Shaulis adds the refinement of *seriousness of cold injury* saying it is the "amount of decrease in fruit production or fruit quality resulting from cold injury". He defines *cold hardiness* as "the survival capability of a specified tissue of a vine following a specified exposure to low temperature".

The Dormant Season as Part of the Annual Cycle

Grapevines are perennial plants, so the challenge of grapevine culture must include more than the achievement of economically acceptable levels of yield and quality in the current season. It must include: *(a)* the maturation of the vine [89,90]; *(b)* the sequestering of stored reserves to initiate and sustain spring growth until the new leaf array can satisfy the need for carbohydrates [18,19,42,43,55]; *(c)* the initiation of vine acclimation to cold [28,91]; *(d)* the maintenance of that cold hardy status [28,29]; and

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Table 1. Seasonal ranking of cultivar hardiness (a) and rootstock influences on cold hardiness of peach twigs (b).

Cultivar	T_{50} of Twig Cambium/Phloem (a) Acclimation			Mid-Winter			Deacclimation		
	Year-1	Year-2	Rank	Year-1	Year-2	Rank	Year-1	Year-2	Rank
Redhaven	-12.7a	-11.1	1,1	-25.3a	-25.5a	1,1	-16.3ab	-19.3b	2,3
Redskin	-12.5a	-10.3	2,2	-24.3b	-24.6ab	3,2	-17.3a	-20.4a	1,1
Cresthaven	-12.0b	-9.9	3,3	-24.7ab	-23.7b	2,3	-15.0b	-19.5b	3,2
F-test	**	*		*	**		*	**	
Cultivar	T_{50} of Twig Cambium/Phloem (b) Acclimation			Mid-Winter			Deacclimation		
	Year-1	Year-2	Rank	Year-1	Year-2	Rank	Year-1	Year-2	Rank
Halford	-10.5	-12.5	2,2	-24.0	-25.5	2,2	-20.0	-17.0	1,1
Siberian-C	-11.5	-13.5	1,1	-25.0	-26.5	1,1	-19.0	-16.0	2,2

*,** Statistically significant at the 0.05 or 0.001 levels of probability respectively.

(e) the slow loss of that hardiness as the late winter cold gives way to the spring warmth [28,29].

Data in Figure 1 have led us to a view of the dormant season that is subdivided into at least three periods: *acclimation,* characterized as a period of transition from the non-hardy to the fully hardy state; *mid-winter*, characterized as the period of most severe cold and greatest cold hardiness; and *deacclimation,* characterized as the period of transition from the fully hardy to the non-hardy state and active growth.

Further, the data in Table 1, on seasonal ranking of cultivar hardiness (Part a) and rootstock influences on cold hardiness of peach twigs (Part b), provide justification for this subdivision [29]. Genotype response differs depending on the portion of the dormant season when hardiness is measured. This may explain different experiences with varietal hardiness. Anecdotal inputs about cultivar differences in cold hardiness liven many discussions in the Great Lakes Region of North America. As one includes other viticultural regions where cold damage can have economic consequences (the Pacific Northwest, the Lower Midwest, Eastern Europe and northern Germany), the degree of difference in opinion becomes even wider. Individuals in different areas have different experiences. An ordering of cultivars might be as A > B > C > D in one area, while experience will be B > C > D > A in another, and D > C > B > A in yet another. These data on peach provide one explanation of why that may be so. Depending on the timing of the temperatures most likely to cause damage for that region, the key to avoiding damage may be early acclimation, while for another region the dangerous time might be associated with the most severe winter cold, or too rapid a response to warm dehardening temperatures in mid-winter or in early spring. This is the reason why "test winters" have limited utility beyond the location of the experienced stress.

In conclusion, we can say that hardy vines must have the capacity to acclimate early, achieve and maintain resistance to severe cold in the mid-winter period, be slow to respond to transient warm periods, and also slowly respond to natural spring warming. If a vine possesses these capabilities for the climate of a specific region, it will survive and produce. If the vine lacks the genetic capacity to accomplish any one of these, it will die [28]. If culture prevents the expression of a hardy vine's genes for cold resistance, it will die [28] [see also presentations by Dr. T. Wolf on "Site selection and vine management principles and practices to minimize the threat of cold injury" [85].

Both the genotype (cultivar choice) and methods of viticulture employed are critical every season to maximize cold hardiness for cool climate, short growing season, and low number of growing degree-day viticultural regions. The hardiness challenge for any genotype will depend on the relative hardiness of that genotype with relation to the frequency of temperatures at or near the threshold for injury. Such temperatures may occur during acclimation, during mid-winter, or during deacclimation — depending on the prevailing weather patterns for the macroclimate of culture. Further discussion of the impact of the portion of the dormant season are presented by Dr. R. Wample in his presentation on "The Dynamics of Grapevine Cold Hardiness" [81].

The Physiology of Cold Hardiness in Woody Plants

Mechanisms of freeze stress resistance. ***Freezing point depression and supercooling:*** Resistance to freezing stresses in cold hardy plants is accomplished by two mechanisms in grapevines and other perennial, woody plants. Levitt [37] refers to these as *freeze avoidance* and *freeze tolerance.* The mechanisms differ both in the conditions of ice formation and the plant tissues involved.

Primordial tissues of either flower or vegetative buds survive by avoiding the formation of ice crystals in the tissue. This is accomplished via the mechanism of *supercooling* [58,59]. Supercooling is the process by which a liquid maintains the fluid state below its tem-

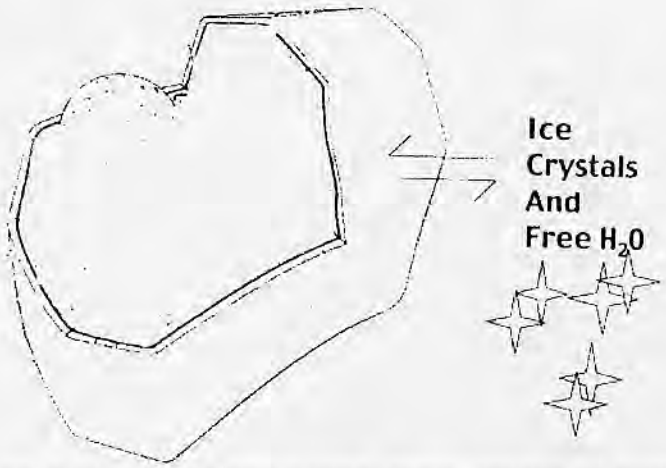

Fig. 2. Diagram of cell and ice crystals showing protoplast: ice crystal equilibrium.

perature for phase change to a solid. Such water can remain liquid at temperatures well below 0°C [66].

Deep supercooling has also been shown to be important for parenchyma tissue associated with the xylem [60,61,62,63,64,65]. Supercooling of this tissue has been shown in hardy perennial trees [64] and *V. riparia* [56] to be as low as -40°C. This supercooling point appears to limit the geographic range of many arboreal trees [10]. The injury to such parenchyma tissues is associated with the "black- heart" disorder observed in fruit trees after a severe winter cold episode [10].

Freezing point depression: In differentiated tissues characterized by large areas of noncellular freespace (apoplast), supercooling in limited. In this case, freeze resistance is primarily the result of a capacity to tolerate both ice in the tissue and the concentration of solutes in the cell. Cells in such tissues achieve protection via subsequent freezing-point depression. Increasing the dissolved solutes in the cell's aqueues solution lower the freezing point as salt does with sea water.

In the freezing of plant tissues, ice forms first where water has least dissolved solutes (the apoplast), as the result of a "nucleating event" [6]. Depending on the tissue and the amount and purity of water in the apoplast, this commonly occurs between tissue temperatures of -1°C and -5°C.

As temperatures decline further, a vapor pressure equilibrium/gradient is established between the ice crystal exterior to the cell and the water and solutes within the cell, and water will move out of the cell to the site of the ice. As tissue temperature warms the process is reversed (Fig. 2).

This can produce several effects potentially lethal to the cells around the ice: *(1)* cells can suffer dehydration stress; *(2)* the rate of temperature decline can be too rapid and the cell supercool and then flash as ice crystals form within the cell; *(3)* ice crystals may penetrate the cell and initiate within-cell freezing; and *(4)* loss of membrane integrity and within cell compartmentalization mixes cellular contents which can result in cell and local tissue mortality. Dr. M. Goffinet discusses this area in greater detail in his presentation "The Anatomy of Low Temperature Injury of Grapevines" [23].

Freeze resistance at the molecular level: Little of this work has been done on grapevines, and most has been done on herbaceous annuals possessing very low levels of freeze resistance. It is worth our attention, however, because: *(1)* they are the earliest reports probing the specific impact of compounds produced or influenced by the same environmental stimuli known to induce hardiness (acclimation); and/or *(2)* they are compounds which influence genetic response to those environmental stimuli by the production of specific hardiness promoting compounds.

Abscisic acid: The plant hormone abscisic acid (ABA) appears to be involved in acclimation. This is based on data showing increasing levels of endogenous ABA during acclimation [13,14,15], as well as demonstrations that applied exogenous ABA increased tissue cold resistance [13]. Recent efforts with *Arabidopsis* have shown: *(1)* ABA deficient mutants cannot acclimate [26]; *(2)* ABA insensitive mutants cannot acclimate [22]; and *(3)* ABA can activate genes for acclimation that are commonly activated by low temperature exposure [36].

Proteins. ***Stress-related proteins:*** An active, exciting area of current research is the evaluation of proteins produced by genes induced by exposure to cold. While there is much yet to be learned, it is interesting to note that many of the encoded proteins share similar characteristics. These include the "heat-shock" proteins [47], the "late embryogenesis-abundant" proteins [69], and the "boiling-stable" and "dehydrin" proteins [1,2,3,4,5,24,51]. There is also the suggestion that "heat-shock" proteins are involved as "chaperonins" [24]. These protein "chaperonins" reportedly are involved in the folding of specific other proteins to achieve its final structure. The final three-dimensional structure and attendant positioning of "active sites" is crucial for enzyme function, and "chaperonins" are suggested to ensure the proper "folding" of protein molecules to achieve appropriate three-dimensional struc-

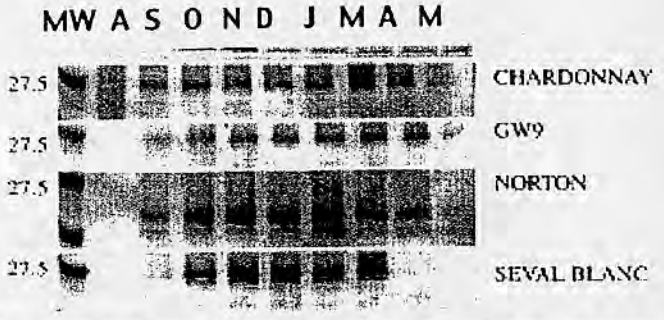

Fig. 3. Immunoblots of dehydrin proteins produced form Chardonal, Chardonnay, Norton, and Seyval blanc grapevines during different parts of the dormant season. After Wisnewski *et al.* [84] (reproduced with permission).

ture for its function. "Chaperonins" are present at "normal" temperatures, but increase sharply after a temperature shock [80].

Grape related efforts have also been reported for stress related proteins [45,69,84]. These reports suggest that grapes produce these compounds and their production is greatest during the winter months. The immunoblots presented in Figure 3 [84] support this conclusion. There are density gradients based on known differences in cultivar hardiness, and band density within each cultivar appears to increase as hardiness increases.

That similar proteins can result from a range of differing environmental stresses (heat, water) and also from cold stimulus suggests that there may be a common basis for resistance to such stresses at the cellular level.

Structural proteins. ***Integral and peripheral proteins in membranes:*** The properties of these proteins vary, but the former are associated with membrane transport, while the latter facilitate interactions between the membrane and microtubules (important in cell division) and the protein actin (a subunit of a microfilament). Microfilaments and microtubules (composed of the globular protein subunits tubulin) operate within the cell to create a network; the cytoskeleton. The cytoskeleton is involved in spatial orientation of organelles and their movement with in the cytoplasm, mitosis, meiosis, differentiation of cell structure, deposition of the cell wall and maintaining cell shape [80].

Enzymes/Isozymes: Specific enzyme changes have been observed to correlate with changes in hardiness state [25]. It is suggested that these enzyme isomers (isozymes) have: *(1)* greater stability under conditions of stress; *(2)* lower activation energies and are thus able to function more effectively at colder temperatures; or *(3)* possess both characteristics. It is interesting to speculate that one role of the "chaperonins" mentioned above might be to ensure that isozymes more functional at lower temperatures are folded to produce the critical 3-dimensional structure necessary for enzyme activity.

Carbohydrates: Carbohydrates have also been widely implicated as important compounds associated with cold hardiness. Indeed, the hardiness literature has more references to carbohydrate status of plants, especially perennial plants like grapevines, than any other type of compounds. The suggested role for carbohydrate is at two levels.

Energy source: The transition from the non-hardy condition to the hardy condition and the maintenance of that resistant condition requires energy, as in the form of carbohydrates elaborated in photosynthetically functional leaves. Insufficient carbohydrates result in inadequate expression of hardiness potential in grapevines [28,31,32,33,57,67,68,79,82].

Cryoprotectant: Protein denaturation results in the loss of those enzymes whose activity is necessary to provide energy and prevents the expression of genes for hardiness and the hardiness related protein syntheses mentioned above. As mentioned above also, water losses can result in the "salting out" of the proteins. Such denaturation is injurious if transient in nature and can be lethal if irreversible. Specific sugars and sugar alcohols [9] have been suggested to replace H-bonded water and prevent such denaturation and cell death. When one considers the range of proteins and their functions mentioned in the section above, the significance of conditions which can help avoid protein denaturation becomes great.

Freeze resistance at the cellular level. ***Membranes:*** As noted previously, water movement from cells to centers of ice nucleation in the apoplast is a mechanism by which cells of hardy tissues avoid intercellular ice formation and its lethal consequences. Since the rate of temperature decline can vary in nature, cells possessing membranes most capable of rapidly exporting water to the intracellular ice would be less likely to suffer damage [39].

Plant membranes are phospholipid bilayers containing transport proteins embedded in the lipid matrix (integral proteins) and additional proteins attached to the membrane surface (peripheral proteins) These proteins are part of the plasmalemma. Protecting these proteins from irreversible denaturation may be important in maintaining selective permeability and the maintenance of membrane integrity [38].

All membranes play a role in cold hardiness at the cell level. Since there are a number of membranes, the following has been organized to provide a listing of them, to give the function of each, and a brief list of potential roles in cold resistance and freeze injury and damage (Fig. 5).

Plasmalemma: The plasmalemma is only one of an array of membranes which may be influenced by changes as the cell acclimates to cold. Space prevents detailed discussion here, but the functions of endoplasmic reticulum, golgi apparatus, tonoplast and mitochondrial and chloroplast membranes are all subject to the changes occurring as acclimation progresses. A series of good reviews concerning membrane activity and cold hardiness has been provided by Steponkus [73,74,75]. The plasmalemma's primary roles are to maintain protoplast integrity, control solute influx and efflux, and to control the movement of ions into the cell.

Vacuolar membrane (tonoplast): Mature cells have most of their volume taken by the vacuole. The vacuole is a key component of the cell's osmotic apparatus and as such strongly influences cellular turgor. It is also a "dumping" area where materials that would otherwise be injurious are sequestered. The tonoplast serves a crucial role in preventing the undesirable mixing of these toxic substances within the protoplast.

Mitochondria and chloroplast: These are the sites of energy conversion. Even in the winter when low temperatures reduce metabolic activity, the functioning of energy producing organelles is necessary, albeit

Table 2. Structure and saturation status of different fatty acid components of phospholipids in cell membranes.

1. Saturated

16 carbon - Palmetic
$CH_3(CH_2)_{14}COOH$

18 carbon - Steric
$CH_3(CH_2)_{16}COOH$

2. Unsaturated

16:1 - Palmetic
$CH_3(CH_2)_5CH = CH(CH_2)_7COOH$

18:1 - Palmetic
$CH_3(CH_2)_7CH = CH(CH_2)_7COOH$

18:2 - Linoleic
$CH_3(CH_2CH = CH)_3(CH_2)_7COOH$

18:3 - Linolenic
$CH_3(CH_2)_3(CH_2CH = CH)_2(CH_2)_7COOH$

3. Desaturation is commonly as:

18:0 Steric → 18:1 Oleic → 18:2 Linoleic → 18:3 Linoleic

at very low rates.

Endoplasmic reticulum: This membrane system is the major network of the cell's internal membranes. A major role of these membranes is involved as a step in protein synthesis.

Golgi apparatus: After the initial step on the endoplasmic reticulum, golgi continue and complete the process of protein synthesis.

Peroxisomes: This organelle plays an important role in oxidative functions of the cell. The enzyme catalase can occupy up to 40% of the volume. Membranes controlling the contents of this organelle insure that important oxidative reactions to detoxify harmful metabolites can occur, while preventing content leakage that could be lethal.

Membrane fatty acid saturation status: The membrane changes that occur which prevent or reduce the hazard of membrane leakage involve the substitution of fatty acid molecules with less saturation for those saturated moieties within the membrane. This results in membranes more capable of carrying out their functions under conditions of colder temperatures. Table 2 provides a listing of fatty acids and their saturation status. These are compounds commonly part of phospholipids shown in Figure 4.

Membrane fluidity: In addition to permeability changes, the insertion of unsaturated fatty acids for saturated structures influences the flexibility of the membrane [80]. In both chilling and freeze sensitive cells, membranes possessing a large percentage of saturated phospholipids will solidify into a semicrystalline state upon exposure to low temperatures, losing properties necessary for the membrane's function. Thus, the plasmalemma and tonoplast can lose selective permeability and mitochondria and chloroplast membranes lose their capacity for energy transduction. This would also influence protein synthesis on endoplasmic reticulum and golgi apparatus, and potentially lead to leakage from peroxisomes.

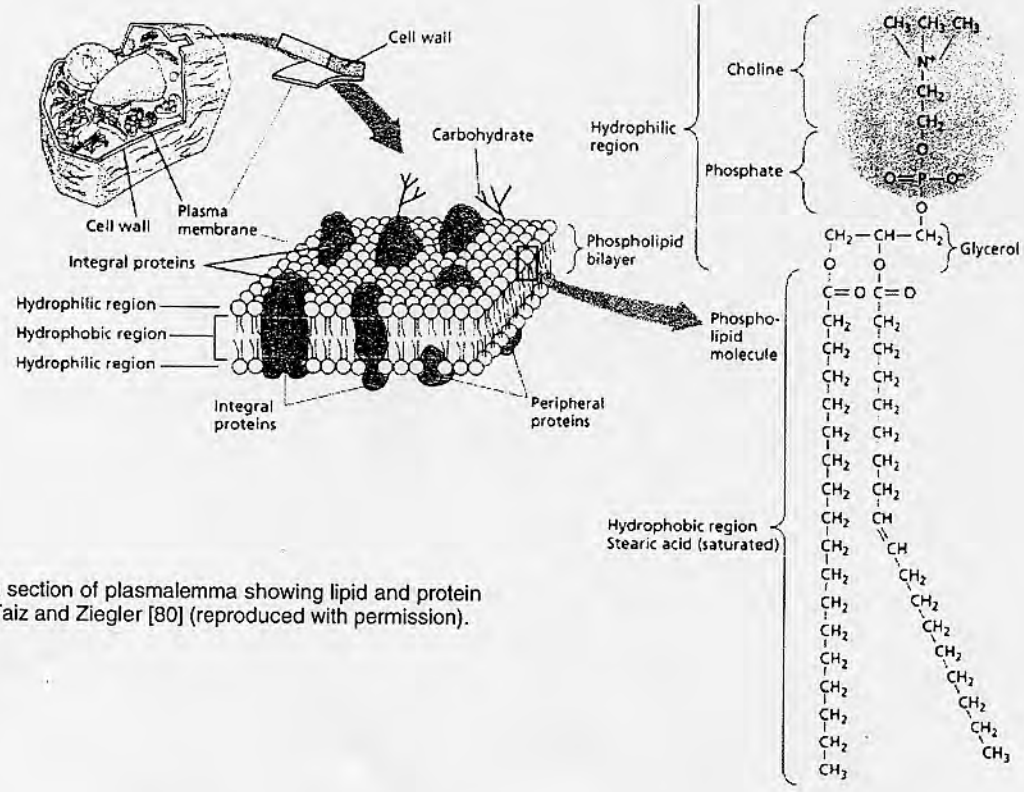

Fig. 4. Diagram of a section of plasmalemma showing lipid and protein components. After Taiz and Ziegler [80] (reproduced with permission).

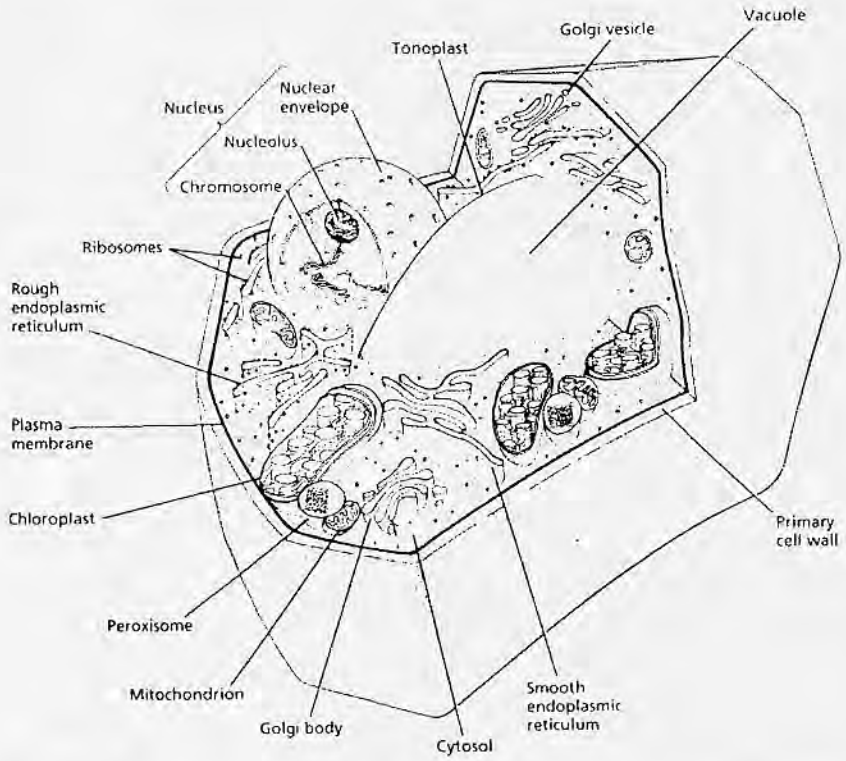

Fig. 5. Plant cell with organelles and membranes. After Taiz and Ziegler [80] (reproduced with permission).

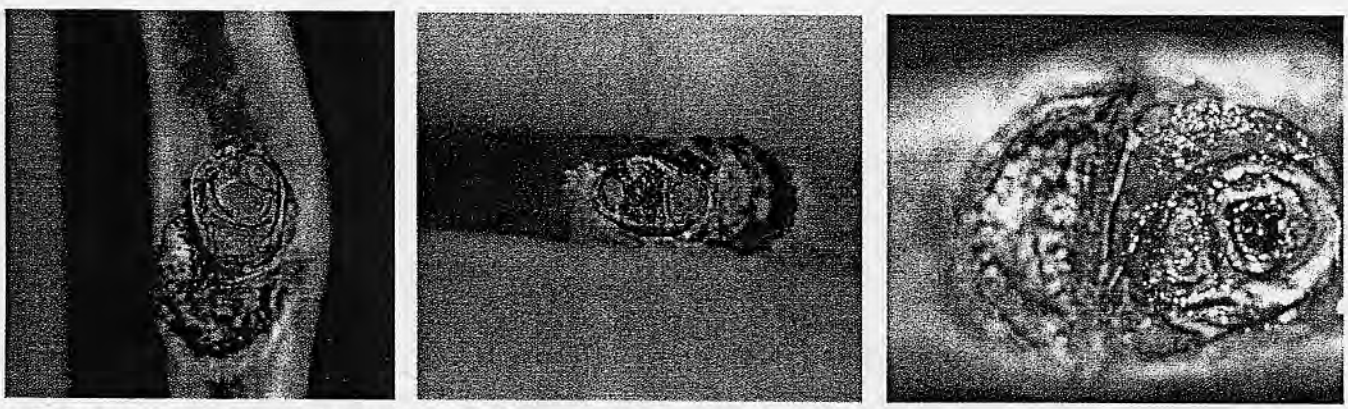

Fig. 6. Cross section of primary and secondary grape buds and primary bud mortality.

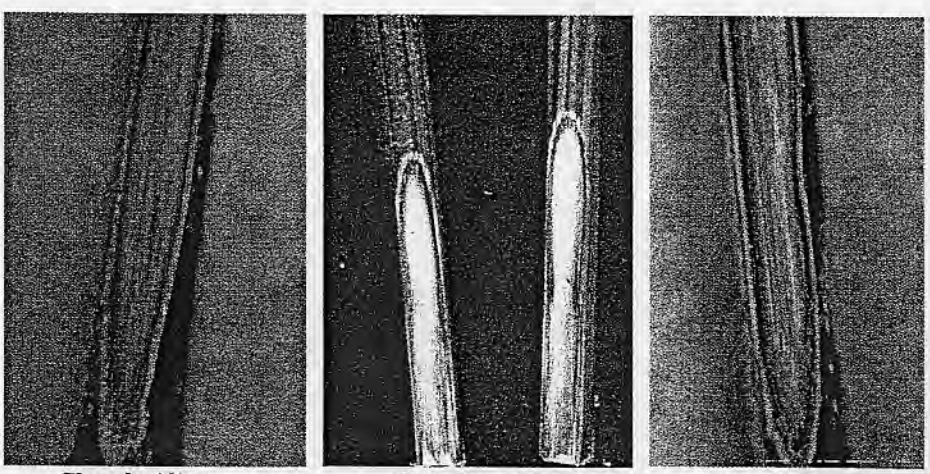

Fig. 7. Differences in live phloem and cambium and freeze-killed phloem and cambium.

Tissue and organ level: There are numerous tissues of importance as one evaluates cold hardiness. Further, individual tissues vary both in their cold hardiness at any given date, and vary in relative cold hardiness depending on the portion of the dormant, hardy season in which the assessment is being made. Specific discussion of the anatomy of freeze injury is in an accompanying presentation by Goffinet [23].

Meristems: The significance of meristematic tissues seems obvious. These are the tissues which give rise to shoot apices, flower structures, and add girth to the trunk and other perennial woody structures. The economic importance of these tissues is shown by the frequency that they are used as the indicators of the extent of injury resulting from a freeze stress episode [77]. We collect buds after a freeze and either force them, or cut them to see whether there is damage as indicated by a brown-black area at the location of the shoot apex or flower primordium (Fig. 6). In grapevines the shoot apex and flower cluster(s) have a common structure, and loss of one means loss of the other.

Cambium, phloem, and xylem: The cambium is the meristematic tissue responsible for secondary thickening of canes and trunks, and the production of the transport tissues phloem (exterior to the cambium and responsible for both apical and basal movement of elaborated organic compounds either stored in the perennial root, trunk, or cane tissues, or produced in leaves by photosynthesis).

Interior to the cambium is xylem tissue whose transport function is from base to apex, and is for water, inorganic nutrients, and several important growth regulating compounds including cytokinins, ABA, and possibly gibberellins (GA).

Freeze damage to the transport tissues has serious consequences. From a practical point, injury and damage is seldom of concern for xylem transport. Injury can occur, commonly to the parenchyma associated with the xylem (see deep supercooling above), but the damage occurs less frequently and with less detriment to the vine than does damage to phloem and cambium (Fig. 7).

The death of phloem creates a girdle response. If the cambium survives, phloem transport can be reestablished as new phloem is differentiated. The importance of cambium survival is obvious; its mortality presages death. The extent of the damage depends on the position of the cambium killed. If near a cane apex, basal tissues will not be negatively influenced. By contrast, if on the trunk near the crown, the vine may begin growth (xylem not impaired), bloom, and even set fruit before the roots die for lack of carbohydrates. This is followed by vine wilt and collapse.

In cold regions, injury may occur on only one portion of the trunk, the equator-facing side and slightly west. In the northern hemisphere, southwest injury is common in fruit trees such as apples and peaches, and I have observed it in grapevines as well. The vine can survive such exposures if there is sufficient cambium and phloem to maintain transport and secondary disease infections such as crown gall (*Agrobacterium tumefaciens*) are not expressed. The subsequent growth of the trunk girth will be asymmetric, reflecting the localized cambial death.

For additional information on crown gall and other freeze-associated disorders see Eastwell [17].

Tissue water content: As noted above the dynamics of water in the cellular-apoplast interaction is a key factor in freeze resistance at the cell level. The water status of tissues and organs also undergoes change during the transitions of acclimation and deacclimation. In grapevines, cold hardiness level of canes and buds increases as water content declines during autumn acclimation [89,90]. Hardiness declines as water content increases during deacclimation in the late winter and spring (Fig. 8) [Howell, unpublished research].

With other hardy, woody genera, artificial dehydration of hardy tissues increased their cold hardiness [12], while rehydration of hardy tissues reduced their hardiness [8,53].

The control of changes in tissue water content with the onset of cold acclimation has been addressed [40]. They observed that water content declined in dogwood tissues as a result of reduced resistance to water loss by stomata and a decreased permeability of root cells to water and suberization of the root surfaces reduced the ability of roots to take-up water. The result was a net drying of the woody portions of the plant. This has also been demonstrated in grapevines [Wolpert and Howell, unpublished research].

Extracellular inhibitors to ice formation and propagation: The mechanisms of cold resistance in tissues and organs includes those mentioned above for cells, but do include others. In work on the crown tissues of barley, Olien [48,49,50] demonstrated the presence and impact of complex polysaccharide freezing inhibitors existing in the apoplast which influenced both the rate of ice formation and the crystal size and lattice formation of the ice. To my knowledge, these have not been reported in grapes, but their presence in tissues of less hardy genera suggests that investigation is desirable.

Bud versus wood hardiness: The mechanisms of freeze resistance of bud and wood tissues have been addressed above and will be mentioned again here only by means of comparison. In typical vineyard conditions, bud and root tissues will possess less hardiness than wood tissues. Within the compound grape bud, the order of hardiness is primary < secondary < tertiary. This is most likely due to differences in developmental status as the primary becomes considerably less hardy than the secondary as early spring development progresses [33,78].

There has been a tendency to use bud hardiness as an indicator of vine hardiness with mixed results. The major impetus for that has been the ease of collecting large numbers of test tissues and the development of rapid, objective methods for determining tissue mortal-

ity via differential thermal analysis (See *Assessing vine cold hardiness).* While it is true that there is a general relationship between bud and wood hardiness, we have seen that the mechanisms of resistance are different (supercooling *vs* freezing point depression), and Wample [83], and we [Howell, unpublished research] have also observed that the relationship can be imprecise.

Root hardiness: It is also generally observed [20,35,54] that root tissues are less cold resistant than their above ground counterparts. Whether this is a true expression of tissue difference or confounded by the warmer environment of roots in soil during winter is not clear. It has been observed that when freeze episodes occur in open vineyards with very low soil water potential, that roots can be killed and above ground tissues survive [Wample; personal communication] until vine collapse due to root loss.

Within-vine differences in cold hardiness: As noted above, there are differing levels of cold resistance among different tissues being evaluated on the same date and for the same tissue at different dates during the dormant, winter season. The comments here will focus on differences that can exist for a given tissue or organ growing on the same vine when sampled on the same date. These differences exist, and they can be sizeable.

Grape producers know that selection of training system, crop control, and canopy management practices can have major impact on vine productivity and fruit composition, and the development of varietal character. These practices have similar impact on bud and cane cold hardiness. This work [31] demonstrates that a large range of hardiness that can exist for a single organ on the same vine on the same date, and provides explanations for the observed differences. A series of studies involving Concord, Vignoles, Cabernet Sauvignon, Pinot noir, and White Riesling grapevines were conducted and showed the following: *(1)* differences as large as 12°C existed for canes on the same vine; *(2)* exposure of canes and their leaves to sunlight during the growing season favored hardiness; *(3)* the differences observed could exist on crowded-shoot vines and on well-exposed shoot vines; the impact was on

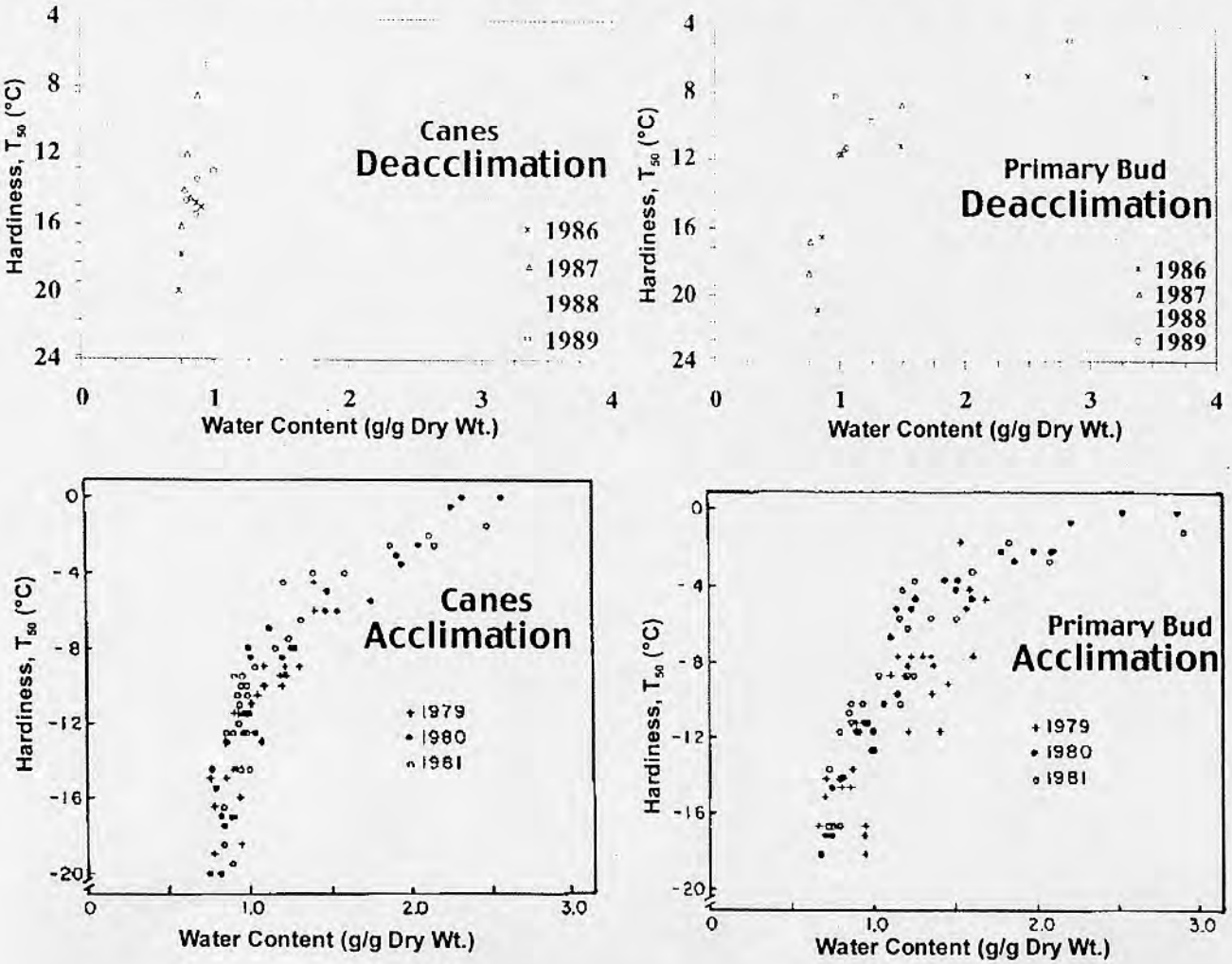

Fig. 8. Influence of cane and primary bud water content on cold hardiness of those tissues during acclimation and deacclimation.

Table 3. Relative impact of imposed carbohydrate stress on % soluble solids accumulation from verasion until harvest in Year-1 and cold hardiness the following winter, and fruitfulness of buds in Year-2.

Treatment	% SS[z]	% of Control-F	% Primary bud hardiness	% of control-D	Fruitfulness gm/node	Fruitfulness % control-F
Control-F	7.4	100	6.5 d	7	270 a	100
Cordon-F	7.6	103	13.5 c	15	237 a	88
Shoot-F	7.2	97	11.5 cd	13	253 a	94
Node-F	6.8	92	8.0 d	9	238 a	88
Node-D	6.9	93	8.0 d	9	233 a	86
Shoot-D	6.6	89	15.0 c	17	239 a	89
Cordon-D	5.1	69	41.5 b	47	153 b	56
Control-D	2.5	34	88.5 a	100	23 c	9
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[z]Amount of change in % ss between verasion and harvest.

,* - Indicate statistical significance at 0.01 and 0.001 level of probability.[After Mansfield and Howell, 1997.]

frequency of the desired cold hardy canes; (big, dense canopy vines had a smaller percentage of maximally hardy canes than did smaller vines with less dense and therefore well exposed canopies); *(4)* canes with either long internodes and/or large internode diameter for that cultivar were less cold hardy than canes of moderate length and diameter; and *(5)* on a given cane, the presence of large persistent laterals was associated with cane hardiness levels less than similar canes with no persistent laterals.

The impact of these within-vine hardiness differences has practical, as well as scientific, importance. The methods of culture employed to produce best fruit quality in Year-1 and return production the following year also favor bud and cane hardiness during the intervening dormant period. For scientists it is important to note that the within-vine cold resistance of canes and dormant buds is not random, and sampling procedure can profoundly influence the answer derived. Stratification of sampling [72] is crucial to effective measurement of cold hardiness of grapevine tissues [31]. The data reinforce the ideas presented earlier about carbohydrate status.

The data in Table 3 suggest that fruit ripening is less influenced than either cold hardiness or Year-2 bud fruitfulness. Simply said, superior culture is more critical if one is to achieve maximum expression of a vine's hardiness capability than is necessary to achieve maximum fruit production and/or crop maturation.

By way of conclusion, we can see that the mechanisms of cold hardiness are *freeze avoidance* via *supercooling* for buds and *deep supercooling* for parenchyma associated with the xylem, and *freeze tolerance* via ice formation in the apoplast of mature vegetable tissues at temperatures just below 0°C result in water export from the cell to an ice crystal in the apoplast and subsequent *freezing point depression* and tolerance of solute concentration within the protoplast.

Specific chemical compounds and compound groups are related to cold hardiness at the cellular level including: ABA, stress related proteins, carbohydrates, isozymes, and membrane proteins and phospholipids.

Structures and organelles of the cell are important to cell survival of a transient freeze stress. Of singular importance are the membranes including the plasmalemma, tonoplast, mitochondrion and chloroplast, endoplasmic reticulum, the golgi apparatus, and the peroxisome.

Genotypic and Cultural Influence on Grapevine Cold Hardiness

All expressions of grapevine growth and productivity are under genetic control as influenced by the environment. This is true of vine cold hardiness as well. The information provided to this point has had a goal of pointing up the large array of molecular, cellular, tissue, organ, and vine organizational morphology on the ability to survive a freeze episode. Cold hardiness in grapevines and other woody fruit species is complex [11,16]. There is no "master reaction" for grapevine cold hardiness.

Assessing vine cold hardiness: There are a number of ways to evaluate a given cultivar's cold hardiness.

Field trial: One may plant a range of selections in a replicated trial and wait for natural freeze events to create the stress circumstances and then evaluate the responses to that winter dormant season. This is commonly done as a hierarchy of injury ranging from shootless node percent to 100% of vines killed. This is the most widely employed method of assessing a vine's cold resistance, and for reasons that we have noted above, it is useful, but it does have limitations.

Field trial - systematic assessment: In this case, the assessment of vine tissues takes place at predetermined intervals so that the impact of natural freeze episodes may be assessed. This is most commonly applied to buds.

Laboratory assessment: There is a large body of literature on methods to determine the cold resistance of perennial woody plants. This literature applies in most cases to grapevines as well. The interest of this presentation is focused on responses and factors which influence hardiness, so we will not explore that area. Suffice it to say this: *(1)* an artificial freezing method is required that will allow discrimination among the cultivar/treatments to be tested; *(2)* the methods should be relevant to differences occurring in the field; *(3)* the freeze program should reasonably express freezing rates occurring in nature; *(4)* a precise method for determining viability of the organ or tissue of interest is required, preferably an objective method; and *(5)* suitable statistical methods both in terms of sampling for the evaluation and for the determination of real, statistically valid differences must be employed. Readers seeking more detail are referred to the following [7,21,34,56,58,76,77,88].

This approach removes the variability inherent in natural freezes and substitutes a controlled temperature freezer. The advantages are clear: *(a)* similar freeze conditions at each assessment date; *(b)* ability to assess at any desired frequency over time; *(c)* allowance for an assessment of seasonal differences both within and among cultivars; and *(d)* allowance for a cold hardiness assessment of the influence of cultivar, cultural management practices, or both in a common experiment.

Attempts to shorten the process of hardiness assessment have been attempted using grapevine callus. Difficulties have been encountered as the unorganized state of the callus did not respond to acclimating programs and cultivars of known hardiness difference in vineyards were not different when subjected to freeze stresses [46]. This is also important as we look at cold hardiness from the whole-vine perspective. All cells on a grapevine, save a few that have mutated, have the same genetic makeup. That some genes are switched on and/or off in a prescribed manner for that cultivar is why we have leaves, shoots, tendrils, flowers, berries, cluster raci, trunks, and roots. The callus tissue experiments reported above point out the importance of tissue type and organization in the expression of a vine's genotype with relation to cold hardiness. Thus, while differences at the cellular level are of considerable interest as we seek to have full understanding of the grapevine hardiness phenomenon, the relative contribution of tissue organization seems to be of greater practical importance.

Cultivar and rootstock: There are considerable differences across the genus *Vitis* in the cold resistance of different species. As noted with dogwood [71], the geographic origins of an ecotype has considerable impact upon the growth and hardiness of the plant in a given location. This is true of grapevines as well. North America is native habitat of a large range of grape species. The response of these to culture in any given macroclimate is closely linked to the geographic origins of the species. As noted above, *V. riparia* has an ability to tolerate severe low temperatures [56]. It is seldom injured by freeze episodes during acclimation or mid-winter. It is can be injured in late winter when unseasonably warm periods are followed by a sharp temperature decline (Fig. 1). *V. riparia* rapidly responds to conditions favoring growth.

Its origin explains the response. Grapevines have evolved numerous methods to achieve the prime reason for its survival — the production and dispersal of grape seeds. *Riparia* is the most northerly adapted of native grape species. Accepting the prime directive of a species as sexual reproduction and progeny dispersal, northern adaptation means reproduction in a very short growing season. In these northern regions, the transitions of weather supporting dormancy to conditions favoring active growth occur rapidly. Under such conditions, rapid vine response favors rapid response to warm conditions, an early leaf array, and ultimately more seeds produced and dispersed.

However, when the same genotype is cultivated further south, under conditions which show much milder mid-winter cold, the vines are sometimes injured. This has been observed in apricots [Howell, 1966; unpublished data]. In peach, the hardy rootstock Siberian C was shown to result in superior scion hardiness during acclimation and mid-winter, but was comparatively less hardy during deacclimation (Table 1b) [29]. The data suggest that the response is due to a hastening in the onset of growth as a response to late winter/early spring warm temperature episodes. Similar rootstock responses have been found with sweet cherry [30].

There is considerable disagreement among scientists and producers concerning the impact of grape rootstock on the cold hardiness of the scion cultivar. The problem is associated with methods of sampling and the questions being asked. It is easy to statistically confound responses. Too often such experiments do not keep crop level, vine size, or canopy management factors similar, and sampling procedures are not stratified. Our work to date suggests that while there are small direct effects on scion cultivars that may be attributed to the rootstock, most observed responses are secondary in origin and mediated through well understood processes such as canopy density and crop load [27,28,41,44,79,86,87].

The data in Table 4 reinforce the view that scion exposure to the above-ground environment has a greater impact on budbreak date, ripening date, productivity, and winter cold hardiness than does the rootstock. Issues of crop control, canopy management, training system, vine nutrition [86,87], and irrigation also bear on the question of vine culture and cold hardiness. Dr. Wolf presents information on these topics [85].

Future

Herein we have considered what changes in vine cold hardiness might be forthcoming. There is much that is promising. The efforts to understand cold hardi-

Table 4. Response of reciprocal grafts of Marechal Foch and Vidal blanc on a range of viticultural factors. All data are mean of five years data.

Treatment Scion/Rootstock	Vine size	Nodes retained	Yield T/A	Fruit composition % SS	pH	TA
Foch/Foch	0.61b	33 ab	5.04 b	20.0	3.43 a	1.11 b
Vidal/Vidal	1.00 a	28 b	6.41 ab	19.6	3.29 b	1.32 a
Foch/Vidal	1.10a	40 a	6.35 ab	19.3	3.33 b	1.21 ab
Vidal/Foch	0.90a	25 b	7.16 a	20.1	3.38 ab	1.12 b
	**	*	**	ns	*	*
Main effects scion cultivar						
Foch	0.86	37	5.70	19.9	3.34	1.16
Vidal	0.95	27	6.79	19.7	3.38	1.22
	*	**	**	ns	ns	ns
Rootstock cultivar						
Foch	0.76	29	6.10	20.1	3.41	1.12
Vidal	1.05	34	6.38	19.5	3.31	1.27
	**	**	ns	ns	ns	ns

Influence on vine performance	Budbreak [z]	Verasion [y]	% Blind nodes [x]	Vine mortality
Foch/Foch	1.6	91	5	0
Vidal/Vidal	11.7	103	15	0
Foch/Vidal	1.4	88	3	0
Vidal/Foch	12.5	105	17	

[z] Foch is earlier than Vidal on own-rooted vines by 8 to 15 days depending on spring weather, Foch was given 1 and Vidal then nu ber of days delayed.
[x] Days from bud burst to verasion.
[y] Number of shootless nodes ÷ number of nodes retained.

ness at the molecular and gene level hold the potential for the ultimate identification of each of the myriad of characters related to vine cold hardiness at the various levels of gene, cell, tissue, organ, and organism. It will be complex, but it can be done.

Of serious consideration will be the public response when it does happen. We can get a hint of the response by a quick assessment of our written and broadcast news media. While we are all more circumspect as we digest the news in the era of tabloid journalism, the key is that controversy sells papers and improves viewer ratings. Both bring money to the presenter. With relation to genetic improvement, there is an interesting coalition of neo-Luddites, religious fundamentalists, and the economic interests with holdings in the *status quo* that will make the path toward this progress a rocky one.

The science is the least of the difficulties facing the future. Already there are interests lining up to say that Chardonnay, with an added gene for disease resistance, cannot be called Chardonnay. Never mind that bigger genetic differences may exist among current clones all being called Chardonnay. As one who trusts the judgement of informed people, one becomes appalled at the fundamental ignorance about the simplest rudiments of genetics that exists among otherwise well-educated people. Especially lacking is the understanding and implications of how much genetic material all life on this planet has in common.

In spite of this, we must not lose sight of the goal — to produce hardy genotypes *and* to culture them under regimes that produce maximal expression of those genes while maintaining the characters of economic consequence in the vine. It seems strange that we could soon face a situation where we have the scientific ability to improve genotype for vine cold hardiness and be losing the underpinnings of quality viticultural research that could make this basic research relevant. The reality is that we require both.

That is sometimes a hard sell when funding sources for the "cutting edge" research carries large percentages of overhead for the scientist's home institution, and sources for efforts important to grape growers and others who care about food production do not. One cannot blame administrators, hard-pressed financially to maintain high quality institutions, for encouraging a greater amount of the former at the potential expense of the latter.

But I remain optimistic about the future and grapevine cold hardiness. I believe that we are now having the best experience at growing cold tender grape cultivars in marginal climates that we have ever had. Thirty years ago I was challenged by a senior, internationally recognized scientist at Michigan State University concerning my desire to initiate cultivar evaluation plantings of French-American hybrids in Michigan. "A waste of time", he said, "we can't grow them here, they don't have enough hardiness." I thought about that one

night a few weeks ago as I enjoyed a glass of my favorite Michigan Pinot gris. I remain optimistic.

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