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THE USE OF CONTROLLED FREEZING TO EVALUATE FACTORS INFLUENCING CRITICAL TEMPERATURES FOR FREEZE INJURY IN DEVELOPING GRAPEVINE BUDS

By

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ABSTRACT

THE USE OF CONTROLLED FREEZING TO EVALUATE FACTORS INFLUENCING CRITICAL TEMPERATURES FOR FREEZE INJURY IN DEVELOPING GRAPEVINE BUDS

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A controlled freezing technique was developed and used to determine critical temperatures for developing grapevine buds. Critical temperatures were estimated for 'Concord' grapevine buds at defined stages of development under both wet and dry surface conditions. Tissue surface moisture strongly influenced freeze resistance. Hardiness was similar for developing 'Concord' buds whether on greenhouse forced cuttings, on cuttings taken directly from the field during spring development, or on outdoor grown whole potted vines. Pre-freeze temperature had no apparent effect on hardiness of developing buds. While freeze resistance always decreased with advancing phenological development, morphological characteristics prior to bud development had no effect on bud hardiness or rate of development. Cultivar differences affected both the rate of bud development, and the hardiness at a given stage, of buds forced from stored cuttings.

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LITERATURE REVIEW

Introduction

Grapes are the leading horticultural crop in terms of world-wide production (Harlan, 1976). Among the United States, Michigan is fourth in production, with 15,800 acres of vines (Lovett and Collins, 1978), and a 1979 crop valued at over $10 million (W.R. Grevelding, personal communication). Freezing injury represents a major economic loss to United States agriculture and 63% of this loss is to fruit crops (Mayland and Cary, 1970), with an estimated 10% of the grape crop being lost annually (Reingold, 1960). Spring freeze damage can be devastating to grapes, and no viticultural area in the United States is completely free of this hazard. In Michigan, substantial crop reduction from spring freeze has occurred in 11 of the 21 years from 1957 to 1977 (Howell and Wolpert, 1978). In two recent years the Michigan grape industry has been officially declared a "disaster area" as a result of spring freeze damage (Howell, 1976).

Spring freeze damage can be reduced by site selection, site modification, cultural practices, and microclimate modification (Rogers and Swift, 1970; Ballard and Proebsting, 1972). Certain aspects of these techniques, as they relate

specifically to grapes, are presented by Dethier and Shaulis (1964). Methods of frost protection not currently used on a large scale, but under investigation, include modification of pruning techniques in grapes to retard spring bud development (Howell and Wolpert, 1978), application of insulating stable foam (Bartholic et al., 1970) and the use of growth regulators and systemic cryoprotectants (Burns, 1974; Ketchie and Murren, 1976).

Frost protection may be divided into two categories; passive or preventative measures such as site selection and cultural manipulation, and active or palliative measures such as heating or sprinkling. The former are taken well in advance of possible frosts, while the latter require a critical decision to begin protective measures. The consequences of error in the decision to start protective measures are excessively high operation costs on the one hand, and crop loss on the other. The decreasing availability and increasing cost of fuel, has stimulated interest in reducing energy consumption for freeze protection. Increasing the accuracy and availability of hour to hour weather forecasts with infrared thermal imagery, and developing protective systems with rapid start-up capabilities, may signigicantly aid this effort (Proebsting, 1975). The final decision to start protective measures must be based on an understanding of the temperature likely to cause injury or the "critical temperature" (Proebsting and Mills, 1978).

Critical Temperatures

Critical temperature, defined as the lowest temperature endured for 30 minutes or less without injury, was apparently introduced by Young (1920). LT_{50}, or the temperature estimated to cause 50% kill, (Timmis, 1977; Dennis et al., 1975) as well as LT_{10} and LT_{90} (Proebsting et al., 1978) have also been used as indicators of the critical temperature. Critical temperatures for several fruit species, including grapes, are given by Young (1947) and Rogers and Swift (1970). Both publications point out the deficiencies in the data used to arrive at these temperatures. Critical temperatures for tree fruits and small fruits in Michigan during spring development were listed by Eichmeier et al. (1965), but the source of the data and methods used to determine these temperatures were not given. More detailed information on critical temperatures has been given for tart cherries in Michigan (Dennis and Howell, 1974), and for 6 deciduous tree fruit species in Washington (Ballard et al., 1971). Critical temperatures for developing grape buds have not been as extensively studied. Clore et al. (1974) suggest that grape buds in full swell can tolerate -5.5°C. Preliminary estimates of critical temperatures for grape buds at several stages of spring development were reported by Proebsting et al. (1978). The concept of critical temperature was extended from fruit species to cone buds in

coniferous seed orchards by Timmis (1976).

Critical temperatures are affected by several factors. The most important factor is the stage of phenological development, (Ballard and Proebsting, 1972). Early studies on the relationship of developmental stage and frost hardiness in peach buds were presented by Blake and Steelman (1944), and Proebsting and Mills (1961). Generally, the more advanced the stage of development, the higher is the critical temperature, although this is not always strictly true (Rogers and Swift, 1970). For example, developmental stage had no effect on freeze resistance in the frost tender reproductive tissues of developing wheat ears after emergence from the leaf sheath (Single and Marcellos, 1974). Suitable definition of the stages of development can be a problem. Young (1947) used 3 descriptive stages for all fruit species. Most recently, Proebsting and Mills (1978) used 7 to 9 stages separately defined for each of 6 tree fruit species studied. Stages of grape bud development were described and given letter designations by Baggiolini (1952). Proebsting et al. (1978) used descriptive terms with definitions, and an effort has been made to coordinate these definitions with other investigators (E.L. Proebsting, personal communication).

Species and cultivar are also important when considering critical temperatures. Early studies indicated that apple blossoms were more tender than peach,

which were more tender than cherry (Chandler, 1913). Varietal differences may be related to developmental rate. Early blooming cultivars of apricot showed 3 times the injury from a naturally occurring frost than late blooming cultivars (Layne, 1966). At one site in Michigan, during the severe frost of April 27, 1976, trace primary bud kill occurred in the grape cultivar 'Vidal-256' whose buds did not reach full swell until May 4, 1976. However, primaries of 'Foch' were 5-7cm long when frosted and suffered 95% kill (Howell, 1976). Variability in bud development within a cultivar can also be a factor. Flowers in late blooming lateral buds of 'Golden Delicious' apple may survive a frost that kills buds on the spurs (Ballard and Proebsting, 1972). Some cultivar differences appear to be inherent rather than being related to development rate. Buds of several peach cultivars showed varying degrees of injury at the same temperature and stage of development (Blake and Steelman, 1944). 'Earliril' apricot and 'Chinook' cherry are early blooming cultivars which appear to be hardier than late blooming cultivars (Ballard and Proebsting, 1972).

In addition to cultivar differences, characteristics of grape vines such as sunlight exposure of the leaf at a given node, periderm color, cane diameter, presence of persistant laterals, and node position on the cane relative to base and apex have been related to winter hardiness of dormant grapevine buds (Howell and Shaulis, 1980).

Preliminary observations by N.J. Shaulis (personal communication) have suggested that poor sunlight exposure during the previous growing season may increase spring frost injury in 'Concord' grapevines.

The duration of the low temperature may be important. Generally, the longer the temperature stays below the critical level, the greater the damage (Rogers and Swift, 1970). Ballard and Proebsting (1972) suggest that duration is important as a factor in tissue-air temperature equilibrium but that the minimum temperature is relatively more important than the duration.

Humidity, usually measured as dew point, can be a factor in spring freeze damage. Rogers and Swift (1970) suggest that in deciduous fruits, more damage results from a given temperature at lower humidities. Ellison and Close (1927) concluded that when the dew point is low, severe injury occurs to apple blossoms at temperatures causing only slight injury when the dew point is high. Ballard and Proebsting (1972) state that dew point probably has no effect on actual tissue hardiness, but that low dew points favor radiation to the sky and evaporative cooling that may result in tissue temperatures $3^{o}$to 4^{o}F lower than the air temperature on calm nights, while winds of 2 miles per hour or more tend to keep tissue temperatures close to air temperatures. The latent heat released when dew or frost forms as the temperature falls to the dew point may considerably retard the rate of

fall (Rogers and Swift, 1970). This, coupled with the fact that no visible frost forms under conditions of sufficiently low dew point, may have led early investigators to conclude that low dew points result in greater injury at a given temperature. Recent results on citrus (Young, 1969) and mulberry leaves (Kitaura, 1967) suggest that the reverse is true. External ice such as hoar frost can readily nucleate plants via entry sites such as stomates, lenticels, and wounds (Burke et al., 1976).

Conditions prior to the frost may also affect the temperature necessary to cause injury. Ballard and Proebsting (1972) state that temperatures during the 24 hours preceding the freeze can have profound effects on hardiness of tree fruit buds prior to bloom, although they do not describe the nature of these effects. Hewett et al. (1978) were not able to find any clear relationship between damage to blossom buds at a given temperature following warm weather, and damage following cool weather. Storage up to 6 days at 1°C did not increase the freeze resistance of developing wheat ears (Single and Marcellos, 1974). Longer term effects may be important, as Ballard and Proebsting (1972) state that apple blossoms developed in cool weather have been shown to be more frost resistant than those developed in warm weather. Under conditions of severe soil moisture shortage, water content of apple blossoms was reduced and damage from frost was condiderably less than that to blossoms of well watered trees (Modlibowska, 1961). Hewett et al. (1978) noted severe

spring freeze damage to developing grapevine buds following sprinkler irrigation while adjacent unsprinkled vines were unaffected. These authors also found that buds from sprinkled peach and apricot trees had higher moisture content, and suffered more damage from artificial freezing, than unsprinkled buds. They suggest that if buds have been subjected to recent sprinkler irrigation or rain, critical temperatures must be revised and that care should be taken to avoid sprinkling before predicted freezes.

Freezing in Cold Tender Plant Tissue

New shoots and leaves of non-acclimated woody perennials are injured by temperatures only slightly below 0°C. Supercooling and freezing point depression are means by which some resistance is achieved, but when ice nucleation occurs, rapid intracellular freezing takes place, resulting in destruction of membrane continuity (Burke et al., 1976). Injured foliage appears flaccid and water soaked, as cell membranes have lost their semipermeability and intracellular compartmentalization has been destroyed. Intracellular freezing is nearly always fatal (Scarth, 1944; Evert, 1967). In herbaceous tissues that tolerate ice formation, (e.g., hardened winter wheat, cabbage, turf grass, etc.), ice appears to form in extracellular spaces. During freezing in hardened barley crown tissue, water readily diffused from the protoplast to extracellular nucleation sites, while freezing in cold tender tissues was characterterized

by explosive ice formation resulting in the rupture of plasma membranes and release of cell contents into extracellular spaces (Olien, 1964). In mature moss shoots water moved to sites of extracellular ice formation allowing survival, while the water in actively growing shoots supercooled, then froze intracellularly; in addition, cells of the mature shoots lost water faster than cells of young shoots when plasmolyzed (Hudson and Brustkern, 1965). These findings indicate that the plasma membrane of cold tender tissues is less permeable and will not readily allow movement of water to external nucleation sites. Increases in cell permeability have been associated with the hardening process (Scarth, 1944; Evert, 1967). In some frost susceptible plants such as Solanum tuberosum L., the quantity of fluid lost by cells, or its rate of loss may be insufficient, causing ice to form at many points throughout the tissue (Hudson and Idle, 1962), rather than at sites which accomodate ice with little damage to the plant, as is the case with hardy woody tissue (Burke et al., 1976).

Exotherm studies have shown that azalea flower primordia (George et al., 1974), peach flower primordia (Quamme, 1978) and blueberry buds (Bittenbender, 1974) are killed at the onset of ice formation, and rely on supercooling for survival even during midwinter. Buds of the wild grapevine Vitis riparia Michx. apparently behave similarly (Pierquet, et al., 1977). This is not the case for apple buds during midwinter (Burke et al., 1976). The extent of supercooling

apparently is related to the number and quality of ice nucleating sites. The freezing temperature of conifer needles rises with increasing needle length and the quantity of ice nucleators, rather than the quantity of water, may be critical (Kaku and Salt, 1968). A similar relationship exists in mature Buxus leaves, although in immature leaves, age was the important factor regardless of size (Kaku, 1971). Changes in hardiness within a tissue that relies on supercooling may be related to the abundance of nucleating centers within cells and or the presence of effective barriers to nucleation (Burke et al., 1976). Supercooling apparently provides some spring frost resistance to the developing buds of deciduous fruit species. Modlibowska (1962) observed supercooling of apple blossoms in the orchard during natural spring frosts. Ballard and Proebsting (1972) report trace injury vs. 100% kill in adjacent peach trees and attribute this to supercooling that did not persist in the damaged tree. Hewett et al. (1978) state that the increased spring freeze injury to sprinkled grape buds may have resulted from lower bud temperatures due to evaporative cooling or from reduced supercooling, but they state elsewhere that the wet bulb temperatures were not low enough to account for the injury. It therefore appears that supercooling was a factor in the superior resistance of the unsprinkled buds.

Controlled Freezing

There has been widespread use of controlled freezing techniques in studies on cold hardiness. Controlled freezing of fruit buds to determine critical temperatures has been used for decades (Proebsting and Mills, 1971). All controlled freezing studies assume a close relationship between the behavior of artificially frozen material and material frozen under natural conditions. This assumption has proven accurate enough for these tests to be very useful (Levitt, 1951; Lapins, 1961; Proebsting and Mills, 1978). Injury to 'Montmorency' cherry from naturally occurring spring freezes tended to be greater in early stages of bud development and less in advanced stages than predicted by controlled freezing (Dennis et al., 1975). Most freezing studies have made use of a standard, or somewhat modified, chest freezer. Scott and Spangelo (1964) described the design and construction of a portable cold stress unit for in situ freezing of whole fruit trees. Quamme et al. (1972) described a technique utilizing the vaporization of liquid nitrogen for freezing biological materials. A control system and solenoid valve are used to either regulate release of nitrogen vapor or activate an electric heater. Advantages are accurate temperature control, rapid response, and a wide range of possible temperatures, (+50°C to -100°C), although capacity is rather limited. Whatever the system employed there are several

important factors that should be considered in any controlled freezing experiment:

1. Temperature stratification within the freezing chamber can cause some material to be subjected to temperatures different from other material or recorded temperatures. A fan within the freezer improves temperature distribution but also adds heat (Proebsting and Mills, 1971).

2. Fluctuations in freezer temperature, caused by thermostatically controlled on-off cycling, can be significant. True temperatures are alternately above, then below intended temperatures. This problem has been circumvented by setting the freezer to a constant low temperature and insulating the plant material within the freezer, resulting in a gradual decline in tissue temperature as equilibrium is established (Howell and Weiser, 1970). Temperature within a conventional freezer can be lowered by controlling the coolant flow rather than by on-off cycling of the entire system. This can be accomplished using an expansion pressure regulator (EPR) valve (Lumis et al., 1972).

3. Rate of temperature change, particularly rate of fall, is generally considered important (Lapins,1961; Proebsting and Mills, 1971; Daniel and Crosby, 1971). However, the rate of cooling did not alter the freeze resistance of guayule (Mitchell, 1944), and early studies have shown that cold tender tissues are not affected by the

rate of freezing (Levitt, 1951). Perhaps this is related to the lack of significant movement of intracellular water to sites of extracellular ice nucleation. This may be the reason that Proebsting and Mills (1971) state that, for fruit buds during dormancy, temperatures should be lowered at 2°F per hour, while during the prebloom period, rate of fall may be increased to 5°F per hour. The same authors point out that if the tissue mass is so large that its heat capacity and release of heat of fusion causes tissue temperature to lag measurably behind air, (box), temperature, very slow rates of fall may be necessary. Cold tender beet root tissue frozen to -4°C suffered less damage from ultraslow cooling (0.2°C/hr.) than from moderately slow cooling (3.3°C/hr.), while ultrafast rates produced no more damage than the moderate rate (Finkle et al., 1974). This may indicate some movement of water out of the cells to extracellular nucleation sites when rates of fall are slow enough. Rates of fall of 0.2°C per hour probably seldom occur in natural freezes. Rates of tissue thawing may be important (Siminovitch and Briggs, 1953) but Ballard and Proebsting (1972) suggest that for developing fruit buds, this is a very minor factor if it has any influence at all.

4. Supercooling was prolonged, and damage from controlled freezing was reduced, in apple blossoms when spurs or single flowers were cut from trees, while blossoms on whole potted trees seldom supercooled throughout a freezing

experiment (Modlibowska, 1962). Mulberry leaves supercooled more when detached from the stem (Kitaura, 1967). If excised plant samples tend to supercool more than whole plants, erroneous conclusions may be made about critical temperatures for natural freezes. To help prevent this, Proebsting and Mills (1971) suggest wetting the surface of all samples before placing them in the freezer. This reduces apparent cold resistance somewhat and Hewett et al. (1978) state that under these conditions, test injury correlates well with field injury. Because the excising of tissues prolongs supercooling, it would seem particularly important to reduce this by wetting the tissues if the test temperatures are not maintained for long periods.

Use of χ^2 and the Rx2 Contingency Table in the Analysis of Hardiness Data

Horticulturists often misuse the analysis of variance (Evert and Howell, 1979). This and other parametric tests require random sampling from a population with normal distribution and homogeneous variance for all treatments. Most parametric statistics also require an interval scale of measurement, that is, a scale that reflects the size of differences between measurements (Conover, 1971). Discrete data, such as living vs. dead buds, are not interval measurements, but rather, they are nominal measurements. Most nonparametric tests assume either a

nominal scale or an ordinal (ranked) scale to be appropriate, and that the distribution function of the random variable producing the data is unspecified (Conover, 1971). There are several nonparametric tests that may be appropriate in the analysis of hardiness data. The modified Friedman test as described by Evert and Howell (1979) appears well suited to certain hardiness studies where the amount of injury among the various treatments can be ranked.

The Rx2 contingency table as described by Cramer (1944), Steel and Torrie (1960), Conover (1971) and Meddis (1975) provides another technique of evaluating hardiness data. A contingency table is an array of natural numbers in matrix form in which the numbers represent counts or frequencies. The Rx2 table consists of R rows of catagories or treatments divided into 2 classes (e.g., alive vs. dead). The question to be answered is "Do the treatments or categories significantly alter the proportion of objects or results in each of the 2 classes?" The null hypothesis (H_o) would then be: $P_1=P_2=P_3=...P_R$, or that the proportion of buds in a given class is the same for, or independent of, the various categories or treatments. The alternate hypothesis (H_1) would be that in at least 2 of the categories or treatments, the proportions are not the same. The calculation of the test statistic used to examine these hypotheses is given in different but equivalent forms by Conover

(1971, page 152), and Steel and Torrie (1960, page 371). The test statistic is distributed approximately as χ^2 with (R-1) degrees of freedom (Cramer, 1946). Large values of χ^2 indicate that the deviation from the null hypothesis is signigicant. Cramer (1946) and Steel and Torrie (1960) discuss methods of determining the degree of dependence indicated by the sample. Because the asymptotic distribution, (χ^2), is used, the approximation of significance levels may be poor if the expected values of a given contingency table are small. The expected value for any cell in a contingency table is the product of the row and column totals for that cell, divided by the grand total. The approximation is considered poor if 20% or more of the expected values are less than 5 (Meddis, 1975; Conover, 1971). If some of the expected values are too small, several categories may be combined provided they are similar in some respects such that the hypotheses retain their meaning (Cramer, 1946; Conover, 1971). Steele and Torrie (1960) indicate that data such as the numbers alive and dead at several test temperatures may be pooled into one contingency table.

A 2x2 contingency table can be used to determine significant differences between any 2 of the various categories in an Rx2 table. If 5 cultivars are subjected to freezing stress, the 5x2 contingency table can be used to determine whether the frequencies of living and

dead buds are dependent on cultivar. A signigicantly high value of the test statistic indicates only that the frequencies for at least 2 of the 5 cultivars are different. To determine whether any given 2 of the 5 cultivars differ significantly from each other, the appropriate 2x2 table must be used. In discussions of 2x2 contingency tables, a correction for continuity, ("Yates correction") is sometimes recommended (Steel and Torrie, 1960) to compensate for the use of a continuous distribution function (χ^2) to approximate the discrete distribution function of the test statistic. Conover (1971) cites several authors with whom he is in agreement in recommending against the use of "Yates correction" as being overly conservative.

The use of a one-tailed test, with its increased sensitivity, is appropriate for the 2x2 contingency table if one category is expected to have a greater proportion in a given class (Conover, 1971). Therefore, when comparing 2 cultivars, or 2 developmental stages, where one cultivar or stage is expected to be less hardy, the one tailed comparison should be used.

PURPOSES

With the ever increasing cost of fuel, more emphasis will probably be placed on frost protection methods other than high energy consuming microclimate modification. This study was initiated for 3 primary purposes. The first was to develop a suitable controlled freezing technique for developing grapevine buds that would facilitate the investigation of new frost control measures by repeated and readily controlled evaluations of their effects. The development of this technique would also make possible the accomplishment of the second purpose, that of determining actual critical temperatures for developing grapevine buds. This would allow more efficient use of existing microclimate modification techniques. The third purpose was to investigate various factors that might affect critical temperatures and therefore influence further investigations into frost protection as well as the use of current frost control methods.

MATERIALS AND METHODS

Plant Materials. During midwinter of 1977 and 1978, cane cuttings were collected from a vineyard of 6-year-old 'Concord' (Vitis labruscana Bailey) grapevines at the Michigan State University Horticultural Reasearch Center. In preparation for the 11 April 1978 experiment, cuttings were stratified in two groups at the time of collection. One group consisted of cuttings with light periderm color taken only from the interior of the vine canopy. The other, with dark periderm color, was from the vine canopy exterior. Cuttings for the 13 April 1978 experiment were stratified as to collection from either main canes or laterals and only cuttings of medium diameter (5.0-7.5mm) were used for forcing and hardiness evaluations. Each of the cuttings collected for the 17 April 1978 experiment included nodes 1 through 16. This allowed stratification based on nodal position as well as cane diameter. In November 1978, additional cuttings of the cultivars 'Baco-1' and 'Vidal-256' (interspecific hybrids of Vitis) were also collected from "Tabor Hill" vineyard in Berrien county, Michigan. Cuttings were stored in moist peat at 1-2°C until used. Storage time was sufficient to satisfy rest and the length of time depended upon material needed for forcing in the greenhouse. Buds were forced on a greenhouse mist bench unless otherwise

stated. Mist cycle varied from 1 to 4 three second mistings every half-hour depending on outside conditions as this affected greenhouse temperature and evaporation rate. For the 11 December 1977 freezing experiment, buds were also forced in the same greenhouse without misting (cane bases in water), and in a growth chamber on a 14 hr-13°C/10 hr-8°C day/night cycle. These conditions approximate the mean max./min. temperature and photoperiod occurring during bud expansion in the spring of 1977 at the Michigan State University Horticultural Reasearch Center. Controlled freezing studies were also carried out on naturally developing buds taken directly as cuttings from a block of 12-year-old 'Concord' grapevines and on non-excised buds on 2-year-old potted 'Concord' vines, grown at the MSU Horticultural Reasearch Center.

Grapevine buds were classified into 5 developmental stages based on those described by Proebsting et al. (1978). Stages are as follows:

Scale Crack. This is the first visible indication that growth has begun. A small crack occurs between the hard outer most bud scales as the bud begins to swell. If these scales have been removed or damaged, as often happens during handling of stored cuttings, this stage can not be accurately assessed. This stage would be intermediate between A and B as defined by Baggiolini (1952).

First-swell. This stage is approximately the equivalent of stage B or "cotton tip" swell of Baggiolini and of "first swell" of Proebsting et al. At this stage the bud has swollen out of the hard outer bud scale and is globular, doe colored, and fuzzy in appearance. No green or pink color is visible.

Full-swell. This stage corresponds to stage C or "green tip" swell of Baggiolini and "full swell" of Proebsting et al. The bud has elongated, being roughly 1.5 to 2 times as long as wide. One or more bulges of leaf tissue are visible and appear green or pink. The bud remains closed around the growing point.

Bud-burst. This stage is roughly equivalent to stage D or "first-leaf" of Baggiolini, (1952) and to "burst" of Proebsting et al. (1978). Here the leaves have separated at the tip, usually exposing the growing point. No leaf has, as yet, made a right angle with the stem.

Expanded-shoot. Here the young shoot is 4-6cm in length with 1-3 small leaves at right angles to the stem. This stage most closely approximates stage E or "leaf expansion" of Baggiolini et al. (1952), and "2nd leaf" of Proebsting et al. (1978A).

While bud development is a continuum, only buds judged to typify a given defined stage were used for hardiness evaluations. In all developmental studies, buds were recorded as being at the stage they most closely fit.

Hardiness Evaluations. Freezing technique was a modification of that used by Howell and Weiser (1970). Buds were cooled, and at each of several test temperatures a portion of the buds was removed from the freezer and allowed to thaw at 2°C. Test temperatures were selected such that the warmest temperature produced no injury and the coldest was lethal for all buds. The temperature interval was 1.5°C.

Freezing was conducted in a special walk-in freezing unit at the MSU Horticultural Research Center. This unit is equipped with several large blowers to minimize temperature stratification within the freezer box. Temperature fluctuation from freezer on-off cycling does not occur as this unit is equipped with an expansion pressure regulator valve which regulates coolant flow. Box temperature is lowered by the gradual opening of this valve controlled by a time clock. Rate of temperature fall was at 3°C/hour. Box temperature was monitored via several 24 guage copper-constantan thermocouples distributed around and among the plant material. Bud tissue temperature was monitored by thermocouples inserted into several extra buds not being used for hardiness evaluation. Preliminary evaluation showed only minor ($\leq$0.2°C) differences between tissue and air temperature. Air temperature therefore served as recorded test temperatures. Because developing grape buds are very susceptible to mechanical damage, they could not be bundled together for

freezing as suggested by Proebsting and Mills (1971). The bases of one node cuttings were instead stuck into pieces of moist floral foam to provide both mechanical support and a continuing moisture supply.

Depending on availability of buds of appropriate stage and or treatment, buds were randomly assigned to 1 group of 8 to 15 buds, (early experiments), or to 3 groups of 6 to 10 buds, (later experiments), per test temperature. A group from every stage or treatment was placed at random on a piece of moist floral foam. Therefore, either 1 foam piece, (early experiments), or 3 foam pieces, (later experiments), were removed at each test temperature. The number of foam pieces per temperature and the number of buds per treatment per foam piece are recorded in Table A1.

In whole vine studies the entire potted vines were placed in the freezer with 3 vines selected at random being removed at each test temperature.

To reduce supercooling, buds in the 27 April 1977 freezing experiment were moistened prior to being placed in the freezer, as suggested by Proebsting and Mills (1977). Results suggested this to be insufficient moistening and in the 13 May 1977 experiment buds were moistened in the freezer just prior to the beginning of freezing, (approximately $0^{o}C$). To reduce variability resulting from moistening, and to further reduce supercooling, buds were regularly moistened throughout freezing experiments unless stated otherwise. In all cases moistening was via a fine

H_2O mist from a hand held spray bottle.

Bud viability was evaluated after 1 week of regrowth on a greenhouse mist bench. Buds were sectioned with a razor blade and lack of growth and tissue browning were used as criteria for determining the live-dead status. Buds were recorded as dead if the growing point or vascular tissues had browned but not if portions of leaves had browned while the main axis was green and growing. All results were recorded as numbers of dead buds out of the total number for each treatment or developmental stage at each test temperature, (see Table A1). Significance of the differences in the frequencies of dead buds of the various treatments was determined using the χ^2 test for Rx2 contingency tables, (see Literature Review p. 14). Temperatures resulting in either 0% or 100% kill in all treatments in any one comparison were not used in the calculation of χ^2. LT_{50} calculations were made via the Spearman-Karber equation (Bittenbender and Howell, 1974).

Developmental Studies. Data on developmental rates of grapevine buds when excised from the parent vine were obtained by forcing stored dormant cuttings. Prior to the 3 August 1977 freezing experiment, 20 six-node 'Concord' cuttings were selected at random from the stored material and placed on the mist bench. The stage of development of the buds at each node on each cutting was recorded at 2-day intervals. Prior to the 11, 14, and 17 April 1978 freezing experiments 4 groups of 32 one-node cuttings were

selected at random from each category of 'Concord' cuttings and were randomly arranged on the mist bench. The stage of development of each bud was recorded at 2 day intervals for all categories of cuttings.

Prior to the 29 June 1978 freezing experiment, 3 groups of 24 one-node cuttings were selected at random from each of the 'Concord', 'Baco-1', and 'Vidal-256' cuttings, and bud development was recorded at 2 day intervals. Buds remaining dormant at the end of all developmental studies were sectioned to determine live-dead status and data were recorded based on the number of live buds.

Bud Weight and Moisture Content. Prior to the 29 June 1978 freezing experiment, 3 replicates of 5 buds each were randomly selected from 'Baco-1' and 'Vidal-256' cuttings at the full-swell stage of bud development, and from the 'Concord' cuttings at first-swell, full-swell, and bud-burst stages of bud development. Primary buds were excised and each group of five placed quickly into seperate air-tight glass vials (25x50mm) with ground glass tops. Vials were weighed on a Mettler H31 single pan balance, opened, and tissues dried at 70°C for 72 hours. Vials were closed and weighed again, emptied and reweighed. Fresh weight, dry weight, and water loss were calculated by difference. Moisture content was expressed as grams of water per gram of tissue dry weight.

RESULTS AND DICUSSION

Controlled Freezing Technique

Preliminary experiments showed that the use of a "Partlow", cam programmable temperature control device was unsatisfactory for planned experiments. True freezing chamber temperature varied by greater than 2°C alternately above and below the set temperature as a result of on-off cycling of the entire unit. The use of an expansion pressure regulator valve (EPRV) remedied this problem, resulting in smoothly declining chamber temperatures. Because coil temperature was very near box temperature with EPRV control, and because of substantial air circulation via 2 large blowers, within-chamber temperature stratification was very much reduced. Temperature seldom varied more than 0.25°C between thermocouples distributed in and around the plant material. Because the cuttings were not bundled together, air circulation kept tissue temperatures very near air temperatures. When grapevine buds were moistened in the freezing chamber before freezing commenced, tissue temperatures were oftern 0.5°-1.0°C below air temperature as a result of evaporative cooling. When the applied surface moisture froze, tissue temperatures rose briefly above air temperatureture as a result of the release of the latent heat of fusion. Critical temperatures should be based on air

temperature because the grower generally has no accurate means of measuring tissue temperatures (Rogers and Swift, 1970). For this reason, and because of the modest variation between tissue and air temperature under these experimental conditions, test temperatures were based on air temperature.

Regrowth of the cold stressed grapevine buds on a greenhouse mist bench proved satisfactory for evaluating freezing injury. Because actively growing, cold tender, tissues tend to supercool, (see Literature Review, p.8), and because freezing under these conditions is very injurious (Burke et al., 1976), cold damaged buds were easily separated from uninjured buds. Well advanced buds suffering freeze injury appeared wilted and water soaked within several hours. Nearly all injured buds, at all stages of development, became desiccated and brown within 3 to 6 days, while buds that escaped injury remained green and continued development. On rare occasions a bud showed no visible injury, but did not continue development. When sectioned, these buds showed browning of the vascular cylinder while peripheral tissues remained green. Because ice grows most rapidly through vascular tissue (Burke et al., 1976), these buds had probably begun to freeze just prior to removal from the freezer. These buds were considered functionally dead.

Tissue surface moisture during freezing had a dramatic effect on survival (Table 1). At full-swell, the LT_{50}

of grapevine buds that were misted regularly during freezing (treatment C) was 3.7°C higher than similar buds that were not misted (treatment A). A similar relationship was found for buds at the first-swell and bud-burst stages as well. The LT_{50}s of buds of a given stage and moistening treatment were very similar in the 27 April 1977 and 14 May 1978 experiments, while buds on 13 May 1977 received an intermediate moistening treatment (treatment B) and were intermediate in hardiness. Moisture on the surface of buds may reduce apparent hardiness several ways. First, evaporative cooling from a wet surface may lower tissue temperatures. Hewett et al. (1978) obtained results very similar to those in Table 1 for developing peach and apricot buds. Because dormant peach buds were not rendered more cold sensitive by a wet surface unless they remained wet for extended periods prior to freezing, Hewett et al. (1978) concluded that evaporative cooling within the freezing chamber was not responsible for the increased injury. The previously discussed deviations of bud tissue temperatures from chamber air temperatures after misting can not explain the freezing injury differences found here. Secondly, a wet surface could increase bud tissue moisture content, resulting in lowered freeze resistance by lowering cell sap concentration (Modlibowska, 1962) or by eliminating barriers to ice nucleation (Quamme, 1978). Hewett et al. (1978) were, in fact, able to demonstrate increased bud

tissue moisture levels after extended periods of wetting. However, buds in the 14 May 1978 experiment (Table 1) were all forced under mist and differed only in the within-freezer treatment. However, during the period in the freezer, the relative water content of the unmisted buds may have decreased and contributed to their greater freeze resistance. Finally, in this study, the most important effect of a wet surface on increased injury was probably the reduction of supercooling via ice crystal inoculation. Kitaura (1967) found that a supercooled mulberry leaf would freeze when touched by an ice crystal.

Results of the 10 June 1978 freezing experiment (Table 1) do not support the view (Modlibowska, 1962) that supercooling is greater in small excised pieces of tissue than in whole plants. However, because the chance of ice nucleation increases with the time at a given subfreezing temperature, and because test temperatures were not maintained for extended periods in this study, supercooling should be minimized if injury at a given temperature is to reflect injury likely to occur in the field at that temperature. Therefore the "standard" freezing technique should include regular misting of the buds during freezing to promote ice nucleation.

The use of artificially forced grapevine buds, rather than naturally developing buds from the field, for controlled freezing experiments, would greatly facilitate development of non-microclimate modifying frost control

techniques. For this reason, two experiments were conducted in which buds forced from dormant cuttings on a greenhouse mist bench were compared, in the same freezing experiment, to buds developed naturally in the field during the spring. No significant differences in hardiness were found at 3 different stages of development (Table 2; 14 May 1978 and 20 May 1978 experiments). Hardiness in these 2 experiments was similar to that of buds of the same stage on whole potted vines in separate experiments (Tabel 2; 10 June 1978 and 13 June 1978 experiments). These data indicate that the hardiness of grapevine buds forced from cuttings in a greenhouse is similar to that of buds developing in the field.

Factors Determining Critical Temperatures in Developing Grapevine Buds

Stage of phenological development is generally considered the most important factor determining the temperature that a developing fruit bud will tolerate (Ballard et al., 1972). Hardiness of developing grapevine buds decreases with advancing stage of development (Table 3) whether the buds develop naturally in the field or are artificially forced on a greenhouse mist bench. Hardiness differences between stages of development may be related to quantitative factors. A simple volume effect may be important in that the large size of more advanced buds would make them, by chance, more likely to contain

more and better ice nucleators. Kaku and Salt (1968) found that the freezing temperature of conifer needles increased with increasing needle length and attributed this to increased numbers of ice nucleators. Because of both size and shape, the area in contact with surface ice will tend to be larger in more advanced buds. Modlibowska (1962) found that apple blossoms supercooled more when closed than when open. Differences in freeze resistance between developmental stages may also be related to qualitative differences in tissue hardiness. Kaku (1971) found that hardiness in immature Buxus leaves was related to age, independent of leaf size. Changes in maturity, or stage of development, may involve structural changes that affect the efficiency of nucleating sites.

It is critical to any frost control technique designed to retard development, that the relationship between stage of development and freeze resistance be independent of the rate of development. One such technique (Howell and Wolpert, 1978) makes use of apical dominance to retard development of buds at nodes that will be kept for fruiting. If however, the slowly developing basal buds are no hardier than the more rapidly developing apical buds, the technique loses its effectiveness. Apical buds suppress the development of buds basal to them even in excised cane sections (Table 4). Therefore, by selecting buds from 2 groups of 6-node cuttings placed on the greenhouse mist bench several days apart,

it was possible, in one experiment, to cold stress full-swell buds that had developed slowly (i.e., basal buds from the first group) vs. rapidly (i.e., apical buds from the second group). The buds whose development had been retarded by buds apical to them, and therefore exposed longer to dehardening conditions, were no less hardy than rapidly developing buds at the same stage (Table 5; 3 August 1977 experiment). Supporting this finding is the fact that the hardiness of buds developing naturally in the field in the spring of 1978 did not differ appreciably at a given stage over 3 sampling dates (17, 20, and 23 May 1978; Table 3).

Pre-freeze conditions, particularly the temperature during the 24 hours prior to freezing and during the entire period of spring bud development, reportedly influence critical temperatures (see Literature Review, p.7). The 3 August 1977 and 5 May 1978 freezing experiments (Table 5) both indicate that exposure of full-swell buds to temperatures of 1°C for 3 days proir to freezing did not result in rehardening. The hardiness of full-swell buds allowed to develop entirely at cool temperatures did not differ significantly from that of full-swell buds developed at warm temperatures (Table 5, 12 October 1977 experiment). Data for field vs. greenhouse grown buds support this conclusion in that mean max./min. temperatures from May 1-20 were 17°/8°C in the field and 30°/17°C in the greenhouse (Table 2; 14 and 20 May 1978 experiments).

These observations agree with Hewett et al. (1978) and indicate that for developing grapevine buds, pre-freeze temperatures do not significantly affect resistance to freezing stress at a given stage of development. This contrasts with a report on apple blossoms (Ballard and Proebsting, 1972) indicating that blossoms developed in cool weather were hardier than those developed in warm weather.

Misting vs. no misting during development did not affect hardiness of full-swell buds forced simultaneously in the greenhouse (Table 5; 12 October 1977 experiment). This conflicts with the report of Hewett et al. (1978) who found reduced hardiness and increased moisture content in developing peach buds exposed to prolonged misting. The misting both groups of grapevine buds received during controlled freezing may, however, have obscured any differences due to prior treatment.

Characteristics of grapevine buds and canes prior to spring development were investigated as to their effect on the hardiness of developing buds. Light periderm color and large cane diameter have been associated with reduced winter hardiness of dormant grape buds (Howell and Shaulis, 1980). Preliminary observations by Nelson Shaulis (personal communication) have suggested that poor leaf exposure to light during the previous summer may result in increased spring frost injury to 'Concord' buds. Pre-development characteristics could affect spring frost

injury in two general ways. First, they may alter the rate of phenological development (Anderson et al., 1980) and second, they may affect the actual hardiness of buds at a given stage of development.

No differences were found in the hardiness of either first-swell or full-swell buds based on pre-development sunlight exposure status (Table 6). In addition, no differences were found in the days to first-swell of one-node cuttings from either group under greenhouse forcing conditions (Table 7). These data indicate that exposure status during the previous summer does not affect freezing damage to developing buds the following spring.

Partridge (1925) warned against the retention of laterals for fruiting canes because of their generally smaller cane diameter, suggesting that buds on small canes develop more rapidly in the spring than buds on large canes, resulting in more damage from spring frost. With cane diameter restricted to medium size, (5.0-7.5mm), no differences were found in the rate of development (Table 8) or in first-swell and full-swell bud hardiness (Table 6) between buds arising from laterals and those arising from main canes, when forced from cuttings.

In a separate experiment, buds were stratified as to the diameter of the cane section from which they arose and to their original nodal position to prevent confounding these characteristics, as small cane sections tend to occur at more apical portions of the original cane. This

allowed comparisons of developing buds arising from large vs. small canes, stratified as to node position; and of buds from apical vs. basal positions, stratified as to cane diameter. Although insufficient numbers of buds on large diameter, apical cane sections prevented their evaluation, no differences were found in the hardiness of full-swell buds (Table 6) or in developmental rates of the buds from the other 3 categories (Table 9). These data indicate that, for buds forced from one-node cuttings, main cane vs. lateral origin, cane diameter, and original nodal position do not affect the hardiness of developing buds or the rate of development. Cutting canes into one-node cuttings may, however, prevent the influence of cane diameter on bud development rate in a manner analagous to the loss of apical dominance. It is interesting to note that Antcliff and May (1961) observed that development of buds on one-node cuttings did show the pattern of original apical dominance if the cuttings were taken one month or less before normal spring bud-burst.

The importance of the data in the 3 experiments presented in Table 6 and Tables 7-9 lies in sampling considerations for future evaluations of frost protection measures. If 'Concord' buds are forced from cuttings for controlled freezing, the cuttings need not be stratified as to exposure status (periderm color), lateral vs. main cane origin, cane diameter, or original nodal position.

Cultivar differences could affect spring freeze resistance through variations in developmental rate, and through actual differences in tissue hardiness at a given phenologic stage of development. Buds of different cultivars are known to develop at different rates under field conditions (Anderson et al., 1980). Because of this, an experiment was conducted to determine relative development rates for cuttings forced under greenhouse conditions. The reported order of field development was 'Baco-1', 'Concord', 'Vidal-256' (Howell, 1976; Anderson et al., 1980). When buds were greenhouse forced, the order of development was 'Baco-1', 'Vidal-256', 'Concord' (Table 10). This apparent reversal of the normal relative development rates of 'Concord' and 'Vidal-256' buds may have resulted from differences in either threshold temperature to induce bud development, or in whole vine vs. forced cutting response. Further investigation of this may lead to an understanding of, and possibly to the manipulation of, variability in bud development rates in the spring.

Knowledge of relative greenhouse forcing rates permitted programming so that buds of these 3 cultivars could be subjected to controlled freezing while at the same stages of development. For first-swell and full-swell buds, freeze resistance is not the same in these 3 cultivars (Table 11). Fifty-six per cent of the 'Baco-1' buds, 40% of the 'Vidal-256' buds and only 22% of the 'Concord' buds were killed by the same test temperatures. A possible explanation

of these findings lies in the data presented in Table 12. While moisture content does not differ significantly, the larger size (fresh or dry weight) of the less cold resistant buds may simply provide more opportunities for fatal ice nucleation. Alternatively, hardiness differences may actually reflect innate differences in tissue hardiness.

One possible explanation for the superior resistance of 'Concord' buds is the tomentose nature of young leaf tissue surfaces, while those of 'Vidal-256' and 'Baco-1' are more glabrous. The felt-like surface of developing 'Concord' buds may reduce the probability of surface ice contacting the supercooled tissue water, causing flash crystallization of cellular water. These hardiness differences can be important, particularly in determining critical temperatures for existing cultivars and in breeding new grape cultivars for spring freeze resistance. However, differences in natural development rates are apparently more important, as Howell (1976) attributed the 60-65% primary bud kill in 'Concord' vs. only trace kill in 'Vidal-256' at the same site after a natural spring freeze, to differences in developmental rates.

Estimation of Critical Temperatures

One of the primary aims of this investigation was to develop a reliable technique for the estimation of practical critical temperatures for developing grapevine buds at various stages of development. Results indicate

that these temperatures may depend on conditions other than developmental stage. While cultivar differences appear to be of some importance, the presence or absence of surface moisture or ice is crucial. Because the presence of surface moisture or hoar frost during a spring freeze may vary from one growing region to another or from one natural freeze to another, critical temperatures may have to be adjusted accordingly. Estimated critical temperatures of developing 'Concord' grape buds for wet and dry tissue surface conditions are presented in Table 13. Temperatures for wet conditions were obtained by averaging, for each stage of development, LT_{50} values for 5 freezing experiments (Table 3). In all of these experiments, the buds were regularly misted throughout controlled freezing. Critical temperatures for dry conditions were obtained by averaging LT_{50} values for buds not misted during freezing, (Treatment A, Table 1). Critical temperatures (Table 13) for buds with surface moisture or frost may well be relevant to Michigan or Eastern viticultural conditions while the lower values listed for dry buds, or the likewise lower values given by Proebsting and Mills (1978), may be more relevant to the more arid regions such as Washington's Yakima Valley. Extensive comparisons with field injury from natural spring frosts over several years is the only method of assessing their accuracy and the natural conditions under which they apply.

Summary and Conclusions

The results of this study indicate that the controlled freezing technique employed is capable of detecting small hardiness differences between the stages of developing grapevine buds or between developing buds of different cultivars. The surface moisture levels must be controlled during freezing, as this markedly affects hardiness.

Buds on forced cuttings, on cuttings taken directly from the field, and on whole vines behave similarly when exposed to controlled freezing. Therefore, the use of stored cuttings forced in a greenhouse should provide a continuous source of developing grapevine buds for the evaluation on non-microclimate modifying techniques of frost protection, such as use of surface and systemic cryoprotectants and breeding of frost resistant cultivars. These cuttings do not have to be stratified for previous exposure status, lateral vs. main cane origin, cane diameter, or original nodal position. All of these morphological conditions will have an effect on whether the primary bud survives that dormant season (Howell and Shaulis, 1980), but once growth begins, a living primary bud's cold resistance is not further influenced.

There appears to be cultivar difference in cold resistance at a common stage of phenological development. This should be further studied to assess the range of resistance extant and to assess the potential value for genetic

improvement of bud resistance to low temperature stress during spring freezes.

The stage of phenological development appears to be the most important endogenous factor affecting bud hardiness. This relationship is independent of the rate of development, temperature during development, and temperature immediately prior to controlled freezing. It would appear that developing grapevine buds can not be rehardened by exposure to temperatures just above freezing.

Knowledge of critical temperatures can be helpful to growers in efforts to use current frost control measures more economically. These critical temperatures may have to be based on prevailing weather conditions such as dew point and precipitation probabilities, as well as the overall bud development status.

Table 1. Effect of surface moisture during controlled freezing on primary bud hardiness in 'Concord' grapevines at several stages of phenological development. Treatments were: (A) moistened prior to freezing only; (B) moistened at the beginning of freezing(0°C); and (C) moistened at regular intervals throughout freezing. Values are LT_{50}(°C) as calculated by the Spearman-Karber equation (Bittenbender and Howell,1974).

	Freezing dates and source of buds					
	27 April 77 Field[z]	13 May 77 Field	14 May 78 Greenhouse[y]		10 June 78 Whole vines[x]	
Treatment / Stage	A	B	A	C	A	C
Scale-crack	-9.4 a[w]					
First-swell	-8.1 b		-7.7 a a	-4.4 a b		
Full-swell	-7.1 bc	-4.6 a	-7.1 b a	-3.4 b b	-7.2 a	-3.5 b
Bud-burst	-6.6 c	-4.1 a	-5.8 c a	-3.0 c b		
Expanded-shoot		-2.9 b				

[z]Cuttings taken from field the day of freezing experiment.

[y]Buds forced on cuttings under mist in the greenhouse.

[x]Whole 2-year-old potted vines were subjected to controlled freezing.

[w]LT_{50} separation by χ^2 analysis of live-dead proportions at critical test temperatures. Letters following values indicate significance within columns and letters below values indicate significance within rows on a given date, p=0.05.

Table 2. Effect of cane excision and pre-freeze conditions on 'Concord' primary bud hardiness during controlled freezing.[z] Treatments were: (A) cuttings taken directly from field, (B) cuttings forced on a greenhouse mist bench; and (C) whole potted 2-year-old vines grown out-of-doors. Values are LT_{50} as calculated by the Spearman-Karber equation (Bittenbender and Howell, 1974).

	Date of freezing experiment					
	14 May 78		20 May 78		10 June 78	13 June 78
Stage \ Treatment →	A	B	A	B	C	C
First-swell	-4.1 a	-4.4 a[y]	-4.9 a	-5.0 a		
Full-swell		-3.4 b	-3.8 b	-3.6 b	-3.5	
Bud-burst		-3.0 c	-3.3 c			-3.4

[z]Standard freezing technique with buds moistened via a fine mist at regular intervals throughout the freezing process.

[y]LT_{50} separation by χ^2 analysis of live-dead proportions at critical test temperatures. Letters following values indicate significance within columns and letters below values indicate significance within rows on a given date, p=0.05.

Table 3. Effect of stage of phenological development on primary bud hardiness of 'Concord' grapevine cuttings exposed to controlled freezing.[z] Values are LT_{50}(°C) as calculated by the Spearman-Karber equation (Bittenbender and Howell,1974).

	Source of buds and freezing date				
	Greenhouse[y]		Field[x]		
Stage	18 July 77	14 May 78	17 May 78	20 May 78	23 May 78
Scale-crack	-5.8 a[w]		-5.6 a		
First-swell	-4.0 b	-4.4 a	-4.1 b	-4.9 a	
Full-swell	-3.1 c	-3.4 b	-3.5 c	-3.8 b	-3.8 a
Bud-burst	-2.9 c	-3.0 c		-3.3 c	-3.3 b
Expanded-shoot					-2.6 c

[z]Standard freezing technique with buds moistened via a fine mist at regular intervals throughout the freezing process.

[y]Buds forced on cuttings under mist in a greenhouse.

[x]Cuttings taken from field the day of freezing.

[w]LT_{50} separation by χ^2 analysis of live-dead proportions at critical test temperatures. Letters following values indicate significance within columns (freezing dates), p=0.05.

Table 4. Effect of node position on development of primary buds on 6-node 'Concord' grapevine cuttings. Twenty cuttings were forced on a greenhouse mist bench. Values are per cent of live buds at a given stage.

Bud stage	Node position					
	Basal					Apical
	1	2	3	4	5	6
7-27-77						
Dormant	65	40	44	44	26	43
Scale-crack	35	60	50	50	63	50
First-swell	0	0	6	6	11	7
Full-swell	0	0	0	0	0	0
7-29-77						
Dormant	45	35	25	31	11	29
Scale-crack	45	65	38	19	32	21
First-swell	10	0	31	50	58	50
Full-swell	0	0	6	0	0	0
7-31-77						
Dorment	50	35	13	25	11	14
Scale-crack	30	30	25	19	0	14
First-swell	10	35	25	13	26	7
Full-swell	10	0	38	44	63	64

Table 5. Effect of pre-freeze treatments on hardiness of full-swell 'Concord' grapevine primary buds subjected to controlled freezing.[z] Values are LT_{50} as calculated by the Spearman-Karber equation (Bittenbender and Howell, 1974).

Pre-freeze treatment		LT_{50}
3 August 77 Experiment		
Days in greenhouse	Days held at 1°C	
14	0	-2.9
11	0	-2.4
11	3	-2.3
		N.S.[y]
12 November 77 Experiment		
13 days in greenhouse (27°/19°C)		
on mist bench		-4.0
no mist		-3.7
17 days in growth chamber (13°/8C)		
no mist		-4.3
		N.S.
23 May 78 Experiment		
Cuttings taken from field		
directly frozen		-3.8
held 3 days at 1°C		-4.0
		N.S.

[z]Standard freezing technique with buds moistened via a fine mist at regular intervals throughout the freezing process.

[y]N.S.- Not significant. Significance determined by χ^2 analysis of live-dead proportions at critical test temperatures. Comparisons within one freezing date only, p=0.05.

Table 6. Effects of cane characteristics and bud position on hardiness of developing 'Concord' grapevine primary buds. Buds were forced as 1-node cuttings on a greenhouse mist bench for all controlled freezing[z] experiments. Values are LT_{50}(°C) as calculated by the Spearman-Karber equation (Bittenbender and Howell, 1974).

	First-swell	Full-swell
11 April 78 Experiment		
Cane exposure status:		
Well exposed[y]	-4.3	-3.7
Poorly exposed[x]	-4.3	-3.8
	N.S.[w]	N.S.
13 April 78 Experiment		
Buds arising from:		
Main canes(5.0-7.5mm diam)[v]	-4.9	-3.3
Laterals(5.0-7.5mm diam)	-4.8	-3.4
	N.S.	N.S.
17 April 78 Experiment		
Cane diam(mm) / Node position		
Large(6.7-10.0) Basal(2-6)		-3.2
Small(4.0-6.5) Basal(2-6)		-3.0
Small(4.0-6.5) Apical(11-15)		-3.0
		N.S.

[z]Standard freezing technique with buds moistened via a fine mist at regular intervals throughout the freezing process.

[y]Only cuttings collected from exterior of vine canopy and with dark periderm color were considered "well exposed."

[x]Only cuttings collected from interior of vine canopy and with light periderm color were considered "poorly exposed."

[w]N.S.- Not significant. Significance determined by χ^2 analysis of live-dead proportions at critical test temperatures. Comparisons within one freezing date only, $p=0.05$.

[v]Cane diameter measured at mid-point of internode below bud.

Table 7. Effect of exposure status on rate of primary bud development in 1-bud 'Concord' grapevine cuttings. One hundred twenty-eight cuttings of each type were forced on a greenhouse mist bench. Values are per cent of live buds at or past first-swell.

Exposure status	No. of live buds	Days on mist bench				
		12	14	16	18	20
Well exposed[z]	121	0	17	67	90	98
Poorly exposed[y]	109	0	17	72	95	99

[z]Only cuttings collected from the exterior of the vine canopy and with dark periderm color were considered "well exposed."

[y]Only cuttings collected from the interior of the vine canopy and with light periderm color were considered "poorly exposed."

Table 8. Effect of cutting origin on rate of primary bud development in 1-bud 'Concord' grapevine cuttings. One hundred twenty-eight cuttings of each type were forced on a greenhouse mist bench. Values are per cent of live buds at or past first-swell.

Origin of cuttings	No. of live buds	Days on mist bench				
		8	10	12	14	16
Persistent laterals	122	0	5	72	93	97
Main canes	122	0	6	66	93	97

Table 9. Effect of cane diameter and original nodal position on rate of primary bud development in 1-bud 'Concord' cuttings. One hundred twenty-eight cuttings of each type were forced on a greenhouse mist bench. Values are per cent of live buds at or past first-swell.

Cane diameter Nodal position	No. of live buds	Days on mist bench				
		8	10	12	14	16
Large(6.7-10.0mm) From nodes 2-6	82	0	56	94	98	100
Small(4.0-6.5mm) From nodes 2-6	125	0	65	91	99	100
Small(4.0-6.5mm) From nodes 11-15	122	0	42	84	98	99

Table 10. Effect of cultivar on primary bud development rate in 1-bud grapevine cuttings. Seventy-two cuttings of each cultivar were forced on a greenhouse mist bench. Values are per cent of live buds at or past the stage indicated.

		Days on mist bench							
		4		8		12		16	
Cultivar	No. of live buds	Bud burst	First swell	Bud burst	First swell	Bud burst	First swell	Bud burst	First swell
'Baco-1'	63	0	90	95	100	100	100	100	100
'Vidal-256'	67	0	0	0	99	91	100	100	100
'Concord'	65	0	0	0	58	22	95	93	98

Table 11. Effect of cultivar on hardiness[z] and per cent mortality[y] of developing grapevine primary buds subjected to controlled freezing.[x]

	'Baco-1'	'Vidal-256'	'Concord'
LT_{50} (first-swell)	-2.9 a[w]	-3.6 a	-4.1 a
	a	b	b
LT_{50} (full-swell)	-2.3 b	-2.7 b	-3.4 b
	a	a	b
% Mortality	56.3	39.6	21.9
	a	b	c

[z]Hardiness given as LT_{50}(°C) calculated by the Spearman-Karber equation (Bittenbender and Howell, 1974).

[y]Per cent mortality is per cent of first-swell and full-swell buds killed at -2.0° and -3.5°C.

[x]Standard freezing technique with buds moistened via a fine mist at regular intervals throughout freezing process.

[w]Separation by χ^2 analysis of live-dead proportions at critical test temperatures. The same letter within a column or row indicates that respective values are not significantly different, p=0.05.

Table 12. Effect of cultivar on tissue weight and moisture content of developing grapevine buds forced from cuttings in a greenhouse.

Bud-stage	Fresh weight (g/5 buds)	Dry weight (g/5 buds)	Total H_2O (g/5 buds)	Moisture content[z]
'Concord'				
First-swell	0.177 a[y]	0.034 a	0.143 a	4.19 a
Full-swell	0.619 b	0.091 b	0.528 b	5.82 c
Bud-burst	1.273 d	0.200 d	1.073 d	5.36 b
'Baco-1'				
Full-swell	1.274 d	0.180 d	1.094 d	6.07 c
'Vidal-256'				
Full-swell	0.826 c	0.115 b	0.711 c	6.18 c

[z]Grams water/gram tissue dry weight.

[y]Mean separation in columns by Duncan's Multiple Range test. Means followed by the same letter are not significantly different, p=0.05.

Table 13. Estimated critical temperatures for developing 'Concord' grapevine primary buds. Values are LT_{50} (°C) as calculated by the Spearman-Karber equation (Bittenbender and Howell, 1974).

Stage of development	Surface moisture status	
	Wet[z]	Dry
Scale-crack	-5.7	-9.4
First-swell	-4.4	-7.9
Full-swell	-3.5	-7.1
Bud-burst	-3.1	-6.2
Expanded-shoot	-2.6	

[z]Indicates the presence of frost, dew, ice, or water from precipitation or irrigation.

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APPENDIX

Table A1. Raw mortality data for freezing experiments on 'Concord' buds frozen on 1-node cuttings. Values are the number of buds killed out of the total number per test temperature per stage of development (Bud total). Pre-freeze status includes any pertinent treatment to, or characteristic of, the buds before freezing. Freeze treatments were: (A) moistened prior to freezing only; (B) moistened at the beginning of freezing (0°C); and (C) moistened intermittently throughout freezing.

Freeze date	Pre-freeze status (Bud source)	Freeze tmt. (Bud total)	Test temp.(°C)	Stage of 1° bud development: Scale crack 1°	Scale crack 2°	First swell 1°	First swell 2°	Full swell 1°	Full swell 2°	Bud burst 1°	Bud burst 2°	Exp. shoot 1°	Exp. shoot 2°
4-27-77	(Field)	A	Control	0	0	0	0	0	0	0	0		
			-3.0	0	0	0	0	0	0	0	0		
		(8)	-4.5	0	0	0	0	0	0	0	0		
			-6.0	0	0	1	1	1	0	3	3		
			-7.5	1	0	3	3	6	4	6	4		
			-9.0	1	0	5	5	7	7	8	8		
5-13-77	(Field)	B	Control					0	0	0	0	0	0
			-2.0					0	0	0	0	1	0
		(8)	-3.5					1	0	1	0	6	0
			-5.0					5	4	8	6	8	2
			-6.5					8	6	8	8	8	8
			-8.0					8	8	8	8	8	8
7-18-77	(Greenhouse)	C	Control	0	0	0	0	0	0	0	0		
			-3.0	0	0	0	0	5	3	8	3		
		(12)	-4.5	2	2	10	8	12	4	11	5		
			-6.0	7	4	12	10	12	10	12	12		
			-7.5	11	10	12	12	12	12	12	12		
			-9.0	12	12	12	12	12	12	12	12		

Table A1.(cont'd)

Freeze date	Pre-freeze status (Bud source)	Freeze tmt. (Bud total)	Test temp.(°C)	Stage of 1° bud development: Scale crack 1°	Scale crack 2°	First swell 1°	First swell 2°	Full swell 1°	Full swell 2°	Bud burst 1°	Bud burst 2°	Exp. shoot 1°	Exp. shoot 2°
8-3-77	Early developing	C	Control	0	0	0	0	0	0				
			-1.5	0	0	0	0	0	0				
	(11 days in greenhouse)	(12)	-3.0	1	0	5	4	11	1				
			-4.5	5	3	9	7	12	11				
			-6.0	10	10	12	12	12	12				
			-7.5	12	12	12	12	12	12				
	Late developing	C	Control			0	0	0	0	0	0		
			-1.5			0	0	0	0	1	0		
	(14 days in greenhouse)	(12)	-3.0			2	2	7	0	12	3		
			-4.5			8	7	12	12	12	11		
			-6.0			12	12	12	12	12	12		
			-7.5			12	12	12	12	12	12		
	Early developing-rehardened	C	Control					0	0				
			-1.5					1	1				
	(11 days in greenhouse plus 3 days at 1°C)	(12)	-3.0					11	4				
			-4.5					12	9				
			-6.0					12	12				
			-7.5					12	12				

Table A1.(cont'd)

Freeze date	Pre-freeze status (Bud source)	Freeze tmt. (Bud total)	Test temp.(°C)	Stage of 1° bud development: Scale crack 1° 2°	First swell 1° 2°	Full swell 1° 2°	Bud burst 1° 2°	Exp. shoot 1° 2°
8-6-77	Late developing	C	Control				0 0	0 0
			-1.0				0 0	0 0
	(14 days in greenhouse)	(12)	-2.5				4 0	6 0
			-4.0				10 2	12 4
			-5.5				12 12	12 11
			-7.0				12 12	12 12
	Very late developing	C	Control		0 0			
			-1.0		0 0			
		(12)	-2.5		0 0			
	(17 days in greenhouse)		-4.0		9 6			
			-5.5		12 11			
			-7.0		12 12			
11-12-77	Not forced on mist bench	C	Control		0 0	0 0		
			-2.0		0 0	0 0		
		(15)	-3.5		1 1	6 0		
	(Greenhouse 27/19°C)		-5.0		14 11	15 13		
			-6.5		15 15	15 15		
	Not forced under mist	C	Control		0 0	0 0		
			-2.0		0 0	0 0		
		(15)	-3.5		3 1	6 0		
	(Growth chamber 13/8°C)		-5.0		14 13	9 8		
			-6.5		15 15	15 15		

Table A1.(cont'd)

Freeze date	Pre-freeze status (Bud source)	Freeze tmt. (Bud total)	Test temp.(°C)	Stage of 1° bud development: Scale crack 1° 2°	First swell 1° 2°	Full swell 1° 2°	Bud burst 1° 2°	Exp. shoot 1° 2°
11-12-77 (cont'd)	Forced on mist bench (Greenhouse 27/19°C)	C (15)	Control			0 0		
			-2.0			0 0		
			-3.5			3 2		
			-5.0			15 14		
			-6.5			15 15		
4-11-78	Poorly exposed (Greenhouse)	C (18)[z] (30)[y]	Control		0 0	0 0		
			-2.0		0 0	0 0		
			-3.5		1 0	9 1		
			-5.0		17 8	30 16		
			-6.5		17 16	30 29		
			-8.0		18 18	30 30		
	Well exposed (Greenhouse)	C (18)[z] (30)[y]	Control		0 0	0 0		
			-2.0		0 0	0 0		
			-3.5		2 0	11 1		
			-5.0		16 11	30 27		
			-6.5		18 15	30 30		
			-8.0		18 18	30 30		

[z]18 buds per test temperature for first-swell buds only.

[y]30 buds per test temperature for full-swell buds only.

Table A1.(cont'd)

Freeze date	Pre-freeze status (Bud source)	Freeze tmt. (Bud total)	Test temp.(°C)	Stage of 1° bud development: Scale crack 1° 2°	First swell 1° 2°	Full swell 1° 2°	Bud burst 1° 2°	Exp. shoot 1° 2°
4-14-78	Laterals only	C	Control		0 0	0 0		
			-2.0		0 0	0 0		
	(Greenhouse)	(18)[z]	-3.5		2 0	17 0		
			-5.0		9 5	30 17		
		(30)[y]	-6.5		18 18	30 30		
	Canes only	C	Control		0 0	0 0		
			-2.0		0 0	0 0		
	(Greenhouse)	(18)[z]	-3.5		0 0	20 0		
			-5.0		10 3	30 21		
		(30)[y]	-6.5		18 18	30 29		
4-17-78				All 1° buds at full-swell				
				A 1° 2°		B 1° 2°		C 1° 2°
	A: large(6.7-10.0mm) basal(nodes 2-6)							
	B: small(4.0-6.7mm) basal(nodes 2-6)	C	Control		0 0	0 0		0 0
			-2.0		0 0	0 0		0 0
	C: small(4.0-6.7mm) apical(nodes 11-15)	(30)	-3.5		22 1	27 0		27 2
			-5.0		30 18	28 18		29 21
			-6.5		30 30	30 30		30 30

[z]18 buds per test temperature for first-swell buds only.

[y]30 buds per test temperature for full-swell buds only.

Table A1.(cont'd)

Freeze date	Pre-freeze status (Bud source)	Freeze tmt. (Bud total)	Test temp.(°C)	Stage of 1° bud development: Scale crack 1°	Scale crack 2°	First swell 1°	First swell 2°	Full swell 1°	Full swell 2°	Bud burst 1°	Bud burst 2°	Exp. shoot 1°	Exp. shoot 2°
5-14-78	(Greenhouse)	A	Control			0	0	0	0	0	0		
			-2.0			0	0	0	0	0	0		
		(24)	-3.5			0	0	1	0	1	0		
			-5.0			0	0	1	1	12	6		
			-6.5			4	3	4	4	11	11		
			-8.0			13	13	21	21	24	24		
	(Greenhouse)	C	Control			0	0	0	0	0	0		
			-2.0			0	0	0	0	0	0		
		(24)	-3.5			5	0	14	2	20	5		
			-5.0			16	12	24	23	24	24		
			-6.5			24	24	24	24	24	24		
	(Field)	C	Control			0	0						
			-2.0			0	0						
		(24)	-3.5			8	2						
			-5.0			18	15						
			-6.5			24	24						
5-17-78	(Field)	C	Control	0	0	0	0	0	0				
			-2.0	0	0	0	0	0	0				
		(30)	-3.5	0	0	6	2	14	2				
			-5.0	14	12	27	23	30	28				
			-6.5	21	18	30	30	30	30				
			-8.0	28	28	30	30	30	30				

Table A1. (cont'd)

Freeze date	Pre-freeze status (Bud source)	Freeze tmt. (Bud total)	Test temp.(°C)	Stage of 1° bud development: Scale crack 1°	Scale crack 2°	First swell 1°	First swell 2°	Full swell 1°	Full swell 2°	Bud burst 1°	Bud burst 2°	Exp. shoot 1°	Exp. shoot 2°
5-20-78	(Greenhouse)	C	Control			0	0	0	0				
			-2.0			0	0	0	0				
		(30)	-3.5			3	1	14	4				
			-5.0			12	6	29	16				
			-6.5			30	30	30	30				
	(Field)	C	Control			0	0	0	0	0	0		
			-2.0			0	0	0	0	0	0		
		(30)	-3.5			2	0	11	3	19	0		
			-5.0			15	8	29	18	30	18		
			-6.5			30	30	30	30	30	30		
5-23-78	(Field)	C	Control					0	0	0	0	0	0
			-2.0					1	0	4	0	8	1
		(30)	-3.5					10	2	18	3	25	6
			-5.0					28	17	28	27	30	30
			-6.5					30	30	30	30	30	30
	Rehardened(held 3 days at 1°C) (Field)	C	Control					0	0				
			-2.0					1	0				
		(30)	-3.5					11	0				
			-5.0					24	18				
			-6.5					30	30				

Table A2. Raw mortality data for freezing experiments on whole potted 'Concord' vines. Three vines were used per test temperature. Only 1° buds pre-determined to be at the indicated stage of development were assessed for freeze damage. Freeze treatments were: (A) not moistened during freezing; and (C) moistened intermittently throughout freezing.

Freeze date	Freeze treatment	Bud total	Bud stage	Test temp.(°C)	1° buds killed
6-10-78	A	27	Full-swell	Control	0
				-3.5	0
				-5.0	0
				-6.5	1
				-8.0	27
	C	40	Full-swell	Control	0
				-2.0	0
				-3.5	24
				-5.0	37
				-6.5	40
6-13-78	C	56	Bud-burst	Control	0
				-2.0	4
				-3.5	29
				-5.0	56
				-6.5	56

Table A3. Raw mortality data for freezing experiment on buds from 3 different cultivars. Values are the number of buds killed out of 24 buds per test temperature per stage of development. Buds were frozen on 1-node cuttings with intermittent misting throughout freezing. Buds were forced from cuttings under mist in the greenhouse.

			Stage of 1° bud development			
			First swell		Full swell	
Freeze date	Cultivar	temp.(°C)	1°	2°	1°	2°
6-29-78	'Baco-1'	Control	0	0	0	0
		-2.0	6	4	8	2
		-3.5	17	10	23	8
		-5.0	22	17	24	24
		-6.5	24	24	24	24
	'Vidal-256'	Control	0	0	0	0
		-2.0	3	0	6	0
		-3.5	10	7	19	11
		-5.0	21	16	24	24
		-6.5	24	24	24	24
	'Concord'	Control	0	0	0	0
		-2.0	0	0	0	0
		-3.5	7	2	14	3
		-5.0	20	18	24	22
		-6.5	23	23	24	24