THESIS

COLD HARDINESS AND WATER CONTENT DURING DEACCLIMATION OF GRAPEVINE BUD AND CANE TISSUE.

Submitted by

Richard A. Hamman Jr.

Department of Horticulture

In partial fulfilment of the requirements

for the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer, 1988

COLORADO STATE UNIVERSITY

July 1, 1988

WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY RICHARD A. HAMMAN JR. ENTITLED COLD HARDINESS AND WATER CONTENT DURING DEACCLIMATION OF GRAPEVINE BUD AND CANE TISSUE BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

Committee on Graduate Work

Advisor

Department Head

ABSTRACT

COLD HARDINESS AND WATER CONTENT DURING DEACCLIMATION OF GRAPEVINE BUD AND CANE TISSUE.

Computer assisted thermal analysis was used to measure deep supercooling in dormant bud and cane tissue of *Vitis vinifera* L. cv. 'Merlot' during a five week deacclimation time period. The temperature of the Low Temperature Exotherm (LTE), an indicator of hardiness, of both cane (internode) and primary bud tissue responded to weekly increases in air temperatures with bud tissue responding faster than cane tissue. Bud tissue from pruned and unpruned canes retained the capacity to supercool until early bud swell 18 April, 1987, when the mean LTE temperature of -9.8°C became obscured by High Temperature Exotherms (HTEs) occurring between -5 and -8°C. Cane tissue had lower LTEs than bud tissue on each date and at each position. Cane positions nearest the trunk, whether canes were pruned or unpruned, were found to be slightly hardier than those more distally oriented, which was not observed with buds. Pruning treatments did not influence the loss of hardiness in either bud or cane tissue. Water content of canes was more affected by all three factors (date, position, and pruning) than was hardiness. Bud water content was only affected by date, and was

lower than cane water content for every date and each position throughout the study. Canes increased in water content with each more distal position. Pruning slowed the rate of cane hydration during the week it was most rapid, especially at the most distal position. Observations during the most pertinent three weeks of this study indicate that cane tissue hydrates rapidly but dehardens only slowly, while buds deharden more quickly yet have only a small increase in bulk water content. The main effect of pruning was on cane water content.

Richard A. Hamman Jr.
Department of Horticulture
Colorado State University
Fort Collins, CO. 80523
July 1, 1988.

ACKNOWLEDGMENTS

I would like to express my sincere appreciation to my boss and advisor Dr. Rocky Renquist, Associate Professor of Horticulture, Orchard Mesa Research Center, Grand Junction, CO. for his outstanding guidance and constructive criticism of this work.

I am also very indebted to Dr. Harrison G. Hughes for his patient on-campus advising and assistance in completing this degree. Special thanks is due to Virgil Esensee for his friendship and valuable technical assistance with instrumentation setup.

I would like to thank Dr. Stephen J. Wallner and Dr. Whitney S. Cranshaw for their comments and review of this manuscript.

A sincere thanks is due to Dr. Harold Larsen for his often-asked-for computer assistance, statistical advice and his personal understanding of the difficult times that occurred with this study.

Many thanks to Joyce Sexton for her always helpful computer advice and assistance in typing this manuscript.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Literature Review

Introduction

Plants have adapted metabolically, physically and morphologically in order to survive the environmental changes occuring throughout the year. These adaptations allow the plants to withstand varying degrees of environmental stresses, such as heat, water deficit and extreme cold. Low temperatures can be injurious and even lethal to tissues in overwintering plants. The ability to resist freezing stress is crucial to their survival. Since grapevines require this ability, the aspects of acclimation, deacclimation, supercooling, freezing, and water status will each be given consideration. Of additional interest to the viticulturist and plant scientist is the cultural practice of pruning, which may have a significant impact on freezing resistance or water content.

Supercooling and Freezing

The mechanisms by which plants survive freezing conditions under natural circumstances are complex and depend on many factors, such as rate of cooling, moisture content, and stage of acclimation (57, 66, 89, 114).

Depending on the species, plants have evolved to avoid and / or tolerate freezing, thus insuring their survival (57, 66, 88, 97). Certain tissues in numerous woody species avoid freezing by a process referred to as deep supercooling or undercooling (17). Deep supercooling defers the freezing of tissues to a temperature below the anticipated freezing point of the tissue (17, 114). The supercooled water is thermodynamically stable and exists in equilibrium with ice at temperatures approaching the homogeneous nucleation point of pure water (32) and lower (89). With the absence of any heterogeneous nucleating agents, the homogeneous nucleation temperature of pure water is the result of the spontaneous aggregation of water molecules to form ice nuclei and only occurs at temperatures of -37° to -40°C (94). Deep supercooling may play a role in limiting certain Angiosperm species to regions where the winter temperature minima remain warmer than the homogeneous nucleation temperature of water (34). Deep supercooling has been reported in numerous temperate trees (32, 34, 39, 52, 88); in blueberry (14), grape (2, 78, 85, 87), azalea (35), apple (44, 90) and many other families of Angiosperms (89). When plant cells freeze, two distinct types of freezing can occur; extracellular and intracellular (17, 57). Extracellular freezing is the formation of ice outside of the cell wall and intracellular freezing is when ice forms within the

internal cell matrix nearly always resulting in the death of the cell (17, 92). Mazur (67) suggests that intracellular freezing results from seeding by very small extracellular ice crystals that have grown through the presumed nanometer sized aqueous channels in the plasma membrane. The mechanism by which intracellular ice injures is physical, a result of mechanical stress and disruption of intracellular membranes (17, 46, 66, 104). Extracellular freezing, on the other hand, is more complex and involves a dehydration of the cell (6, 9, 72, 73). Mazur (66) and Asahina (6) report that this dehydration produces several physical alterations of the cell: concentration and precipitation of solutes, reduction in cell water content, cell shrinkage, changes in pH and reduction in spacial separation of macromolecules.

In contrast to deep supercooling, a tolerance mechanism characterized by a loss of cellular water to extracellular ice (89, 104) is found in some hardy coniferous species (97). Plants such as the subalpine fir can tolerate freezing to -70°C without deep supercooling (96).

How plant tissues develop the ability to deep supercool is not clearly understood. Tissues that supercool have been described as having small cell size, minimal intercellular space and relatively low moisture content (6, 57, 73). Xylem ray and axial parenchyma (32, 75, 89, 96), floral primordium (8, 78, 82, 87), and phloem (75, 78, 82) are examples of tissues found in hardy woody plants that supercool. Whether or not deep supercooling is influenced by the physical structure of tissue and cell constituents is a question which has prompted several studies concerning this phenomenon. In a number of *Prunus* species, a lack of xylem continuity between floral primordia and

bud axes is common in species capable of supercooling (10) indicating that ice nucleation barriers are organized at the tissue level (8, 46). Supporting this concept, Ashworth (8) and Quamme (93) have reported that water soluble dyes failed to move from peach bud axis tissue into the floral primordium. Quamme et al. (90) showed that finely ground powders of tissues and tissues sectioned at .5 mm thickness did not deep supercool. They also reported that tissues killed by steam, chloroform, and oven drying (with rehydration) supercooled, thus providing evidence that the structural feature does not involve the protoplasm. That deep supercooling may depend upon the integrity of floral structures was demonstrated by mechanical wounding of peach floral buds prior to freezing, this enabled ice formation in the bud axis and nucleation of water in the primordium (8, 93).

Detection of freezing of supercooled tissue is commonly based on thermal analysis (TA) and differential thermal analysis (DTA) (13, 16, 18, 69, 74, 77, 86, 108). Nuclear magnetic resonance (NMR), and differential scanning calorimetry (DSC), have, to lesser extents, been utilized (17, 18, 22, 57). Thermal analysis detects the temporary latent heat of fusion released (exotherm) when water freezes. DTA is similar to TA except that DTA involves use of an additional reference thermocouple junction, and the exotherms represent the temperature difference between the sample tissue and the reference junction. DTA has emerged as a simple rapid technique for determining hardiness since it was first shown that an exotherm is involved in freezing injury of flower bud primordia (35). Exotherms have also been associated with freezing injury of flower buds of blueberry (13), grape (2, 71, 77, 87, 117), and a number of *Prunus* species (3, 4, 7, 84, 91). Usually two

distinct exotherms are observed; the first is the result of extracellular freezing (High Temperature Exotherm or HTE) and the second is thought to be the result of intracellular freezing (Low Temperature Exotherm or LTE) (2, 35, 93) and is considered a lethal event (32, 57, 66, 96).

The presence of compounds which depress the freezing point of a solution can aid in a plant's ability to supercool. These antifreeze like compounds may be secondary cell metabolites such as polyhydroxy compounds or glycoproteins similar to those identified in antartic fishes (28). Winkler (115, 116) reported that with *Vitis vinifera*, starch is hydrolyzed to sugar in the above-ground tissues during dormancy, and serves as a cryoprotectant, affecting the freezing point depression of the tissue.

Several investigators (57, 66, 102, 109, 125) believe that membrane structure may be a factor influencing the freezing resistance of tissue. Protoplasmic stiffening (57) and augmentation of phospholipids in membranes (62, 103) have been associated with freezing resistance. Studies with Black Locust (103) have shown that the degree of unsaturation of cell lipids during the winter remained unchanged. Changes in enzyme activity (38, 56) and protein synthesis (15, 70) induced by exposure to freezing temperatures provides evidence that alterations in cellular constituents may affect ability to resist freezing stress.

Freezing in plants produces physiochemical events which are well understood separately, but, the interaction of various events is complex, and the biological process may never be totally explained.

Acclimation and Hardiness

Widespread research by over 100 scientists in North America (88) has led to increased knowledge of not only freezing injury and mechanisms of hardiness but also the influence of the acclimation cycle and metabolic processes.

In late summer and early fall, plant tissues undergo a dramatic increase in cold resistance so that by early winter, some tissues are able to tolerate temperatures of -40°C and below (114). Some plants (124) can only acclimate a few degrees below 0°C, while others are able to survive the temperature of liquid nitrogen (95, 97). This increase in hardiness, termed acclimation, is the general process whereby a plant changes from the cold tender to the cold hardy condition.

Certain environmental signals which initiate cold acclimation processes are known, i.e. low-temperature (37, 42, 74, 82, 97), reduced photoperiod (42, 45, 105, 110) and declining water content (40, 59, 78, 123). Irving and Lanphear (45) demonstrated that the development of hardiness is a photoperiodic response, which agrees with views of Kacperska (51) and Weiser (114). It has been reported that potted 'Concord' grapevines, when subjected to a night interruption by white light, were generally less hardy than those exposed to naturally decreasing daylengths (119). Investigations with apple revealed that acclimation occurs in two stages (42). The first stage being induced by short days (42, 45, 105) and the latter by low temperature (82, 97). Increases in hardiness during the second stage occur very rapidly and normally coincide with the first freezing temperatures of the fall (42). Schnabel and Wample (100) reported that a weak development of grape cold hardiness occurs when short days and low temperatures are

administered separately. However, when short days or low temperatures are combined, a greater increase in hardiness occurs, indicating a synergistic effect of these two environmental factors. When perennial plants are undergoing active growth, neither short days nor low temperature can induce cold hardiness (111).

Scientists have tried to determine what initial factor causes the changes in metabolism that allow plants to acclimate. Steponkus and Lanphear (105) studied the light stimulation of cold acclimation of *Hedera helix* and concluded that light acted via translocatable promoters of hardiness which were either a fraction of organic acids and phosphorylated compounds or carbohydrates. Van Huystee et al. (110) inferred that during autumn, acclimation is a phytochrome response which triggers a hormone that increases the production of water-soluble proteins. They believe these proteins represent enzymes responsible for the increase of carbohydrates (75) and other organic products in cold acclimating plants.

During early acclimation, hardiness increases are in part associated with tissue water loss (59, 68). The decline in water content serves to protect tissues in two distinct ways. First, it dehydrates the cell and lessens the chance for destructive formation of ice crystals (6, 66) and second, it concentrates the cell sap which effectively lowers its freezing point (6, 57, 73) allowing the cell to escape damage from ice formation. Wolpert (123) has shown that a decline in tissue water of *Vitis* is highly correlated with short day induced acclimation. Pierquet and Stushnoff (78) report that *Vitis riparia* buds are more hydrated during early fall and become dehydrated and more hardy in midwinter.

In addition to these environmental signals, investigators have shown that certain biochemical responses by plants are correlated to cold acclimation. Some examples of these are: ABA accumulation during acclimation (26, 57), osmotic adjustment (75, 99, 104) and structural changes, e.g., membrane augmentation (103, 109), cellular alterations of the plasma membrane (102, 106, 125) and reduced intermolecular distances of molecules in cells (114). Although the precise effect of such changes on tissue and cell hardiness has not been determined, structural or conformational changes involving cell constituents such as proteins (15, 70, 37, 110), enzymes (38, 56), carbohydrates (75, 116), lipids (62) and certain metabolites (28, 99) are associated with increased tolerance to cold.

Compared to biochemical investigations, fewer studies involving the effects of morphology and cultural practices on cold acclimation and hardiness of plants have been conducted, perhaps due to environmental limitations and the difficulty of data collection.

Grapevines possess unique growth habits among plants whose acclimation and hardiness have been researched. They not only produce very long shoots with indeterminate growth and compound buds but also lack growth cessation by terminal bud set (79, 80). As long as shoot elongation is rapid, accumulation of carbohydrate will be delayed until the grapevine passes the period of self support (when carbohydrate from the vine equals that stored from current photosynthesis) and initiates maturation (54). A grapevine's ability to acclimate is related to morphological changes during maturation (40). Overloading grapevines with fruiting buds caused a series of morphological and physiological changes in the shoots and stems of the cultivar `Merlot', resulting in

plant desiccation and shoot die-back (47). Wolpert (121) describes vegetative maturity as a dominant feature in the initial phase of acclimation. Treatments that affect acclimation during this phase appear to do so via their affect on the rate of shoot maturation. Evidence clearly indicates that presence and color of periderm tissue (40, 120, 121), shoot and node location (40, 49), cane diameter (101, 120, 123) and cane exposure (40, 101) are correlated with resistance to cold during the subsequent dormant season.

Efforts to understand the affect cultural practices the previous growing season has on a grapvine's ability to mature and resist freezing stress are well documented. Variables such as cropping stress (41, 107), exposure of leaves to sunlight (40), weed control (20), presence of adequate leaf area (107), method of training (20, 122), proper soil moisture level (101) and growing site (113, 118) all influence the level of cold hardiness.

A factor to consider in any test in which hardiness is measured is sampling uniformity. This has proven to be true in several fruit crops such as blueberry (13, 14) apple (42) peach (82), cherry (4, 5) and grape (40, 49). Tissue maturation (40) as related to position of buds (13, 40, 123) or shoot segments (49) are examples where caution must be exercised to collect uniform samples over the duration of the study.

Chilling

A condition of protection against extremes in moisture and temperature termed dormancy (99) is a key defense mechanism ensuring survival of many perennial plants. Dormancy can be grouped into periods called quiescence and rest. Quiescence is a period of

nonvisible growth controlled by external factors (113); growth will proceed under favorable environmental conditions. Rest, on the other hand, is a period of nonvisible growth controlled by internal factors (63); growth will not occur even under favorable conditions. Rest completion and chilling satisfaction are considered synonymous (63, 113).

One area of both scientific and practical interest is whether or not there exists a chilling requirement for grapevine bud break. Experiments designed to examine the relationships between grapevine bud dormancy and chilling have received little attention. Magoon and Dix (65) exposed 9 cultivars of grapes to ambient air temperatures of less than 7.2°C for various lengths of time. Subsequently, the vines were grown under ambient temperatures of 18-21°C. They found that as the duration of exposure to temperatures below 7.2°C increased there was a decrease in the length of time to bud break. Weaver et al. (112) reported a wide range in the time required for grape buds to end rest. He found that cuttings of cultivar 'Pearl of Casaba', made on 21 March broke bud after 25 days at 25°C, while cuttings of 'Ribier' did not break bud even after 105 days. Martin (63) reported that grapevines appear to require about 200 hours of winter chilling, (commonly temperature above 0°C and below 7°C). With insufficient chilling duration, grapevine shoot growth can be erratic with poor cluster development and irregular berry set (65).

Deacclimation

Deacclimation is an important aspect of development in overwintering perennial plants. It occurs when rest is complete (83);

movement from the quiescent state begins (113), loss of hardiness and increases in tissue moisture and temperature occur (5, 14, 44, 123), followed by bud swell and rapid growth (85).

The transition from the acclimated to the deacclimated state in '*Prunus*' and '*Vitis*' buds is associated with a shift of the LTE to warmer temperatures (2, 4, 5). In addition, an increase in the water content of certain tissues, e.g. flower buds (91, 122) flower bud primordia (3) and stem tissue (122) occurs. As spring progresses and temperatures increase, flower buds of several perennial species lose their ability to deep supercool (2, 4, 52, 91) and become more susceptible to injury by freezing temperatures previously considered harmless (85). Reports in the literature attest to the dehardening effects of warm temperatures in the post-rest period (44, 82).

Experiments directed towards understanding fluctuations in hardiness during deacclimation confirm that once deacclimation begins, irreversible changes in hardiness take place. For example, during deacclimation grape flower buds from the cultivar 'Riesling' showed greater cold resistance when induced at -3°C for 96 hours but failed to reach the original level of resistance (27). This supports the work of Howell and Weiser (44) with apple bark tissues which indicated that the dehardening / rehardening process was only partially reversible. In 1959, Proebsting (81) observed a fluctuation in hardiness of peach while in rest. Later, Proebsting (82) reported that hardiness of peach flower buds fluctuated a few degrees during deacclimation and this hardiness level was closely correlated with the minimum air temperature

of the previous day. Investigations by Howell and Wolpert (43) indicated that conditions which deplete grapevine reserves (inadequate vine maintenance) reduce the bud's ability to regain hardiness during a cold period prior to the onset of growth.

In overwintering flower buds of `Redhaven' peach (91) irreversible changes in the flower bud occur, e.g. vascular xylem differentiation, which prevents supercooling (10). Woolly thread-like tissues (76) surrounding the bud develop upon swelling in the spring and along with bud scales (31) provide grape buds with protection against sudden temperature changes and desiccation (99). Callus pads in the phloem of grapevine sieve plates, that formed to stop movement of food material during dormancy, are dissolved upon swelling in the spring, allowing normal sieve tube operation (116). Parker (75) reports that decreases in the sugars sucrose, raffinose and stachyose during the spring were related to a loss of hardiness of hardwood tree barks. Polyacrylamide electrophoresis has shown slight decreases in several hydrolytic enzymes of alfalfa during deacclimation (56). Photoperiod has received little attention as to its effect on deacclimation of perennial plants, perhaps due to the lack of major photosynthetic organs (leaves). Photoperiodic response during deacclimation is not clear-cut but some authors (99) hypothesize that enough long-day light penetrates the bud scales to bring about the response within the primordial leaf tissues inside the bud.

The evidence reviewed here substantiates that temperature appears to be the key factor critical to the deacclimation process of perennial plants. Investigations with some species also demonstrate the importance of root temperature during deacclimation. The effect of

root temperature on bud break of Cabernet Sauvignon grapevines (55) revealed that, with air temperatures the same for all treatments, bud break occured 4 to 7 days earlier at 20-35°C than at 11°C. Peaches, in contrast, do not show this response (126).

The interactions involved in the biological processes of deacclimation are diverse and complex and efforts to better understand this phase should be intensified.

Pruning

Pruning is a cultural practice which needs further study since it is often assumed that it might influence hardiness and acclimation, especially if executed at an unfavorable time.

Pruning affects the physiological behavior of plants and includes the removal of shoots, leaves, living stems, and other vegetative parts of the plant (48, 116). Pruning involves the mechanical removal of tissue whether by hand, chemically (48) or even burning (47). The history of horticulture has involved attempts to modify the growth of plants by direct manipulation to achieve enhanced growth and efficient production. Before 600 BC, the Phoenicians (and later the Romans) engaged in grapevine pruning activities without understanding the physiological basis for removing 85 to 98 percent of the annual growth (116). More recently, it has been shown that pruning concentrates the activities of the plant into the remaining parts but diminishes the total capacity of the plant for growth and fruit production(48, 113).

A common misconception is that pruning invigorates, when actually it has an overall dwarfing affect with only localized invigoration occuring (48, 113, 127). This can partially be explained by the work of

Lewis (58) and Cameron (21) with citrus trees. They investigated top regeneration of decadent orange trees by severe pruning (skeletonization). They revealed that severely pruned trees required two or more years to re-establish a full complement of leaves while lightly pruned trees (removal of all parts less than 1 centimeter in diameter) re-established a full complement of leaves during the first year after pruning.

The physiological responses of a grapevine to pruning are well documented (76, 113, 116). Several authors (31, 76, 113) describe the effects of pruning grapes as a "stunting effect", i.e. the removal of living tissue decreases the capacity of the vine. This view is supported by Clingeleffer (25) in that traditional pruning limits production. He has shown that minimally pruned (only 15% of the wood was removed) 'Sultana' vines increased yield over 6 seasons by 60 percent compared to that of traditionally pruned (85% of the wood was removed) vines. Similar results were obtained by Baldwin (11) and Kimball (53). However, these views fail to consider other factors such as pest management, vine health and overall vineyard operations, eg. thinning, spraying and harvest execution, that may be altered by this treatment.

Pruning has been reported to cause a delay in acclimation of grape (20), retarded spring bud growth in grape (1, 43, 60, 76) and reduced levels of hardiness of apple (19) peach (127) and grape (29, 101, 107, 122). For example, grape cane tissue at the tips of fall pruned canes showed more injury when frozen to -30°C than either unpruned canes or samples extracted from the basal part of the fall pruned canes (29). Wolpert and Howell (122) report that fall pruned 'Conord' vines had

more bud mortality than unpruned controls. Subsequently, they found more injury of buds at the tips of pruned canes than those closer to the main trunk. This is consistent with reports that buds in those positions develop first (123) and are therefore more likely to be injured by a spring freeze (20). Grapevine buds at greater phenological development were reported to be delayed in growth by pruning which reduced chances of spring frost injury (43, 76). Stergios and Howell (107) revealed that canes and buds failed to reach proper hardiness levels on underpruned vines because too much vegetation shaded interior nodes, producing canes and buds of inferior hardiness. Grapevine buds on fall pruned canes showed more cold injury than non pruned canes when the winter minimum dropped to -26°C (101). Early investigations with apple (19) inferred that pruning per se predisposed the trees to winter damage.

One physiological aspect common to grapevines that receives attention during late pruning is that of "bleeding". "Bleeding", a process that is not fully understood, occurs as a result of the removal of water from the soil by the epidermal cells of the root and which, under sufficient pressure, flows out of the xylem bundles with pruning or injury (76, 116). This process most frequently occurs under conditions of adequate soil moisture and warmer "spring like" soil temperatures (113). The exudate consists of mainly water with minute levels of hormones, cytokinins, gibberellins, sugars and other mineral nutrients (114, 116). Initially, "bleeding" was thought to materially injure the vine by causing irregular growth, delayed bloom and delay in both fruit and wood maturation (31, 76); however, Weaver (113) reports that bleeding has no harmful effects on the vine. Winkler (116) states

that bleeding can occur in mid-dormancy by activation of root growth with warm irrigation water (unpublished data). However, reports by Freeman and Smart (30) indicate that root growth activity of grapevines may be delayed as much as 10 weeks behind renewed shoot activity in the spring, which suggests further investigations are needed.

An important aspect of pruning grapevines, of interest to several scientists, is timing. The view that pruning can occur anytime between full dormancy and bud break is accepted by many authors (1, 31, 76, 101, 113, 116, 118); however, late winter and early spring are times considered optimum for pruning in colder climates (101, 116, 118). This has been primarily based on the assumption that it is then possible to select the most desirable canes for removal avoiding those which might have been poorly matured or winter injured. Shaulis (101) reports that an unpruned and mature vine in early March will show some winter injury and the injury will not be randomly distributed over the vine but will be less on those canes that are most mature. Recommendations for pruning late and selectively to decrease winter injury are recognized by many authors (12, 29, 101, 113, 116). Wolfe (118) noted that early pruning of large tissue such as trunks or "bull" canes should be avoided as the wounds may split from freezing or drying. In addition, he states that pruning cuts should be made about 2.54 cm above the last bud and discontinued at temperatures below -4°C to prevent splitting of frozen canes.

Contrary to the view of optimum pruning time, Loomis (61) and Magoon and Dix (64) report that no significant differences in yield or hardiness were noted between *Vitis labrusca* vines pruned soon after leaf fall and vines pruned just before the buds began to swell in the

spring. Magoon and Dix (64) revealed that the variance due to the different pruning dates was the least of all in magnitude and neither in itself nor in its interaction with other factors showed significant correlation. However, in conclusion, they reported that where winter temperatures seldom fall below -23°C pruning may begin as soon as the leaves abscise in the fall.

A study in Ankara, Turkey (1), involving physiological effects of six different pruning times, revealed that with the grape cultivar `Hafizali' there was a significant effect in yield, berry weight, pruning weight, sugar content and total acidity of the fruit. However, the pruning time effect on blooming period was insignificant.

It is evident from the above findings that pruning time may influence hardiness level, bud swell, yield, and other biological events with the grapevine. The physiological response of grapevines to pruning is recognized in this review as a multifaceted process that ultimately influences the vigor and bearing capacity of the crop.

Water Content

Water in differentiated plant tissue is distributed between the intracellular and extracellular spaces (57, 66, 73). Two populations of intracellular water exist with highly different properties. "Bound" water is water closely associated with macromolecules and is not available for freezing (24). This fraction of water comprises about 0.2 to 0.4 g water/g dry tissue (18). The remainder of the intracellular water is similar to that of a dilute salt solution (6, 17, 57). Extracellular and a portion of intracellular water is termed "free water" and is not bound per se (6, 24, 114). During cold

acclimation there is a reduction in the amount intracellular water (57). The reduction depends on temperature and initial solute concentration of the intracellular water (24). It is known that as extracellular freezing occurs, a portion of intracellular supercooled water moves out of the cell to the growing ice crystals due to its higher vapor pressure (6, 17, 57, 66). The resulting concentration of intracellular solutes produces a small freezing point depression (17).

Water content of overwintering perennial plant tissues nearly always decreases with increasing hardiness and increases as plants deacclimate (17, 57, 73). This phenomenon has been reported with a variety of perennial plants such as azalea (35), rhododendron (36), blueberry (13), 'Red Osier' dogwood (18, 23, 68), peach (91, 93), sweet cherry (3), grape (123), 'Saskatoon' berry (50) and several conifers (97, 98). Changes in water content have been associated with photoperiod (68, 123), hardiness (1, 3, 91, 123), node position (121, 123) and pruning (122). For example, McKenzie et. al. (68) reported with 'Red Osier dogwood (*Cornus stolonifera* Michx) that a significant reduction in stem water content occurred when plants were subjected to 4 weeks of short day photoperiod with day/night temperatures at 20/15°C respectively. Wolpert (123) also, reported that tissue water loss with 'Concord' grape was highly correlated with short day induced acclimation.

The hypothesis that increases in hardiness by dehydration is partially controlled by extracellular freezing is supported by research from Biermann et. al. (13). They artificially hydrated highbush blueberry buds and found they were hardy to -10°C, whereas artifically dehydrated buds were 15° hardier. Fluctuations in hardiness with peach

(91) were correlated with seasonal changes in water content and appeared to result from the redistribution of water during extracellular freezing and thawing. Other investigations with peach (93) and sweet cherry (3) revealed that a withdrawal of water from the bud axis into the bud scales during extracellular freezing caused a dry region at the base of the bud and prevented ice from spreading into the primordium, allowing supercooling. Andrews and Proebsting (3) hypothesized that supercooling of *Prunus* flower buds is a physical process that is inversely related to the water content of the flower primordium . This process may occur with *Vitis species*, which also have bud scales and supercool, but this has not been investigated.

Internal changes in water content can occur. For example, the water content of flower primordia and vascular tissues of peach declined at constant freezing temperatures in plastic bags whereas that of whole flower buds remained stable (91). Microscopic observations of stem sections of 'Red Osier' dogwood revealed that the major reduction in water content during acclimation occurred during maturation and senescence of pith cells (68). McKenzie et. al. (68) also reported self-induced root suberization occurs in 'Red Osier' dogwood during acclimation, which lowered the permeability and absorption of water. These changes may be protective and comprise adaptive physiological response to unfavorable environmental conditions.

The water content of excised buds and cane internode segments for three distinct node positions of 'Concord' grape (121) was reported to have significantly different values. The tissue water content of apical tissues for both bud and cane segments were higher than either middle

or basal tissues of the same cane. The water content of the cane segments in all treatments was lower than that of buds. With `Red Osier' dogwood (68) a similar pattern during midwinter was reported in that the water content of internodes was high near the stem apex. Evidence that a change in water content of grape bud tissue occurs as a result of pruning has been documented by Wolpert (122). They found that on 2 May water content of `Concord' buds of fall pruned vines with 8 node canes were 22% greater than those of buds from the same node position on unpruned vines.

It can be concluded from the above that sampling location (node position), photoperiod, pruning, and temperature may influence the water content of certain perennial plant tissues during the overwintering period.

Research Objective

Because very little is known about deacclimation of grapevines, the objectives of this work were to provide solid quanitative data on key aspects of pruning and deacclimation of grapevines and, specifically to answer the following questions:

1.) Will pruning during deacclimation affect the ability of either bud or cane tissue to supercool or alter the water content of these tissues?

2.) If a reduction is evident, will the effect be more localized near the pruning cut or more generalized throughout the vine?

Demonstrations of winter injury due to early pruning in a hardy cultivar such as `Concord' have serious connotations for more cold tender cultivars such as *Vitis vinifera* `Merlot' (2, 92). `Merlot' was

the cultivar selected for this study because of its economic importance and enological qualities in the colder `border line' viticultural areas. The occurrence and extent of reduced hardiness as a result of early pruning would influence recommendations on vine management. How early one can safely begin pruning is an important viticultural consideration in these locations where late winter injury potential is high.

References

1. Agaoglu, Y. S. 1979. A study on the time of pruning grapevines. Zirrat Fakultesi Yaninları (Ankara, Turkey). 723:63-68.

2. Andrews, P. K., C. R. Sandidge III, and T. K. Toyama. 1984. Deep supercooling of dormant and deacclimating *Vitis* buds. Amer. J. Enol. and Vitic. 35:175-177.

3. Andrews, P. K., and E. L. Proebsting. 1987. Effects of temperature on the deep supercooling characteristics of dormant and deacclimating sweet cherry flower buds. J. Amer. Soc. Hort. Sci. 112(2):334-340.

4. Andrews, P. K., E. L. Proebsting, and G. S. Campbell. 1983. An exotherm sensor for measuring the cold hardiness of deep supercooled flower buds by differential thermal analysis. Hortscience 18(1):77-78.

5. Andrews, P. K. and E. L. Proebsting. 1983. Differential thermal analysis and freezing injury of deacclimating peach and sweet cherry reproductive organs. J. Amer. Soc. Hort. Sci. 108(5):755-759.

6. Asahina, E. 1978. Freezing processes and injury in plant cells. In: Plant Cold Hardiness and Freezing Stress. (eds. P. H. Li and A. Sakai.), pp.17-36. Academic Press, New York.

7. Ashworth, E. N., G. W. Lightner, and D. J. Rowse. 1981. Evaluation of apricot flower bud hardiness using a computer-assisted method of thermal analysis. Hortscience 16(6):754-756.

8. Ashworth, E. N. 1982. Properties of peach flower buds which facilitate supercooling. Plant Physiology 70:1475-1479.

9. Ashworth, E. N., D. J. Rowse, and L. A. Billmyer. 1983. The freezing of water in woody tissues of apricot and the relationship to freezing injury. J. Amer. Soc. Hort. Sci. 108(2):299-303.

10. Ashworth, E. N. 1984. Xylem development in prunus flower buds and the relationship to deep supercooling. Plant Physiology 74:862-865.

11. Baldwin, F. G., G. E. Bleasdale , R. S. Cadman, and I. L. Keens. 1979. Comparison of trellises and pruning levels for Sultana vines in the murray valley. Aust. J. Exp. Agric. Anim. Husb. 19:634-640.

12. Bernstein, Z. 1983. Improving the regularity of bud burst in regions with mild winters. In XVIIIe Congres International de la Vigne et du Vin de l'O.I.V. pp.147-157. Capetown, South Africa.

13. Biermann, J., C. Stushnoff, and M. J. Burke. 1979. Differential thermal analysis and freezing injury in cold hardy blueberry flower buds. J. Amer. Soc. Hort. Sci. 104(4):444-449.

14. Bittenbender, H. C., and G. S. Howell. 1975. Interactions of temperature and moisture content on spring deacclimation of flower buds of highbush bluberry. Can. J. Plant Sci. 55:447-452.

15. Brown, G. N. 1978. Protein synthesis mechanisms relative to cold hardiness. In: Plant Cold Hardiness and Freezing stress (eds. P. H. Li and A. Sakai) Vol. I pp.153-163. Academic Press, New York.

16. Brown, M. S., E. Sa B. Pereira, and B. J. Finkle. 1974. Freezing of nonwoody plant tissues. Plant Physiology 53:709-711.

17. Burke, M. J., L. V. Gusta, H. A. Quamme, C. J. Weiser, and P. H. Li. 1976. Freezing and injury in plants. Ann. Rev. Plant Physiology 27:507-528.

18. Burke, M. J., R. G. Bryant, and C. J. Weiser. 1974. Nuclear magnetic resonance of water in cold acclimating `Red Osier' dogwood stem. Plant Physiology 54:392-398.

19. Burkholder, C. L. 1936. December pruning in 1935 results in severe injury to `Jonathon' and `Stayman' trees in Lafayette, Indiana. Proc. Amer. Soc. Hort. Sci. 34:49-51.

20. Byrne, M. E. and G. S. Howell. 1978. Initial response of Baco Noir grapevines to pruning severity, sucker removal, and weed control. Amer. J. Enol. Vitic. 29:192-198.

21. Cameron, S. H. and R. W. Hodgson. 1941. Effect of severity of pruning on top regeneration in citrus trees. Proc. Amer. Soc. Hort. Sci. 39:67-72.

22. Campbell, R. W. and N. Ghosheh. 1957. Hardiness studies of selected grape varieties. Proc. Amer. Soc. Hort. Sci. 70:161-164.

23. Chen, P. M., P. H. Li, and M. J. Burke. 1977. Induction of frost hardiness in stem cortical tissues of *Cornus stolonifera* Michx. by water stress. Plant Physiology 59:236-239.

24. Chen, P. and L. V. Gusta. 1978. The role of water in cold hardiness of winter cereals. In: Plant Cold Hardiness and Freezing Stress.(eds. P. H. Li and A. Sakai) Vol.I pp.165-174. Academic Press, New York.

25. Clingeleffer, P. R. 1984. Production and growth of minimal pruned Sultana vines. Vitis 23:42-54.

26. Corgan, J. N. and C. Peyton. 1970. Absissic acid levels in dormant peach flower buds. J. Amer. Soc. Hort. Sci. 95(6):770-774.

27. Damborska, M. 1978. The effect of higher winter temperatures on changes of the frost resistance of grapevine buds. Vitis 17: 341-349.

28. Eastman J. T. and A. L. Devries. 1986. Antartic fishes. Scientific American 255(5):106-114.

29. Edgerton, L. J. and N. J. Shaulis. 1953. The Effect of time of pruning on cold hardiness of Concord grape canes. Proc. Amer. Soc. Hort. Sci. 62:209-213.

30. Freeman, B. M. and R. E. Smart. 1976. A root observation laboratory for studies with grapevines. Amer. J. Enol. Vitic. 27:36

31. Fuller, A. S. 1894. The Grape Culturist. Orange Judd Company, New York.

32. George, M. F. and M. J. Burke. 1977. Cold hardiness and deep supercooling in xylem of shagbark hickory. Plant Physiology 59:319-325.

33. George, M. F., S. G. Hong, and M. J. Burke. 1977. Cold hardiness and deep supercooling of hardwoods: Its occurrence in provenance collections of red oak, yellow birch, black walnut, and black cherry. Ecology 58:674-680.

34. George, M. F., M. J. Burke, H. M. Pellett, and A. G. Johnson. 1974. Low temperature exotherms and woody plant distribution. Hortscience 9:519-522.

35. Graham, P. R. and R. Mullin. 1976. The determination of lethal freezing temperatures in buds and stems of deciduous azalea by a freezing curve method. J. Amer. Soc. Hort. Sci. 101(1):3-7.

36. Graham, P. R. and R. Mullin. 1976. A study of flower bud hardiness in azalea. J. Amer. Soc. Hort. Sci. 101(1):7-10.

37. Graham, D. and B. D. Patterson. 1982. Responses of plants to low, nonfreezing temperatures: proteins, metabolism, and acclimation. Ann. Rev. Plant Physiol. 33:347-372.

38. Hellergren, J., S. Widell, and T. Lundborg. 1985. ATPases in relation to freezing injuries in purified plasma membrane vesicles from nonacclimated seedlings of *Pinus sylvestris*. Acta Horticulturae 168:161-165.

39. Hong, S. G., E. Sucoff, and O. Y. Lee-Stadelman. 1980. Effect of freezing deep supercooled water on the viability of ray cells. Bot. Gaz. 141(4):464-468.

40. Howell, G. S. and N. J. Shaulis. 1980. Factors influencing within-vine variation in the cold resistance of cane and primary bud tissues. Amer. J. Enol. Vitic. 31:158-161.

41. Howell, G. S., B. G. Stergios, and S. S. Stackhouse. 1978. Interrelation of productivity and cold hardiness of Concord grapevines. Amer. J. Enol. Vitic. 29:187-191.

42. Howell, G. S. and C. J. Weiser. 1970. The environmental control of cold acclimation in apple. Plant Physiology 45:390-394.

43. Howell, G. S. and J. A. Wolpert. 1978. Nodes per cane, primary bud phenology, and spring freeze damage to Concord grapevines. A preliminary note. Amer. J. Enol. Vitic. 29:229-233.

44. Howell, G. S. and C. J. Weiser. 1970. Fluctuations in the cold resistance of apple twigs during spring dehardening. J. Amer. Soc. Hort. Sci. 95(2):190-192.

45. Irving, R. M. and F. O. Lanphear. 1967. Environmental control of cold hardiness in woody plants. Plant Physiology 42:1191-1196.

46. Ishikawa, M. and M. Sakai. 1982. Characteristics of freezing avoidance in comparison with freezing tolerance: A demonstration of extraorgan freezing. In: Plant Cold Hardiness and Freezing Stress. (eds. P. H. Li and A. Sakai.), pp.325-340. Academic Press, New York.

47. Ivanchev, V. 1984. Desiccation of 'Merlot' grapevines overloaded with fruit. Referativnyi Zhurnal 55:(5)22-24.

48. Janick, J. 1963. Horticultural Science. W.H.Freeman and Company, San Francisco. 472 pp.

49. Johnson, D. E. and G. S. Howell. 1981. The effect of cane morphology and cultivar on the phenological development and critical temperatures of primary buds on grape Canes. J. Amer. Soc. Hort. Sci. 106(5):545-549.

50. Junttila, O., C. Stushnoff, and L. V. Gusta. 1983. Dehardening in flower buds of saskatoon-berry, *Amelanchier alnifolia*, in relation to temperature, moisture content, and spring bud development. Can. J. Bot. 61:164-170.

51. Kacperska-Palacz, A. 1978. Mechanism of cold acclimation in herbaceous plants. In: Plant Cold Hardiness and Freezing Stress. (eds. P. H. Li and A. Sakai.), pp.139-152. Academic Press, New York.

52. Kaku, S. and M. Iwaya. 1978. Low temperature exotherms in xylems of evergreen and deciduous broad leaved trees in Japan with reference to freezing resistance and distribution range. In: Plant Cold Hardiness and Freezing Stress. (eds. P. H. Li and A. Sakai), pp.227-239. Academic Press, New York.

53. Kimball, K. and N. Shaulis. 1958. Pruning effects on the growth, yield, and maturity of concord grapes. Proc. Amer. Soc. Hort. Sci. 71:167-176.

54. Kliewer, W. M. 1982. How does a grapevine make sugar? Vinifera Wine Growers Journal 9:260-271.

55. Kliewer, W. M. 1975. Effect of root temperature on budbreak, shoot growth, and fruit-set of 'Cabernet Sauvignon' grapevines. Amer. J. Enol. Vitic. 26:82-89.

56. Krasnuk, M., F. H. Witham, and G. A. Jung. 1976. Electrophoretic studies of several hydrolytic enzymes in relation to the cold tolerance of alfalfa. Cryobiology 13:225-242.

57. Levitt, J. 1980. Responses of Plants to Environmental Stress. Vol.I. Academic Press, Orlando, Florida. 497 pp.

58. Lewis, L. N., C. D. McCarty, and P. W. Moore. 1963. Effects of skeletonization on the rejuvenation and production of declining orange trees. Proc. Amer. Soc. Hort. Sci. 83:295-299.

59. Li, P. H. and C. J. Weiser. 1971. Increasing cold resistance of stem sections of *Cornus stolonifera* by artificial dehydration. Cryobiology 8:108-111

60. Loomis, N. H. 1939. Note on grape foliation as affected by time of pruning. Proc. Amer. Soc. Hort. Sci. 37:653-654.

61. Loomis, N. H. 1943. The influence of time and method of pruning on yields of *Muscadine* grapes. Proc. Amer. Soc. Hort. Sci. 43:418-420.

62. Lynch, D. V. and P. L. Steponkus. 1987. Plasma membrane lipid alterations associated with cold acclimation of winter rye seedlings *(Secale cereale* L. cv Puma). Plant Physiology 83:761-767.

63. Martin, G. C. 1984. An introduction to bud dormancy. Proceedings of bud dormancy in grapevines: Potential and practical uses of hydrogen cyanamide on grapevines. University of California Davis, CA U.S.A.

64. Magoon, C. A. and I. W. Dix. 1941. Relation of pruning to performance of grapes. Proc. Amer. Soc. Hort. Sci. 38:369-372.

65. Magoon, C. A. and I. W. Dix. 1943. Observations on the response of grapevines to winter temperatures as related to their dormancy requirements. Proc. Amer. Soc. Hort. Sci. 42:407-412.

66. Mazur, P. 1969. Freezing injury in plants. Ann. Rev. Plant Physiology 20:419-448.

67. Mazur, P. 1977. The role of intracellular freezing in the death of cells cooled at supraoptimal rates. Cryobiology 14: 251-272.

68. Mckenzie, J. S., C. J. Weiser, and P. H. Li. 1974. Changes in water relations of *Cornus stolonifera* during cold acclimation. J. Amer. Soc. Hort. Sci. 99(3):223-228.

69. Mcleester, R. C., C. J. Weiser, and T. C. Hall. 1969. Multiple freezing points as a test for viability of plant stems in the determination of frost hardiness. Plant Physiology 44:37-44.

70. Meza-Basso, L., M. Alberdi, M. Raynal, M. L. Ferrero-Cadinanos, and M. Delseny. 1986. Changes in protein synthesis in rapeseed *(Brassica napus)* seedlings during a low temperature treatment. Plant Physiology 82:733-738.

71. Miller, D. P. 1986. Cold hardiness of grapevine rootstocks and their effect on scion cold hardiness and time of bud burst. M.S. Thesis, Michigan State University.

72. Olien, C. R. 1974. Energies of freezing and frost desiccation. Plant Physiology 53:764-767.

73. Olien, C. R. 1967. Freezing stresses and survival. Annu. Rev. Plant Physiology 18:387-408.

74. Palta, J. P., J. Levitt, and E. J. Stadelmann. 1978. Plant viability assay. Cryobiology 15:249-255.

75. Parker, J. 1962. Seasonal changes in cold resistance and free sugars of some hardwood tree barks. Forest Science 8:255-262.

76. Perold, A. I. and B. A. Phil. 1927. A Treatise on Viticulture. Macmillan and Co., Limited. 335 pp.

77. Pierquet, P., C. Stushnoff, and M. J. Burke. 1977. Low temperature exotherms in stem and bud tissues of *Vitis riparia* Michx. J. Amer. Soc. Hort. Sci. 102(1):54-55.

78. Pierquet, P. and C. Stushnoff. 1980. Relationship of low temperature exotherms to cold injury in *Vitis riparia* Michx. Amer. J. Enol. Vitic. 31:1-6.

79. Pratt, C. 1978. Shoot nodes of *Vitis labruscana* Baily cv. Concord. Vitis 17:329-334.

80. Pratt, C. 1974. Vegetative anatomy of cultivated grapes - a review. Amer. J. Enol. Vitic. 25:131-150.

81. Proebsting, E. L. 1959. Cold hardiness of Elberta peach fruit buds during four winters. J. Amer. Soc. Hort. Sci. 74:144-153.

82. Proebsting, E. L. 1963. The role of air temperatures and bud development in determining hardiness of dormant Elberta peach fruit buds. J. Amer. Soc. Hort. Sci. 83:259-269.

83. Proebsting, E. L. 1978. Adapting cold hardiness concepts to deciduous fruit culture. In: Plant Cold Hardiness and Freezing Stess. (eds. P.H. Li and A. Sakai.), Vol.1 pp.267-279. Academic Press, New York.

84. Proebsting, E. L., P. K. Andrews, and D. Gross. 1982. Supercooling young developing fruit and floral buds in deciduous orchards. Hortscience 17(1):67-68.

85. Proebsting, E. L., M. Ahmedullah, and V. P. Brummund. 1980. Seasonal changes in low temperature resistance of grape buds Amer. J. Enol. Vitic. 31:329-336.

86. Proebsting, E. L. and A. Sakai. 1979. Determining T-50 of peach flower buds with exotherm analysis. Hortscience 14(5):597-598.

87. Quamme, H. A. 1986. Use of thermal analysis to measure freezing resistance of grape buds. Can. J. Plant Sci. 66:945-952.

88. Quamme, H. A. 1985. Winter hardiness research in north america on woody perennials. Acta Horticulturae 168:191-194.

89. Quamme, H. A. 1985. Avoidance of freezing injury in woody plants by deepsupercooling. Acta Horticulturae 168:11-29.

90. Quamme, H. A. 1973. The mechanism of freezing injury in xylem of winter apple twigs. Plant Physiology 51:273-277.

91. Quamme, H. A. 1983. Relationship of air temperature to water content and supercooling of overwintering peach flower buds. J. Amer. Soc. Hort. Sci. 108(5):697-701.

92. Quamme, H. A. 1974. An exothermic process involved in the freezing injury of flower buds of several *Prunus* species. J. Amer. Soc. Hort. Sci. 99:315-318.

93. Quamme, H. A. 1978. Mechanism of supercooling in overwintering peach flower buds. J. Amer. Soc. Hort. Sci. 103(1):57-61.

94. Rasmussen, D. H. and A. P. Mackenzie. 1972. Effect of solute on the ice-solution interfacial free energy; calculation from measured homogeneous nucleation temperatures. In: Water Structure at the Water Polymer Interface, ed. H. H. G. Jellinek, pp.126-45. New York, Plenum.

95. Sakai, A and Y. Sugawara. 1973. Survival of poplar callus at super-low temperatures after cold acclimation. Plant & Cell Physiol. 14:1201-1204.

96. Sakai, A. 1978. Low temperature exotherms of winter buds of hardy conifers. Plant and Cell Physiology 19(8):1439-1446.

97. Sakai, A. 1966. Studies of hardiness in woody plants. Plant Physiology 41:353-359.

98. Sakai, A. 1979. Freezing aviodance mechanism of primordial shoots of conifer buds. Plant & Cell Physiology 20(7):1381-1390.

99. Salisbury, F. B and C. W. Ross. 1985. Plant Physiology. Wadsworth Publishing Company, Belmont, California. 540 pp.

100. Schnabel, B. J. and R. L. Wample. 1987. Dormancy and cold hardiness in *Vitis vinifera* cv. White Riesling as influenced by photoperiod and temperature. Am. J. Enol. Vitic. 38(4):265-272.

101. Shaulis, N. 1971. Vine hardiness: A part of the problem of hardiness to cold in N. Y. vineyards. Proc. N.Y. St. Hort. Soc. 116:158-167.

102. Sikorska, E. and A. Kacperska. 1982. Freezing induced membrane alterations: Injury or adaptation? In: Plant Cold Hardiness and Freezing Stress. (eds. P.H. Li and A. Sakai), pp. 261-272. Academic Press, New York.

103. Sminovitch, D., J. Singh, and I. A. De La Roche. 1975. Studies on membranes in plant cells resistant to extreme freezing. I. Augmentation of phospholipids and membrane substance without changes in unsaturation of fatty acids during hardening of black locust bark. Cryobiology 12:144-153.

104. Steponkus, P. L. 1984. Role of the plasma membrane in freezing injury and cold acclimation. Ann. Rev. Plant Physiol. 35:543-584.

105. Steponkus, P. L. and F. O. Lanphear. 1967. Light stimulation of cold acclimation: Production of a translocatable promoter. Plant Physiology 42:1673-1679.

106. Steponkus, P. L. and S. C. Wiest. 1978. Plasma membrane alterations following cold acclimation and freezing. In: Plant Cold Hardiness and Freezing Stress.(eds. P.H. Li and A. Sakai), Vol.I pp.75-91. Academic Press, New York.

107. Stergios, B. G. and G. S. Howell. 1977. Effect of site on cold acclimation and deacclimation of concord grapevines. Amer. J. Enol. Vitic. 18:43-48.

108. Stergios, B. G. and G. S. Howell. 1973. Evaluation of viability tests for cold stressed plants. J. Amer. Soc. Hort. Sci. 98(4):325-330.

109. Sugawara, Y. and A. Sakai. 1978. Cold acclimation of callus cultures of jerusalem artichoke. In: Plant Cold Hardiness and Freezing Stress. (eds. P.H. Li and A. Sakai), pp.197-210. Academic Press, New York.

110. Vanhuystee, R. B., C. J. Wieser, P. H. Lee. 1965. Some postulated relationships among autumn phenomena in woody plants. Nature 208:89-90.

111. Vanhuystee, R. B., C. J. Wieser, and P. H. Li. 1967. Cold acclimation in *Cornus stolonifera* under natural and controlled photoperiod and temperature. Bot. Gaz. 128(3-4):200-205.

112. Weaver, R. J., K. Y. Der, and R. M. Pool. 1968. Relation of plant regulators to bud rest in *Vitis vinifera* grapes. Vitis 7:206-212.

113. Weaver, R. J. 1976. Grape Growing. John Wiley & Sons, Inc., New York. 371 pp.

114. Wieser, C. J. 1970. Cold resistance and injury in woody plants. Science 169:1269-1278.

115. Winkler, A. J., and W. O. Williams. 1945. Starch and sugars of *Vitis vinifera*. Plant Physiology 20:412-432.

116. Winkler, A. J., J. A. Cook, W. M. Kliewer, and L. A. Lider. 1974. General Viticulture. University of California Press, Berkley. 710 pp.

117. Wolf, T. K. 1986. Effects of rootstock and nitrogen fertilization on the growth, yield, tissue nitrogen concentrations, and dormant bud cold hardiness of 'Chardonnay' *(Vitis vinifera* L.) grapevines. Ph.D. Thesis, Cornell University.

118. Wolfe, W. 1979. Minimizing winter damage: Site selection, vineyard establishment, and maintenance. Proc. Wash. St. Grape Soc. 9:67-75.

119. Wolpert, J. A. and G. S. Howell. 1986. Dormancy and cold hardiness in *Vitis vinifera* L. cv. 'White Riesling' as influenced by photoperiod and temperature. J. Amer. Soc. Hort. Sci. 111(1):16-20.

120. Wolpert, J. A. and G. S. Howell. 1985. Cold acclimation of Concord grapevines. I. Variation in cold hardiness within the canopy. Amer. J. Enol. Vitic. 36:185-188.

121. Wolpert, J. A. and G. S. Howell. 1985. Cold acclimation of Concord grapevines. II. Natural acclimation pattern and tissue moisture decline in canes and primary buds of bearing vines. Amer. J. Enol. Vitic. 36:189-194.

122. Wolpert, J. A. and G. S. Howell. 1984. Effects of cane length and dormant season pruning date on cold hardiness and water content of Concord grapevines. Amer. J. Enol. Vitic. 35:237-241.

123. Wolpert, J. A. 1983. Cold acclimation of Concord grapevines. Ph.D. Thesis, Michigan State University.

124. Yelenosky, G. 1976. Ice tolerance of cold-hardened "Valencia" orange wood. Cryobiology 13:243-247.

125. Yoshida, S. 1982. Fluorescence polarization studies on plasma membranes isolated from mulberry bark tissues. In: Plant Cold Hardiness and Freezing Stress. (eds. P. H. Li. and A. Sakai), pp.273-284. Academic Press, New York.

126. Young, E. and D. J. Werner. 1986. 6-BA applied after shoot and or root chilling and its effect on growth resumption in apple and peach. Hort. Sci. 21(2):280-281.

127. Yu, K. S., M. K. Rogoyski, and A. R. Renquist. 1986. Peach Ochard Management. Colorado State University technical report TR86-7.

COLD HARDINESS AND WATER CONTENT DURING DEACCLIMATION OF GRAPEVINE BUD AND CANE TISSUE.

Introduction

The transition from the acclimated to the deacclimated state in 'Prunus' and 'Vitis' buds is associated with the occurrence of Low Temperature Exotherms (LTEs) at increasingly warmer temperatures (3, 5, 6). As spring progresses and environmental temperatures increase, flower buds of several perennial species lose their ability to deep supercool (3, 6, 23, 42) and become more susceptible to injury by freezing temperatures previously considered harmless (39). Reports in the literature attest to the dehardening effects of warm temperatures in the post-rest period (20, 37). Fluctuations in hardiness occur during deacclimation (37) and experiments directed towards understanding these fluctuations confirm that once deacclimation begins, the rehardening process is only partially reversible (13, 20).

Water content of overwintering perennial plant tissues, e.g. flower buds (42, 56) and stem tissue (56), nearly always decreases with increasing hardiness and increases as plants deacclimate (10, 24, 34). This phenomenon has been reported with a variety of perennial plants such as azalea (16), highbush blueberry (9), dogwood (31, 33), peach (42), 'Saskatoon' berry (22) and grape (57). An inverse relationship between tissue water content and cold hardiness has been reported early

in the acclimation period for 'Concord' (55). Water content changes have been associated with photoperiod (31, 57), node position (55, 57) and pruning (56). For example, McKenzie et al. (31) reported with 'Red Osier' dogwood that a significant reduction in stem water content occurred when plants were subjected to 4 weeks of short day photoperiod with day/night temperatures at 20/15°C respectively. Wolpert (57) with 'Concord' grapevines reported that tissue water loss was highly correlated with short day induced acclimation. A change in water content of grape bud tissue as a result of pruning has been documented by Wolpert and Howell (56). They also report more injury of buds at tips of pruned canes than those closer to the main trunk.

The view that grapevine pruning can occur anytime between full dormancy and bud break is accepted by many authors (1, 15, 35, 45, 50, 51, 54); however, late winter and early spring are times considered optimum for pruning in colder climates (45, 51, 54). This has been primarily based on the assumption that it is then possible to select the most desirable canes for removal avoiding those which might have been poorly matured or winter injured. Magoon and Dix (30) recommended that growers in colder sites wait until the danger of heavy freezing is past before pruning, even though they could find no significant differences in yield or hardiness between *Vitis labrusca* vines pruned soon after leaf fall and vines pruned just before the buds began to swell in the spring. Pruning has been reported to cause a reduction in the hardiness of apple (11), peach (58) and grape (45, 47, 56). Stergios and Howell (47) revealed that canes and buds failed to reach proper hardiness levels on underpruned vines because shading produced canes and buds of inferior hardiness. Pruning in the fall can cause a

delay in acclimation of grape (12) whereas spring pruning has been reported to retard bud growth (1, 19, 29, 35).

This study was developed to provide quanitative data on key aspects of pruning and deacclimation of grapevines and specifically to address the following questions:
Will pruning during deacclimation affect the ability of either bud or cane tissue to supercool or alter the water content of these tissues? If a reduction is evident, will the effect be more localized near the pruning cut or more generalized throughout the vine?

Materials and Methods

Plant Materials:

Experiments were conducted on *Vitis vinifera* L. cv. Merlot dormant bud and cane tissue during a 5 week period beginning 21 March and ending 18 April, 1987. Tissues were obtained from vines grown at the Orchard Mesa Research Center, Grand Junction, Colorado (elevation 1397 meters) or from a commercial vineyard 7.2 kilometers east of the research center. The climate of this region typically provides a growing season of 182 frost free days. Vines were of bearing age (6 and 11 years) and recommended viticultural practices (54) were used at both sites. The vines were grown on a deep Mesa clay loam soil with moderate fertility. The primary sampling criterion was cane maturity, based on tan or brown periderm color (17). Canes were from the exterior of the canopy and weak or vigourous "bull" canes were excluded as well as those with any apparent physical defects. The node or internode positions on the cane

from which the sample was taken were numbered from the base of the shoot. The numbers represent either a fruiting bud (node) or cane tissue (internode) which was adjacent to the respective node. The sample material for all artificial freezing tests consisted of cane cross sections split longitudinally in half measuring 1.5-2.0 cm in length and taken from the middle portion of the designated internode. Excised buds included subjacent nodal material up to 3 mm thick and 1.5 cm in length, as recommended by Quamme (41). The sample material for water content (Exp. 2) was the same except that cane cross sections were not split.

Thermal Analysis:

The apparatus used for measuring freezing events by thermal analysis (TA) was similar to that described by Wolf (53) and Andrews et.al. (4) and is shown in schematic diagrams (Fig. 1 and 2). Exotherms were detected by thermoelectric modules (TEM) from MELCOR, (Trenton, N J) which consisted of multiple semiconductor junctions connected in series and fastened between thin ceramic plates (Fig. 3). The TEM modules were designed as thermoelectric heat pumps, providing refrigeration capabilities from very low voltages such as those generated from solar cells. When being used as heat pumps, heat is absorbed at the cold face (Fig. 3) and is pumped to the hot face at a rate proportional to the current passing through the circuit and the number of junctions. The thermoelectric module used was an 18 junction FC series (FC 0.6-18-06L) which was 2.7 mm thick with cold face dimensions of 6.2 x 6.2 mm and hot face dimensions of 6.2 x 8.3 mm (Fig. 3). When being used as a temperature sensor, the live samples were placed on the cold face of the

TEM to provide maximum sensitivity temperature change. The TEMs were connected to an analog to digital converter (ADC-1) from Remote Measurement Systems, Inc., (Seattle, W) and the analog outputs from the eight TEMs were converted to digitized microvolt output. The ADC-1 was controlled by a portable micro-computer (TRS 80 model 100 Radio Shack) programmed to printout the microvolt values every 3.5 seconds. The microvolt printout was converted to temperature (°C) from the National Bureau of Standards thermocouple reference tables (49).

The sample chamber was a 0.75 kg aluminum block and lid (Fig. 2). The sample chamber acted as a temperature stabilizing heat sink. Insulating the sample chamber with styrofoam chips and a cardboard box resulted in a cooling rate of -17.5°C per hour, which has been used for grape thermal analysis (36). Ten equally spaced 15 by 37 mm holes were bored in the block and lined with 15 mm nalgene centrifuge tubes. The TEMs were positioned to hang by the 22 gauge copper lead wires through 3.17 mm drilled holes in the lid, centered over the nalgene lined bored holes. Each 22 gauge lead wire was soldered to 152 cm of 18 gauge copper wire and connected to the ADC-1.

A freezing run consisted of eight excised dormant grapevine buds or canes that were individually fastened with small strips of Parafilm™ to the cold face of the TEM, with dry tissue of similar mass fastened in the same manner to the hot face (Fig. 3). Bud and cane tissues were positioned such that their longitudinal cut surfaces were in contact with the ceramic face of the thermoelectric modules. Unless otherwise stated, the sample tissue was then innoculated with ice nucleation active (INA) bacteria (see Exp. 4) to promote the occurrence of HTEs distinct from LTEs. The eight prepared TEMs were inserted into the

individual bored holes of the block along with one 24 gauge type T thermocouple with dry tissue to measure internal block temperature. The insulated block was placed in an ultra-low Revco (model ULT-659) chest freezer which was held at -75°C. Freezer air temperatures were monitored with a 24 gauge type T thermocouple on the tenth channel of the data aquisition system. The sample chamber was removed from the freezer when sample temperatures reached -35°C, which typically required 3 hours.

Experiment 1: Hardiness of Cane and Bud Tissues in Relation to Pruning, Date and Position.

Bud and cane tissue of four different vines of 'Merlot' were sampled weekly during deacclimation (5 dates) and subjected to artificial freeze tests. Hardiness at four internode positions within a fruiting cane was examined. Positions 4, 8, 11, and 14 of each cane were tested throughout the experiment. To facilitate data entry and presentation of experiments 1 and 2, tissue positions 4, 8, 11, and 14 were respectively assigned numbers 1, 2, 3, and 4. The 5 sampling dates for experiments 1 and 2 were as follows: 21 March, 28 March, 4 April, 11 April, and 18 April, 1987. Pruned canes were cut 6 to 10 mm beyond position 14 one week prior to removal, based on results from experiment 5. A pruning interval of 7 days was used throughout the experiment to allow for any climatic or physiological changes that may occur during deacclimation. Between 6:45 and 7:30 AM on each date, four canes from each vine were removed, wrapped in plastic bags and if not immediately used, held at 1.0 to 2.0°C. Of the four canes removed, two canes (pruned and

unpruned) were used for bud samples and the other two canes (pruned and unpruned) were used for cane tissue samples. On each sampling date, four buds or canes were tested for hardiness within 56 hours of being removed from the vine. Each combination of node position and pruning treatment was replicated four times. The data were analyzed with SPSS (46) as a split plot, four factor factorial design with whole plot factors being "vine" and "date" and subplot factors being "pruning" and "position". Polynomial contrasts were run for any linearity that might be present.

The split plot four factor factorial was used throughout experiment 1 and 2, and herein will be referred to as the statistical analysis or analysis. Significance, if not otherwise stated, represents the $p = 0.05$ level.

Experiment 2: Water Content.

Subsequent to each freeze test in Exp.1, fresh weight of the same canes was determined by removing two buds or cane tissues adjacent to the respective freezing tissue positions, placing in glass vials and immediately weighing them. Tissues were then oven-dried for 36 hours at 75°C and reweighed. This duration was determined by the no change in weight method. Water content was calculated by difference after correction for vial weight and expressed as g water/g tissue dry weight. The data were analyzed as described in Exp. 1 with the response variable being water content.

Experiment 3: Refrigerated Storage.

Sample canes for experiment 1 were routinely cut at 7:00 AM and either subjected to artificial freeze tests immediately or wrapped in plastic bags and held at 1 to 2°C for up to 2 days. To determine if storage for this duration altered hardiness and therefore LTEs, a TA test compared buds of canes immediately cut verses stored canes. On 24 March, 1987 four different grapevines of the cultivar 'Semillon' were selected with two buds of each cane analyzed. Two canes of each vine were tested immediately and two after 56 hours of storage in plastic bags at 1-2°C. Bud LTEs were determined as in experiment 1. The data were analyzed with MSTAT (32) as a randomized complete block design.

Experiment 4: Inoculation with Ice Nuclei.

Inoculation with ice nuclei was necessary (52) and the approach used in all TA tests was to inoculate with ice nucleation active (INA) bacteria *Pseudomonas syringae* 'Cit 7'. This experiment was designed to quantify the effect of INA on the HTE temperatures in buds. Eight excised buds from a cane of the cultivar 'Semillon' (positions 4, 5, 7, 8, 10, 11, 13, 14) were grouped in pairs. Each pair consisted of adjacent buds of which one was inoculated immediately prior to freezing with an aqueous suspension of freeze dried INA bacteria (freshly prepared each day). The buds were subjected to artificial freezing tests and the temperature at which HTEs occurred was evaluated. The data were analyzed with MSTAT (32) as a randomized complete block design.

Experiment 5: Interval from Pruning to Freezing Test.

A test was performed to be confident that the 7 day interval used in experiment 1 didn't fail to detect some impact of pruning on hardiness that would have been detected by use of either shorter or longer intervals. Pruning intervals of 0, 3, 7, and 14 days were compared for their effect, if any, on supercooling. One cane from four different 'Merlot' grapevines was pruned 6 to 10 mm beyond bud 14 on each of the above time intervals prior to the freezing test. LTE values of bud 14 and stem segments of internode 13 of each treatment, the positions closest to the cut, were evaluated with MSTAT (32) as a two way ANOVA using vines as replicates.

Experiment 6: Temperature-Survival Curve (T_{50}).

Since all controlled freezing experiments tested tissue hardiness by use of Thermal Analysis (TA) using the data aquisition system previously described, a test to verify the method and system was needed. Once the TA system was shown to accurately detect exotherms, verifying that the exotherms do in fact indicate tissue hardiness was the next step.

The T_{50} viability test was based on the temperature-survival curve method (40), and was selected for comparison to LTE values in the TA method. The sample material for the T_{50} consisted of 100 mature "Merlot" canes that were randomly selected thoughout the 3 acre Price vineyard. The canes were trimmed to contain 8 buds within positions 4 to 14. Five canes comprised a bundle with 10 bundles per replicate.

experiment was performed twice (21 March and 1 April) with 2 replicates per date. Subsequently, on the same dates, four canes from 4 different vines were selected from the same vineyard for comparative analysis by the TA method. Buds 8 and 14 were evaluated. The bundles were cooled at 2°C/hr. Bundles were removed in 2° decrements bracketing a temperature predicted to be lethal to 50% of the primary buds. Buds were sectioned after being held for 12 hours at 2°C and 48 hours at room temperature and examined for primary bud viability on the basis of tissue browning (99). The temperature lethal to 50% of the sample population (LT^{50}) was determined by curve fitting, a process giving results similar to probit analysis.

Experiment 7: Water Content Uniformity.

Because of the importance of node position in studies in experiment 1, water content of buds and cane segments of the same cane was determined by using tissues other than those used for freezing runs (eg. positions 3 and 5 or 12 and 13). This was based on the fact that some bud scale or bark tissue from freezing runs used in experiment 1 would dislodge from hand manipulation during TEM attachment and fail to give accurate measurements. Using five `Merlot' grapevines, water content for both buds and canes was determined (as described in Exp. 2) for positions 4, 5, 6, 12, 13, and 14. The data were analyzed with MSTAT (32) as a randomized complete block design (using the five vines as replicates).

Experiment 8: Cultivar Comparison.

Low temperature exotherms of two different *Vitis vinifera* cultivars, 'Merlot' and 'Semillon' were monitered over four different dates during deacclimation. LTEs of both bud and stem tissue from position 8 were observed. Differences in hardiness and patterns in deacclimation between cultivars were compared (Fig. 8). The air temperature data was recorded on a CR21 Data Logger. The data were analyzed with MSTAT (32) as a randomized complete block 2 factor factorial split plot design.

Results and Discussion

Experiment 1: Hardiness of Cane and Bud Tissues in Relation to Pruning, Date and Tissue Position.

Prior to a consideration of the effect of pruning on hardiness it would be helpful to consider the influence of bud or internode position on the cane relative to hardiness over the five week time course of this study. The statistical design used was a split plot four factor factorial with whole plot factors being "vine" and "date" and subplot factors being "pruning" and "position". Polynomial contrasts were run to determine if any linear responses with these factors were present. For either bud or cane tissue no interactions involving pruning, date, and vine were observed so the analysis can be simplified by combining some factors when examining others. No independent vine effects were observed, either, so inter-vine variability or variability with interactions of the above need be given no further consideration. Exp. 1 procedures were validated by a series of tests (see Exp. 3, 4, 5, and 6 below).

Low Temperature Exotherms for both bud and cane tissue for 21 and 28 March, and 4, 11, and 18 April, 1987 are presented in Figures 4 and 5. Note the relatively greater influence of position on the cane for internode samples (Fig. 5) compared to buds (Fig. 4). Also note the much greater sensitivity of buds to the subfreezing temperatures

between 21 March and 28 March. To focus on the influence of each of the two factors (sampling date and cane position) on hardiness, Tables 1a and 1b list mean LTEs, combining all levels of one factor when analyzing the other. This simplification was possible due to the lack of significant interaction between the two. Each factor's influence on LTE was almost the same for either pruned or unpruned canes (interaction not significant) but pruning treatments are separated in these tables for completeness, since pruning is the main factor in this study.

Sampling Date Effects:

For cane tissue (Table 1a.) a clear deacclimation trend occurred, which the contrast analysis indicated was linear. The differences between early and later dates were significant ($p = 0.5$).

For grape buds, the hardiness was very dynamic during this deacclimation period. The statistical analysis for bud hardiness (Table 1b.) resulted in a significant separation between each sampling date and the next. March 28 was significantly different from all other dates and had the most negative mean LTE temperature (-23.2°C). This increase in hardiness compared to the previous week was not surprising following six days of unseasonably cold weather. This reacclimation phenomenon is not uncommon and has been observed with apple (43), peach (37), and grape (44). Reacclimation in grape was previously reported (44) to have occurred after a three day cold period in mid February with the cultivar 'White Riesling'. April 18 had the

most positive mean LTE temperature (-9.8°C), following the expected decrease in hardiness which accompanied seasonal warming of air and soil and visible bud swell.

In this study, both cane and bud tissue responded to weekly increases in air temperatures; however, bud tissue appeared to be more sensitive and responded more quickly than cane tissue. One can speculate that either cane tissue responds more slowly than bud tissues to fluctuations in air temperature or some other factor such as increasing photoperiod triggers the loss of hardiness in cane tissue. It has been reported that potted `Concord' grapevines, when given a night interruption of white light, were generally less hardy than those exposed to naturally decreasing daylengths (57).

Buds lost much of their capacity to supercool by early bud swell (18 April) when the LTEs began to disappear or were completely overshadowed by the HTEs which occurred between -5 and -8°C. This was not true with cane tissue, where the mean LTE for 18 April for both pruned and unpruned tissues was -18.2°C. They were beginning to lose hardiness rapidly though, judging from the 23 April measurements for Exp. 8 (see below). This demonstrates that canes are hardier than buds and retain the capacity to supercool further into the time of renewed growth. The combined overall mean for pruned and unpruned buds averaged over all dates was -16.4°C whereas cane tissue was -21.5°C. The fact that buds were less hardy than canes supports previous work with hardiness of `Concord' vines in mid winter (18). Cane hardiness measurements were not continued beyond this date since the information would be of only minor viticultural significance.

Loss of canes to freeze injury at this late developmental period is very unlikely and there are no known observations of impacts on hardiness from pruning then.

Positional Effects

Differences in hardiness along the cane were noted with stem tissue. Mean separation tests for the cane position factor (Table 1a.) showed that positions 1 and 2 (near the trunk) were slightly but significantly hardier than positions 3 and 4, which were more distal. Unlike cane tissue, significance between positions was not demonstrated with bud tissue (Table 1b.). Generally, the influence of position on hardiness appears to be more easily distinguished with canes than buds (Figures 4 and 5) and may be related in part to the overall higher water content of canes (see Exp. 2). From this study it can also be concluded that cane positions nearest the trunk (i.e. position 1) are slightly hardier than those further away, which is consistent with previous work involving grape bud tissue in the fall and mid winter (12, 17, 21, 56). This pattern, however, was not observed with bud tissue in this study and therefore cannot augment those previous observations. The positional bud hardiness observed may represent greater complexity of responsiveness to the environment during the deacclimation period (see Exp. 2).

Pruning Effects

The influence of pruning on cane and bud hardiness, the central subject being studied here, was found to be negligible. Pruned canes, averaging all dates and positions, had an LTE value of -21.6°C and

unpruned canes had a mean value of -21.4°C (Table 6). There was also no observed effect of pruning when each position was examined individually, including position 4, which was adjacent to the pruning cut.

The overall mean bud LTE (Table 7) was -16.5°C for pruned and -16.4°C for unpruned canes which did not differ significantly. This was also true for each cane position, averaged over the five dates; all mean LTEs were within 0.3°C of -16.45°C whether canes were pruned or not.

It was evident from this study that pruning did not significantly influence the loss of hardiness in either bud or cane tissue. Previous reports that pruning decreases hardiness of grape (14, 45, 56) were not confirmed in this study during the time period of greatest interest. It can be concluded from this study that during the time period covered, the hardiness of `Merlot' tissues was not influenced by pruning. This suggests that pruning could possibly begin at least five weeks earlier than currently recommended in seasons like 1987.

Experiment 2: Water Content

As in Exp. 1, it will be helpful to consider the influence of bud or internode position and water content over the five week time course prior to a consideration of the effect of pruning. The statistical design used was a split plot four factor factorial with whole plot factors being "vine" and "date" and subplot factors being "pruning" and "position". Polynomial contrasts were run to determine if any linear

responses with these factors were present. No independent vine effects were observed so inter-vine variability need be given no further consideration.

Percent water content (g water/g dry tissue) * (100) for both buds and canes on 21 and 28 March, and 4, 11, and 18 April, 1987 are presented in Figures 6 and 7. Note the relatively greater influence of cane position on water content of internode samples (Fig. 7) compared to buds (Fig. 6). Also note the much greater rate of increase of cane water content between 4 April and 18 April. To focus on the influence of each of the two factors (sampling date and cane position) on water content, Tables 2a and 2b list mean percent water content, combining all levels of one factor when analyzing the other. Pruning treatments are separated in Tables 3, 4, and 5 for completeness, since the analysis indicates an interaction involving pruning and position with bud tissue and a pruning by date interaction with cane tissue. The date and position effects were each identified by polynomial contrast as being linear. Water content in measured buds and canes may be properly equated with that of adjacent like tissues for purposes of relating it to hardiness, given the results of Exp. 7 (see below).

Sampling Date Effects

Cane water content changes during the five week time course were more dramatic and somewhat more complex than cane hardiness changes in Exp. 1. Percent water decreased 4.3% in cane segments at each position from 21 to 28 March (Fig. 7). This 4.3% nonsignificant decrease in cane water content (Table 2a.) was hypothesized to be a response to a six day cold period. Percent cane water content returned to a level

similar to 21 March on 4 April. This period of relatively stable water content reinforces the results of Wolpert and Howell (56) with 'Concord', where cane water content changed very little between 5 February and 5 April. However, this study revealed that from 4 to 18 April the cane water content for all four positions increased an average of 22.8% and differed significantly between all three dates (Table 2a.).

For buds, water content also underwent significant change with date (Table 2b). Buds on 21 March, had the highest water content (83.9%) and the 7.6% decrease on 28 March was significant and persisted for another week, unlike cane tissue (Table 2a). While bud water content did increase during the final two weeks, the 7.6% gain was much smaller than the 22.8% increase for canes. It may be that deacclimation of buds is associated with a redistribution of water or hydration of the very small bud axis only, rather than a large increase in bulk water content. The larger change during the first week was noteworthy, but probably of more relevance to the behavior of hardy buds during the winter than to the deacclimation process.

Positional Effects

The above observation that the five week time course apparently spanned two physiological processes, a dehydration response to low temperature and then rapid hydration after 4 April, suggests that when examining mean water content in relation to position it might be unwise to combine all five dates within each mean. Since this study focused on deacclimation, only the final three dates, 4, 11, and 18 April, 1987 were combined (Table 2a). The significant influence of cane internode position on water content is evident. Position 1 had the lowest mean

water content (92.9%) and it was higher at each distal position up to 4, which had the highest water content (102.7%). Position 4 also had the greatest increase in water content from 4 to 18 April (30%) and position 1 had the smallest increase (16.6%) (Fig. 7). Position 1 always had lower water content than all other positions, which is consistent with work by Wolpert (57).

The position of buds (Table 2b.) did not affect the water content significantly when averaged over date and pruning treatment. The range was 77.5 to 80.0%. It appears that bud water content is relatively independent of cane tissue water content. This parallels the earlier observation that bud hardiness did not differ with position even though cane hardiness did differ slighty (Table 1a.).

Pruning Effects

The above conclusions on the effect of bud and internode position on water content, where pruned and unpruned samples are combined, would have been the same whether all five dates or just the last three were averaged. With the smaller sample size when pruning treatments are separated (Tables 2a and 2b), the effect of combining data from the dehydration and hydration phases did alter the apparent effect of pruning on both buds and canes. The analysis of pruning effects by position (Tables 4 and 5) therefore used only the last three weeks of data. The overall effect of pruning averaged over all positions and the last three dates was to reduce cane water content 2.1% which was significant (Table 5). Pruning did result in a significant interaction with both position and sampling date. The single significant site of pruning influence was position 4, where pruned tissue had a 5.5% lower water content. That the bud nearest the pruning cut would experience

dehydration seems reasonable; however, it may be more correct in this case to say that pruning slows hydration, just as it appears to have slowed dehydration on 28 March (Table 3). Looking at pruning effect on cane water content over date, the most instructive statistic is the weekly change in water content of pruned verses unpruned canes (Table 3). Note that the change is the difference of each from the unpruned canes of the previous week, since that represents the population from which both unpruned and pruned canes were drawn. On 11 April, the date of most rapid hydration, water content of cane internodes increased an average of 16.6% compared to about half that amount for pruned canes. Then the hydration process slowed and pruning had no effect. Pruned canes were 8.1% lower in water content on 11 April than unpruned canes.

Pruning had no significant effect on bud water content when averaging all observations (Table 4, overall). However, pruned buds at each position were slightly lower in water content, and the difference was even significant at two positions. This may be a reflection of the overall 2.1% reduction in cane water content. The observation by Wolpert and Howell (56) that on 2 May the water content of buds of fall pruned vines with 8 node canes was 22% greater than those of buds from the same node position on unpruned vines appears to contradict this finding. However, it is likely that on canes which had been pruned for months that node 8 buds would behave like distal buds and hydrate sooner than node 8 buds on longer canes.

Conclusions

The time course of this study appeared to span two physiological processes. The first was a re-acclimation response to low temperature from the 21-28 March observations, which is more pertinent to dormant

bud and cane behavior, and the second, which began with the 4 April observations, was deacclimation. Apparently the deacclimation and hydration processes observed are responding to temperature and possibly photoperiod since daylength increased during this investigation. However, to more confidently determine whether these processes do respond to both environmental factors, a study quantifying these parameters in controlled environments would need to be developed.

Experiments 1 and 2 provide evidence that the two tissues studied (buds and canes) respond separately and can maintain differences in hardiness and water content. Cane tissue had lower LTEs than bud tissue on every date and at every position and overall (combining all dates and positions) was 5°C hardier than for buds. The water content for both tissues increased after 4 April and overall (combining all dates and positions) was 13.3% higher for canes than buds. Cane positions more distally oriented, whether canes were pruned or unpruned, were found to be slightly less hardy than those nearest the trunk. The change in cane hardiness levels with position although slight (Table 1a, P + U), was inversely related to the water content levels (Table 2a, P + U). This relationship between water content and hardiness of canes is consistent with previous reports (10, 24, 56) which support the hypothesis that as water content increases, hardiness decreases. For buds, position had no affect on either hardiness or water content of either the unpruned or pruned treatment (Tables 1b, and 2b). Pruning did not influence the loss of hardiness in either bud or cane tissue but did have a slight water content affect (slowing hydration) on cane tissue. On 11 April pruned canes were 8.1% lower in water content than unpruned canes.

This research provides a better understanding of tissue hardiness and water content of *Vitis vinifera* cv. 'Merlot' during the final stages of deacclimation and shows how little influence pruning has on these parameters.

Experiment 3: Refrigerated Storage

Buds from two positions on canes of the cultivar 'Semillon' were subjected to artificial freeze tests as in Exp. 1, except that a direct comparison of LTEs was made between freshly sampled buds and those stored at 1 to 2°C for up to 56 hours. The experiment was designed to duplicate the maximum storage time of 56 hours typically used throughout Exp. 1. The mean LTE value for the 24 March (fresh) tissue was -21.2°C whereas the 26 March (stored) tissue was -21.6°C. These were not significantly different ($p = 0.05$). Therefore, the 56 hour storage time required for adequate freezing test replication was not a source of error.

Experiment 4: Inoculation with Ice Nuclei

Tissue samples in all thermal analysis (TA) tests were inoculated with *Pseudomonas syringae* 'Cit 7'. This epiphytic Ice Nucleating Active (INA) bacteria is reported by Lindow (25) to be a very efficient ice nucleator, typically active between -2 and -5°C. The temperature at which ice formation is initiated within plant tissues is of considerable current interest (2, 7, 8, 25, 26, 27, 28, 38, 52) and was important in this study. Ashworth (7) suggests that seeding a tissue

not fully hardened (e.g. in early spring or early fall) with a nucleating agent prior to TA testing can result in a more direct reflection of the tissue's true capacity to deep supercool.

A study using paired `Semillon' buds was developed to quantify the influence of INA bacteria on High Temperature Exotherm (HTE) temperatures. Inoculation of `Semillon' buds with Ice Nucleating Active (INA) bacteria `Cit 7' significantly ($p = 0.05$) raised the mean bud HTE temperature from -9.2°C for the untreated buds to -6.4°C. This effect occurred at all four positions along the cane. The use of bacterial nucleators was therefore not only very effective but also a simpler method than those previously reported (7, 41, 55).

To conclude, nucleation of excised `Merlot' and `Semillon' bud/cane tissues with INA bacteria improved the ability to distinguish non-lethal HTE temperatures from lethal LTE temperatures. This enabled TA tests to be continued further into the onset of spring growth.

Experiment 5: Interval from Pruning to Freezing

This experiment was designed to identify a pruning interval for Exp. 1 most likely to influence hardiness but not so long as to allow recovery. The 3 and 14 day pruning intervals were tested 27 March and the 0 and 7 day intervals were tested 28 March, 1987. The LTE values obtained for all four pruning intervals did not differ significantly ($p = 0.05$) for either buds or canes. Since canes and buds were tested independently, their values were not compared statistically.

This experiment shows that the pruning interval was not critical for either canes or buds. An interval of 7 days was chosen for convenience.

Experiment 6: Temperature - Survival Curve (T_{50})

The object was to verify that the LTE temperatures detected by the data aquisition system (Fig. 1) do in fact represent tissue hardiness.

The T_{50} curve fitting method for the 21 March data resulted in a value of -18.9°C. A probit analysis, which is somewhat more objective, gave a T_{50} value of -19.0°C for the same data, validating the curve fitting approach. The median LTE temperature for the same date using the Thermal Analysis (TA) method was -16.5°C, a difference of 2.4°C from the T_{50} curve method.

The T_{50} curve fitting method for the 1 April data resulted in a value of -18.9°C, with a probit analysis value of -18.6°C. The median LTE temperature using the TA method for that date was -17.5°C, a difference of 1.4°C from the T_{50} method.

It was evident from this study that the T_{50} values did not precisely match the thermal analysis LTE values, but they were reasonably close. These results are similar to the report (3) that the T_{50} values of *Vitis vinifera* cv. 'White Riesling' were 1.3°C lower than the LTE values. Quamme (41) reports similar findings with several cultivars of grape. The consistently lower T_{50} values obtained in these studies may be due to the influence of cane tissue, which has considerably less mass in the TA method. Only a small section of stem tissue need be included with buds to achieve full expression of supercooling of grape (41) and peach buds (43) with the TA method,

while bundles of 10 four node canes are typically used with the T_{50} proceedure. Furthermore, freezer air temperature rather than tissue temperature was used as a guide for the T_{50} test. So it is probable that the TA method is a more meaningful measure of hardiness. Precision is good, much less tissue is required, execution time is less, and it is at the very least a very good measure of relative hardiness when comparing treatments or cultivars.

Experiment 7: Water Content Uniformity

Water content uniformity among specific positions within canes of 'Merlot' was investigated to determine whether the water content of a bud or cane internode used to measure hardiness could be equated with that of adjacent buds or internodes. Use of adjacent sites for Exp. 2 was necessary because hand manipulation could dislodge some bud scale or bark tissue from Exp. 1 samples during attachment to the thermoelectric sensors (TEMs), and introduce a source of error if the same tissues were used for measuring water contents. Bud and cane node/internode positions 4, 5 and 6 were compared, as were positions 12, 13, and 14. The results of the statistical analysis indicate that there were no significant differences in water content (g H_2O/g dry tissue) among either buds or canes from positions 4, 5, and 6. Bud and cane positions 12, 13, and 14 were also not significantly different at

the p = 0.05 level, providing confidence that node/internode positions adjacent to each other (Exp. 1 vs. Exp. 2) did not differ significantly in water content.

Experiment 8: Cultivar Comparison

This experiment was developed to generalize some conclusions drawn from Exp. 1 beyond the cultivar 'Merlot', since cultivar differences do exist (3). Hardiness differences of the *Vitis vinifera* cultivars 'Merlot' and 'Semillon' (Fig. 8) were compared over four dates (25 March, 8 April, 15 April, and 23 April 1987).

In both cultivars, buds lost hardiness faster than canes. Cane tissue was only slightly hardier than bud tissue on the first date, 25 March (mean cane LTE = -22.8°C vs. -21.7°C for buds). Hardiness of both tissues decreased by the final date, 23 April (mean cane LTE = -13.°C vs. -7.5°C for buds). These changes with time were significant for both 'Merlot' and 'Semillon'. Overall, canes appear to be the hardier tissue (mean cane LTE temperature for both cultivars = -17.5°C while the mean bud LTE temperature of both cultivars = -13.2°C) which parallels findings by Wolpert and Howell (55) that early winter, dormant grape buds are more susceptible to freezing injury than dormant canes. Given that buds are usually the more temperature sensitive of the two tissues, this study found little hardiness difference between 'Merlot' (mean LTE = -13.3°C) and 'Semillon' (mean LTE = -13.1°C) during this deacclimation period. However, a small but significant difference was observed with canes in that 'Merlot' tissue (mean LTE = -18.2°C) was hardier than 'Semillon' tissue (mean LTE = -16.9°C).

Figure 1.

A schematic of the Thermal Analysis (TA) data aquisition system used throughout the study. The analog sensor values (in microvolts) are converted to digitized output via the Analog to Digital Converter (ADC-1). A computer controls operation of the ADC-1 and printer.

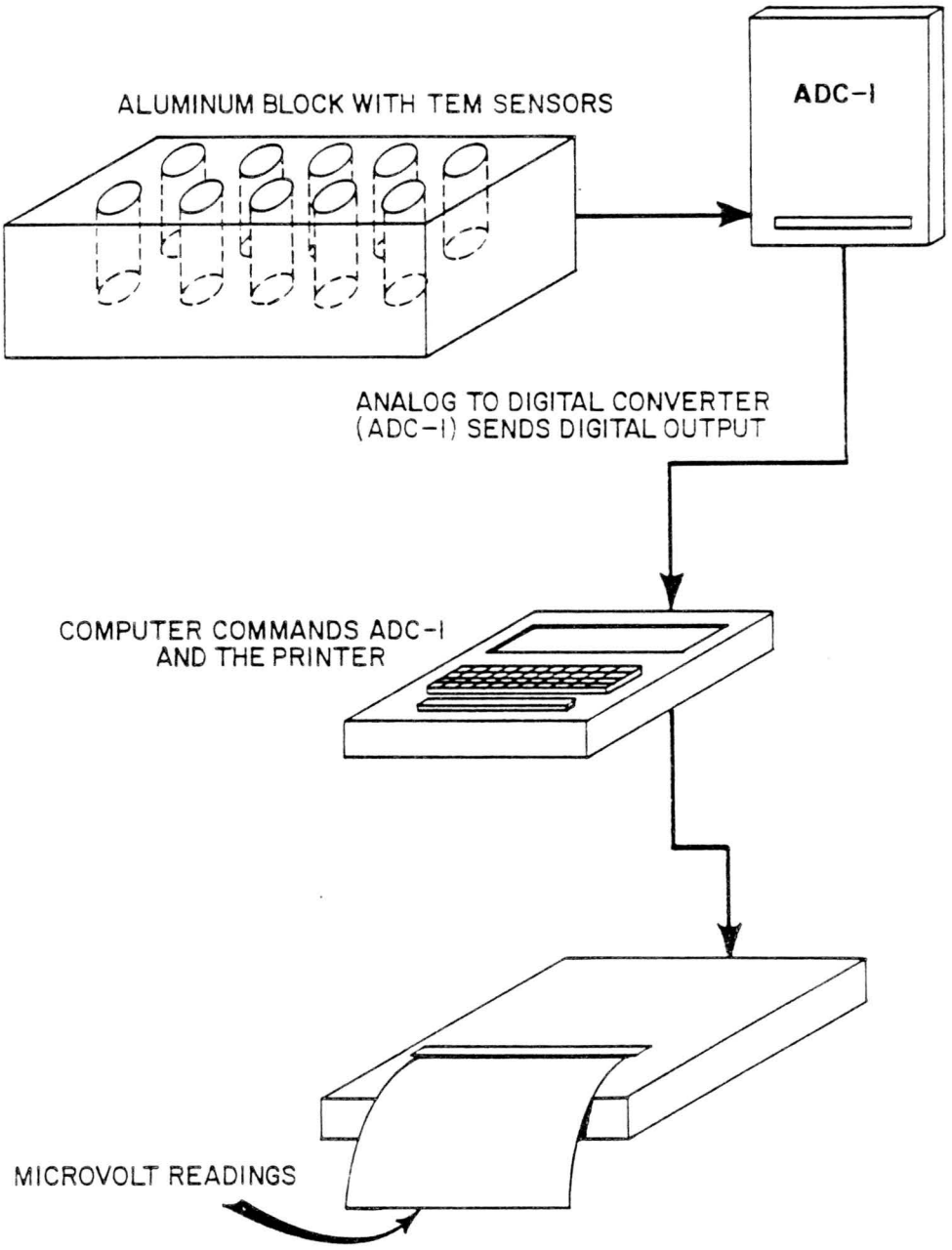

Fig. I. Schematic of data acquisition.

Figure 2.

A diagram of the ten hole aluminum block sample chamber used in all controlled freezing experiments. The Thermal Electric Modules (TEM's) are shown as they would appear before tissue attachment (Fig. 9).

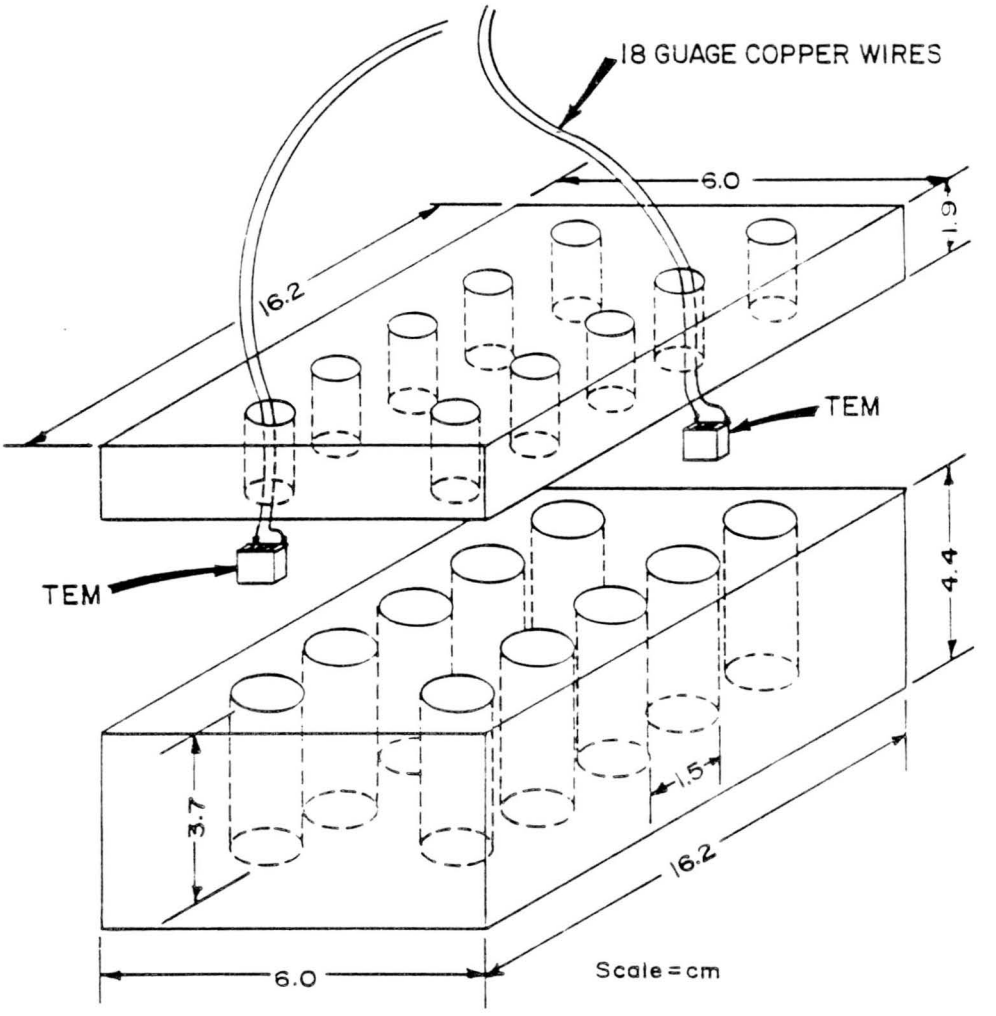

Fig. 2. Sample chamber.

Figure 3.

Tissue attachment to a Thermal Electric Module (TEM) sensor. Sample tissue is attached to the cold face of the TEM (see text) using parafilm.

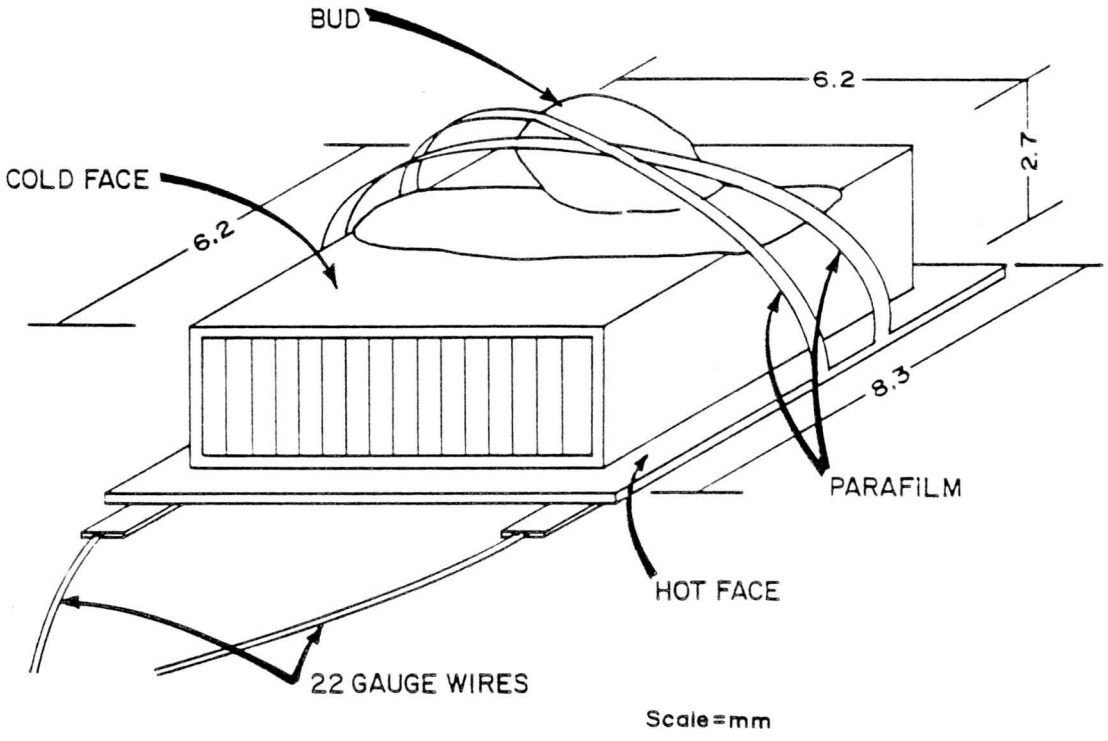

Fig. 3. Thermoelectric module with sample.

Figure 4.

Hardiness (LTE's, where values higher on the y axis are more negative and represent greater hardiness) of 'Merlot' grapevine primary buds. Buds from four positions along the cane were compared during a five week period (21 March to 18 April, 1987) when vines are typically deacclimating. Daily maximum and minimum air temperatures are also shown (T MAX. and T MIN.). Bud positions which were 4, 8, 11 and 14 nodes from the trunk are labelled and referred to in the text as bud positions 1, 2, 3 and 4 respectively. Pruned and unpruned cane tissues were combined and each point represents the mean of 8 replications.

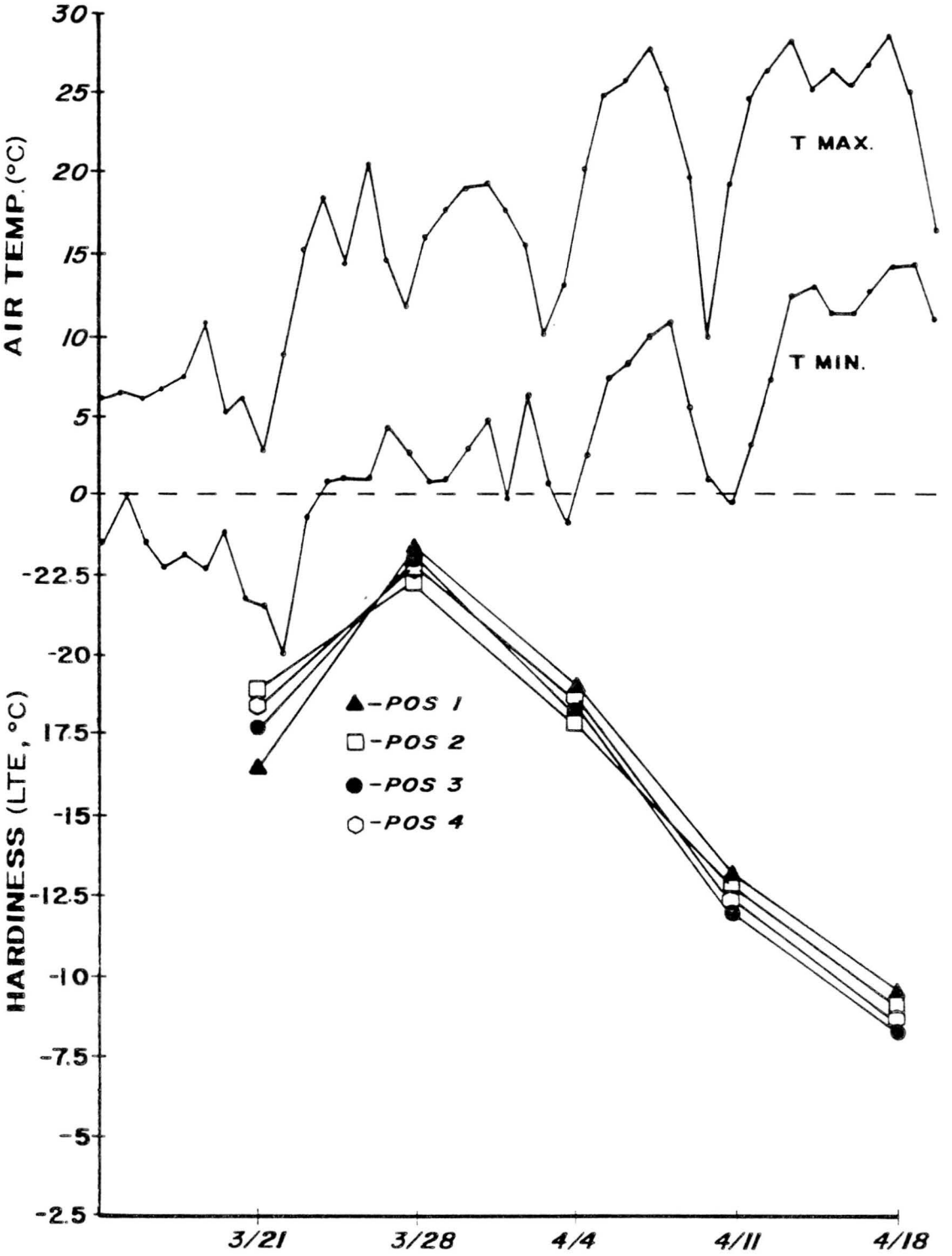

Fig. 4. Merlot primary bud hardiness, 1987.

Figure 5.

Hardiness (LTE's, where values higher on the y axis are more negative and represent greater hardiness) of 'Merlot' grapevine cane tissue. Cane internode tissue from four positions along the cane were compared during a five week period (21 March to 18 April, 1987) when vines are typically deacclimating. Daily maximum and minimum air temperatures are also shown (T MAX. and T MIN.). Internode positions which were 4, 8, 11 and 14 nodes from the trunk are labelled and referred to in the text as internode positions 1, 2, 3 and 4 respectively. Pruned and unpruned cane tissues were combined and each point represents the mean of 8 replications.

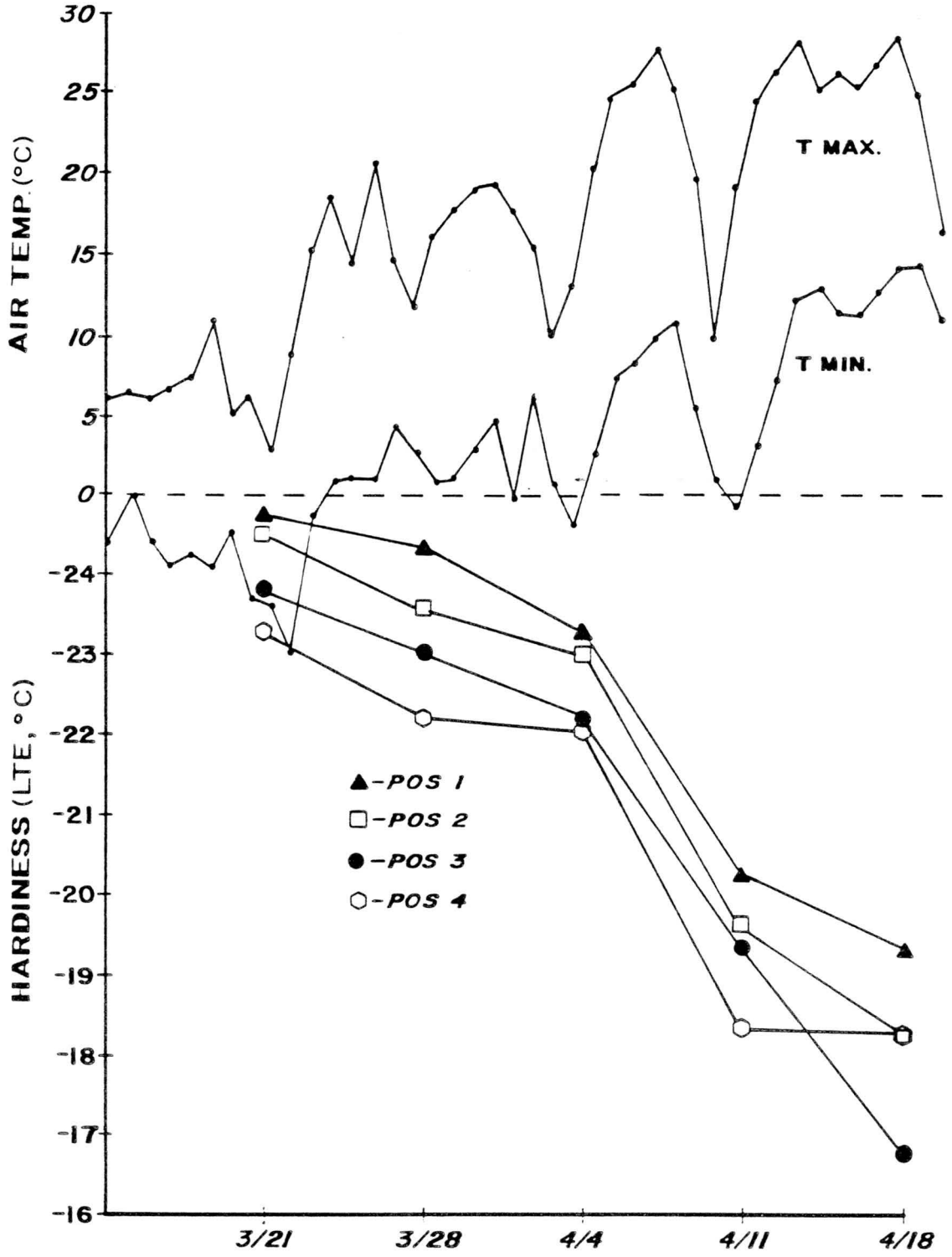

Fig. 5. Merlot cane hardiness, 1987.

Figure 6.

Bud percent water content (g water/g dry tissue) * (100) along four node positions of 'Merlot' grapevines during a five week deacclimation period (21 Mar. to 18 Apr., 1987), plus daily maximum and minimum air temperatures (T MAX. and T MIN.). Bud positions labelled 1, 2, 3, and 4 represent the average water content of pairs of buds adjacent to the bud used for freezing tests. Pruned and unpruned buds were combined and each point represents the mean of 8 replications.

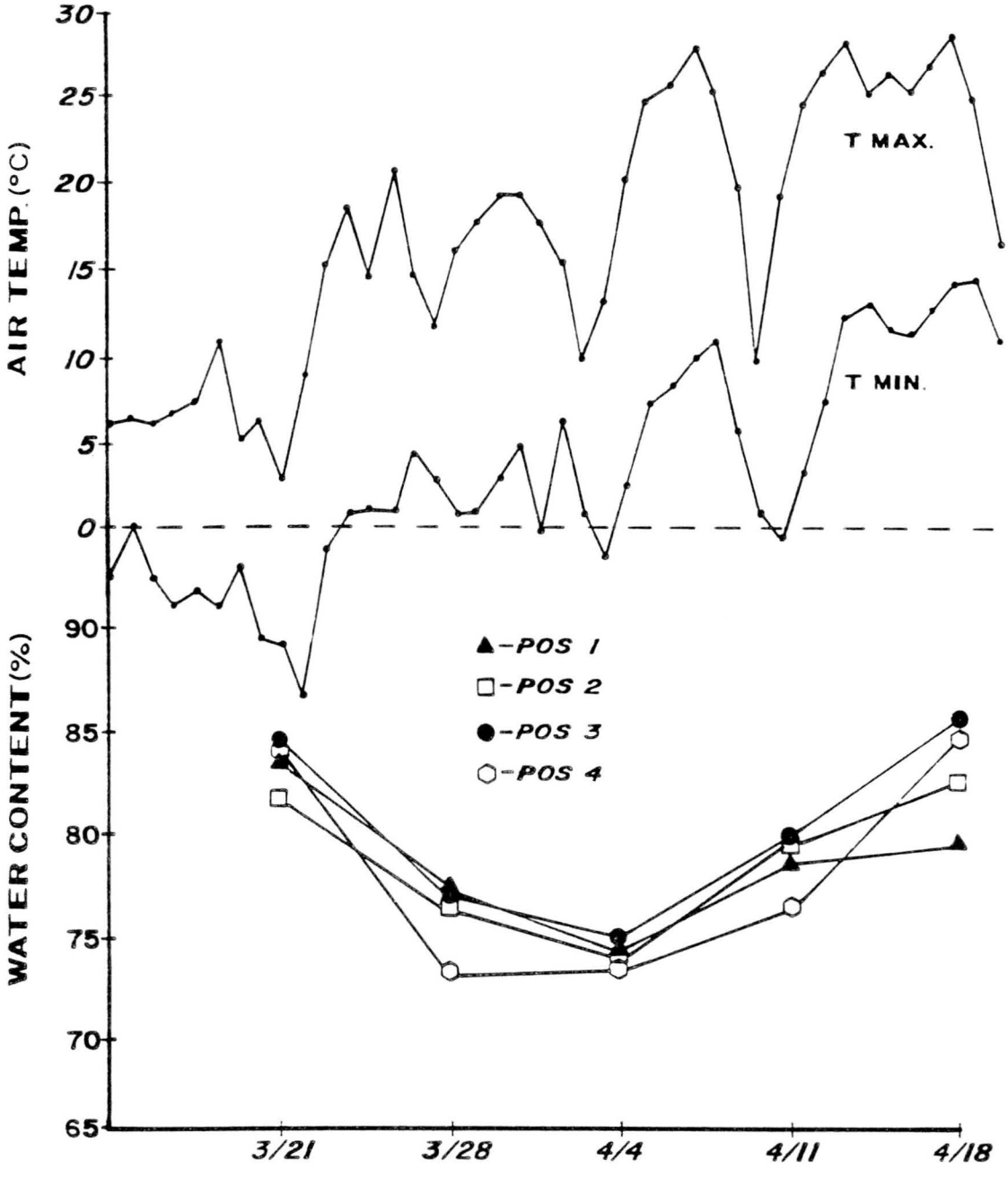

Fig. 6. Merlot bud water content, 1987.

Figure 7.

Cane percent water content (g water/g dry tissue) * (100) along four internode positions of 'Merlot' grapevines during a five week deacclimation period (21 Mar. to 18 Apr., 1987) plus daily maximum and minimum air temperatures (T MAX. and T MIN.). Cane positions labelled 1, 2, 3, and 4 represent the average water content of two internode segments adjacent to the canes used for the freezing tests. Pruned and unpruned cane tissues were combined and each point represents the mean of 8 replications.

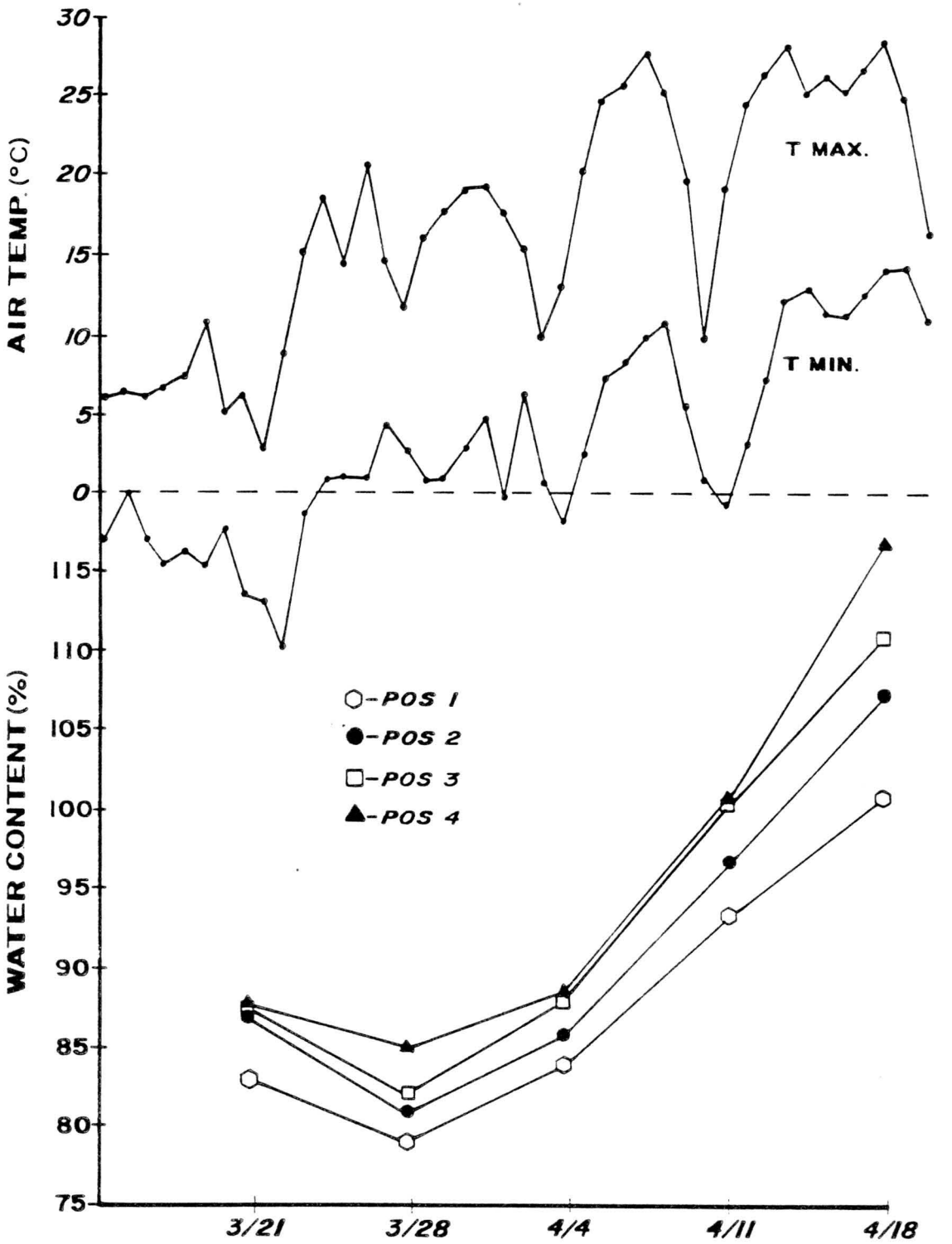

Fig. 7. Merlot cane water content, 1987.

Figure 8.

Bud and cane tissue hardiness (LTE's) of 'Merlot' and 'Semillon' grapevines during the deacclimation period of 25 March to 23 April, 1987 plus daily maximum and minimum air temperatures (T MAX. and T MIN.).

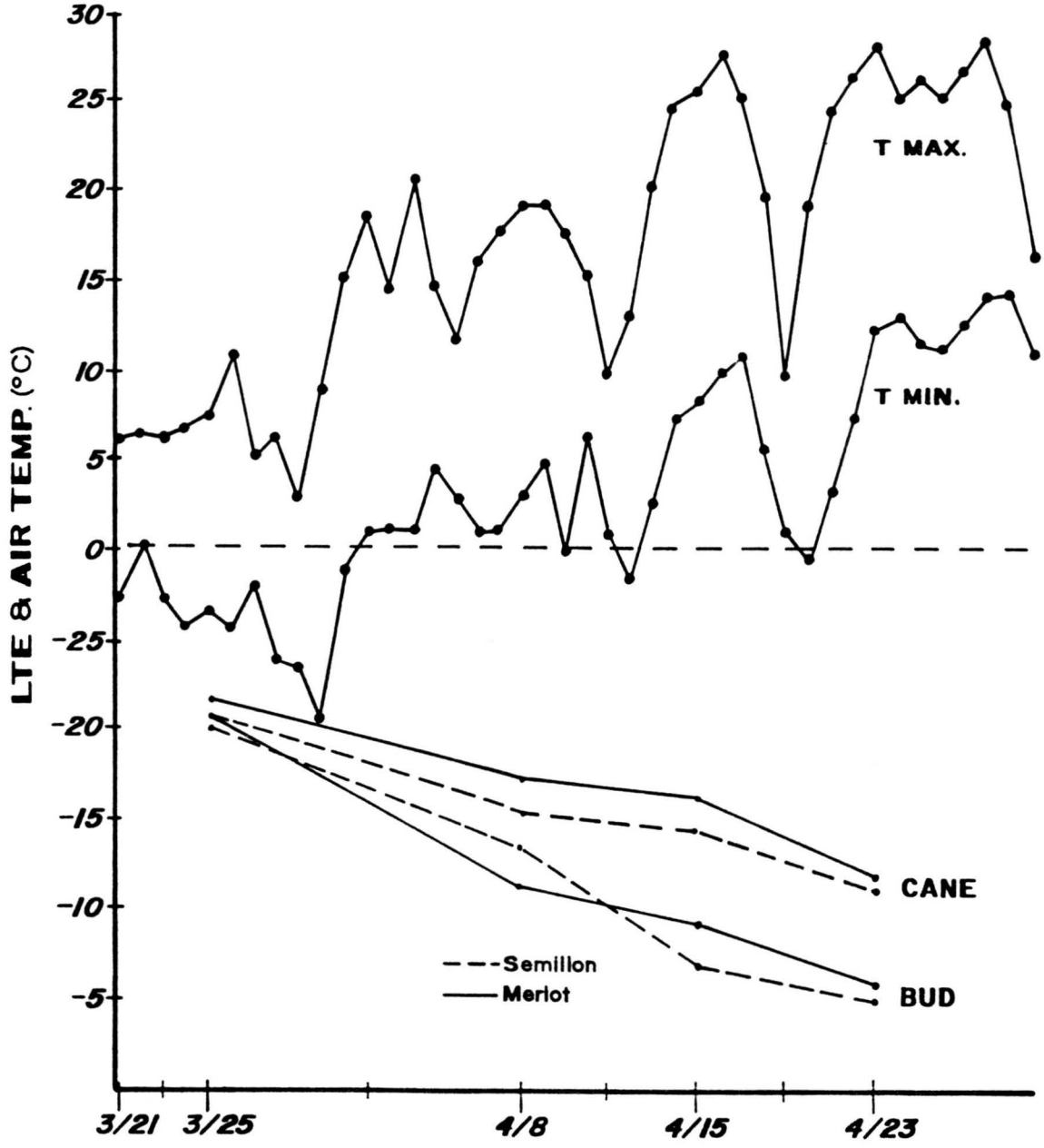

Fig. 8. Hardiness changes of Merlot and Semillon during deacclimation, 1987.

Table 1a. Hardiness expressed as mean Low Temperature Exotherm (LTE, °C) of 'Merlot' cane internodes by date and by position (1 = proximal, 4 = distal on the cane). On each date the Pruned, Unpruned, and averaged (P + U) means included observations from all four positions, so the P + U means each contain 32 observations. The P + U means for position contain 40 observations since they were likewise combined for all five dates.

	Cane Hardiness (LTE, °C)		
By Sampling Date			
Date	P + U	Pruned	Unpruned
21 Mar.	-24.1 c	-24.2 a	-24.0 a
28 Mar.	-23.3 bc	-23.9 a	-22.7 a
4 Apr.	-22.6 abc	-22.8 a	-22.5 a
11 Apr.	-19.3 ab	-19.2 a	-19.4 a
18 Apr.	-18.2 a	-18.1 a	-18.3 a
By Position			
Pos.	P + U	Pruned	Unpruned
1	-22.3 b	-22.2 b	-22.4 b
2	-21.9 b	-21.8 a	-21.9 b
3	-21.0 a	-21.3 a	-20.7 a
4	-20.9 a	-21.1 a	-20.6 a

Means within a section column with the same letter are not significantly different (p= 0.05), according to Duncan's multiple range test using F protection.

Table 1b. Hardiness, expressed as mean Low Temperature Exotherm (LTE, °C) of 'Merlot' buds by date and by position (1 = proximal, 4 = distal on the cane). The number of observations per mean were the same as in Table 1a.

Bud Hardiness (LTE, °C)			
By Sampling Date			
Date	P + U	Pruned	Unpruned
21 Mar.	-17.9 c	-18.0 b	-17.8 b
28 Mar.	-23.2 d	-23.1 c	-23.2 c
4 Apr.	-18.2 c	-18.2 b	-18.3 b
11 Apr.	-13.0 b	-13.0 a	-13.0 a
18 Apr.	-9.8 a	-9.2 a	-10.1 a
By Position			
Pos.	P + U	Pruned	Unpruned
1	-16.5 a	-16.6 a	-16.5 a
2	-16.4 a	-16.6 a	-16.3 a
3	-16.2 a	-16.5 a	-16.2 a
4	-16.5 a	-16.5 a	-16.5 a

Means within a section column with the same letter are not significantly different (p= 0.05), according to Duncan's multiple range test using F protection.

Table 2a. Mean percent water content ((g water /g dry tissue) * (100)) of 'Merlot' cane internodes by date and by position. On each date the Pruned, Unpruned, and averaged (P + U) means included observations from all four positions, so the P + U means each contain 32 observations. The P + U means for position contain 24 observations since they were averaged over the last three dates only.

	Cane Water Content (%)		
By Sampling Date			
Date	P + U	Pruned	Unpruned
21 Mar.	86.4 ab	85.7 a	87.7 ab
28 Mar.	82.1 a	83.7 a	80.5 a
4 Apr.	86.9 ab	87.9 ab	85.9 ab
11 Apr.	98.4 c	94.4 b	102.5 c
18 Apr.	109.7 d	109.6 c	109.9 c
By Position			
Pos.	P + U	Pruned	Unpruned
1	92.9 a	92.4 a	93.5 a
2	97.0 b	96.5 ab	97.7 ab
3	100.6 c	100.2 b	101.0 b
4	102.7 c	100.0 b	105.5 c

Means within a section column with the same letter are not significantly different (p= 0.05), according to Duncan's multiple range test using F protection.

Table 2b. Mean percent water content ((g water /g dry tissue) * (100)) of 'Merlot' buds by date and by position. On each date the Pruned, Unpruned, and averaged (P + U) means included observations from all four positions, so the P + U means each contain 32 observations. The P + U means for position contain 24 observations since they were averaged over the last three dates only.

	Bud Water Content (%)		
By Sampling Date			
Date	P + U	Pruned	Unpruned
21 Mar.	83.9 b	83.5 c	84.2 b
28 Mar.	76.3 a	75.6 a	76.9 a
4 Apr.	75.5 a	75.1 a	75.9 a
11 Apr.	78.5 a	77.9 a	79.0 a
18 Apr.	83.1 b	82.2 b	83.9 b
By Position			
Pos.	P + U	Pruned	Unpruned
1	77.5 a	77.2 a	77.8 a
2	78.8 a	76.9 a	80.7 a
3	80.0 a	79.3 a	80.8 a
4	78.4 a	77.4 a	79.4 a

Means within a section column with the same letter are not significantly different (p= 0.05), according to Duncan's multiple range test using F protection.

Table 3. Effect of pruning on cane water content ((g water/g dry tissue) * (100)) on five dates. Values were averaged over four cane positions and each represents the mean of 16 samples. Weekly change in water content, compared to unpruned canes the prior week, are shown in parenthesis.

	Cane Water Content and Weekly Change (%)				
Treatment	21 Mar.	28 Mar.	4 Apr.	11 Apr.	18 Apr.
Unpruned	87.7 a	80.5 a	85.9 a	102.5 a	109.9 a
Change		(-7.2)	(+5.4)	(+16.6)	(+7.4)
Pruned	85.7 a	83.7 a	87.9 a	94.4 b	109.6 a
Change		(-4.0)	(+7.4)	(+8.5)	(+7.1)

Means within a column with the same letter are not significantly different using an LSD test (p= 0.05).

Table 4. Effect of pruning on bud water content ((g water/g dry tissue) * (100)) at four positions. The values have been averaged over the last 3 dates and each value, other than overall, represents the mean of 12 samples.

	Bud Water Content (%)				
Treatment	Pos. 1	Pos. 2	Pos. 3	Pos. 4	Overall
Unpruned	77.8 a	80.7 a	80.8 a	79.4 a	79.7 a
Pruned	77.2 a	76.9 b	79.3 a	77.4 b	77.7 a

Means within a column with the same letter are not significantly different using an LSD test (p= 0.05).

Table 5. Effect of pruning on cane water content ((g water/g dry tissue) * (100)) at four positions. The values were combined over the last 3 dates and each represents the mean of 12 samples.

	Cane Water Content (%)				
Treatment	Pos. 1	Pos. 2	Pos. 3	Pos. 4	Overall
Unpruned	93.5 a	97.7 a	101.0 a	105.5 a	99.4 a
Pruned	92.4 a	96.5 a	100.2 a	100.0 b	97.3 b

Means within a column with the same letter are not significantly different using an LSD test (p= 0.05).

Table 6. Effect of pruning on cane hardiness levels at each of four cane positions. Values are expressed as mean Low Temperature Exotherm temperatures (LTE's, °C) and each represents the mean of 20 samples over five dates.

	Cane Hardiness (LTE, °C)				
Treatment	Pos. 1	Pos. 2	Pos. 3	Pos. 4	Overall
Unpruned	-22.4 a	-21.9 a	-20.7 a	-20.6 a	-21.4 a
Pruned	-22.2 a	-21.8 a	-21.3 a	-21.1 a	-21.6 a

Means within a column with the same letter are not significantly different using an LSD test (p= 0.05).

Table 7. Effect of pruning on bud hardiness at each of four cane positions. Values are expressed as mean Low Temperature Exotherm temperatures (LTE's, °C) and each represents the mean of 20 samples over five dates.

	Bud Hardiness (LTE, °C)				
Treatment	Pos. 1	Pos. 2	Pos. 3	Pos. 4	Overall
Unpruned	-16.5 a	-16.3 a	-16.2 a	-16.6 a	-16.4 a
Pruned	-16.6 a	-16.6 a	-16.5 a	-16.5 a	-16.5 a

Means within a column with the same letter are not significantly different using an LSD test (p= 0.05).

References

1. Agaoglu, Y. S. 1979. A study on the time of pruning grapevines. Zirrat Fakultesi Yaninlari (Ankara, Turkey). 723:63-68.

2. Anderson, J. A., E. N. Ashworth, and G. A. Davis. 1987. Nonbacterial ice nucleation in peach shoots. J. Amer. Soc. Hort. Sci. 112(2):215-218.

3. Andrews, P. K., C. R. Sandidge III, and T. K. Toyama. 1984. Deep supercooling of dormant and deacclimating Vitis buds. Amer. J. Enol. and Vitic. 35:175-177.

4. Andrews, P. K., E. L. Proebsting, and G. S. Campbell. 1983. An exotherm sensor for measuring the cold hardiness of deep supercooled flower buds by differential thermal analysis. Hortscience 18(1):77-78.

5. Andrews, P. K. and E. L. Proebsting. 1983. Differential thermal analysis and freezing injury of deacclimating peach and sweet cherry reproductive organs. J. Amer. Soc. Hort. Sci. 108(5):755-759.

6. Andrews, P. K., and E. L. Proebsting. 1987. Effects of temperature on the deep supercooling characteristics of dormant and deacclimating sweet cherry flower buds. J. Amer. Soc. Hort. Sci. 112(2):334-340.

7. Ashworth, E. N. and G. A. Davis. 1987. Influence of ice nucleation temperature on the freezing of peach flower buds. Hortscience 22(5):923-925.

8. Ashworth, E. N. and G. A. Davis. 1984. Ice nucleation within peach trees. J. Amer. Soc. Hort. Sci. 109(2):198-201.

9. Biermann, J., C. Stushnoff, and M. J. Burke. 1979. Differential thermal analysis and freezing injury in cold hardy blueberry flower buds. J. Amer. Soc. Hort. Sci. 104(4):444-449.

10. Burke, M. J., L. V. Gusta, H. A. Quamme, C. J. Weiser, and P. H. Li. 1976. Freezing and injury in plants. Ann. Rev. Plant Physiology 27:507-528.

11. Burkholder, C. L. 1936. December pruning in 1935 results in severe injury to 'Jonathon' and 'Stayman' trees in Lafayette, Indiana. Proc. Amer. Soc. Hort. Sci. 34:49-51.

12. Byrne, M. E. and G. S. Howell. 1978. Initial response of Baco Noir grapevines to pruning severity, sucker removal, and weed control. Amer. J. Enol. Vitic. 29:192-198.

13. Damborska, M. 1978. The effect of higher winter temperatures on changes of the frost resistance of grapevine buds. Vitis 17: 341-349.

14. Edgerton, L. J. and N. J. Shaulis. 1953. The Effect of time of pruning on cold hardiness of Concord grape canes. Proc. Amer. Soc. Hort. Sci. 62:209-213.

15. Fuller, A. S. 1894. The Grape Culturist. Orange Judd Company, New York.

16. Graham, P. R. and R. Mullin. 1976. The determination of lethal freezing temperatures in buds and stems of deciduous azalea by a freezing curve method. J. Amer. Soc. Hort. Sci. 101(1):3-7.

17. Howell, G. S. and N. J. Shaulis. 1980. Factors influencing within-vine variation in the cold resistance of cane and primary bud tissues. Amer. J. Enol. Vitic. 31:158-161.

18. Howell, G. S., B. G. Stergios, and S. S. Stackhouse. 1978. Interrelation of productivity and cold hardiness of Concord grapevines. Amer. J. Enol. Vitic. 29:187-191.

19. Howell, G. S. and J. A. Wolpert. 1978. Nodes per cane, primary bud phenology, and spring freeze damage to Concord grapevines. A preliminary note. Amer. J. Enol. Vitic. 29:229-233.

20. Howell, G. S. and C. J. Weiser. 1970. Fluctuations in the cold resistance of apple twigs during spring dehardening. J. Amer. Soc. Hort. Sci. 95(2):190-192.

21. Johnson, D. E. and G. S. Howell. 1981. The effect of cane morphology and cultivar on the phenological development and critical temperatures of primary buds on grape canes. J. Amer. Soc. Hort. Sci. 106(5):545-549.

22. Junttila, O., C. Stushnoff, and L. V. Gusta. 1983. Dehardening in flower buds of saskatoon-berry, *Amelanchier alnifolia*, in relation to temperature, moisture content, and spring bud development. Can. J. Bot. 61:164-170.

23. Kaku, S. and M. Iwaya. 1978. Low temperature exotherms in xylems of evergreen and deciduous broad leaved trees in Japan with reference to freezing resistance and distribution range. In: Plant Cold Hardiness and Freezing Stress. (eds. P. H. Li and A. Sakai), pp.227-239. Academic Press, New York.

24. Levitt, J. 1980. Responses of Plants to Environmental Stress. Vol.I. Academic Press, Orlando, Florida. 497 pp.

25. Lindow, S. E., D. C. Arny, and C. D. Upper. 1978. The role of bacterial ice nuclei in frost injury to sensitive plants. In: Plant Cold Hardiness and Freezing Stress. (eds. P. H. Li and A.Sakai), pp.249-263. Academic Press, New York.

26. Lindow, S. E., D. C. Arny, and C. D. Upper. 1978. Distribution of ice nucleation-active bacteria on plants in nature. Applied and Environmental Microbiology 36(6):831-838.

27. Lindow, S. E. and J. H. Connell. 1984. Reduction of frost injury to almond by control of ice nucleation active bacteria. J. Amer. Soc. Hort. Sci. 109(1):48-53.

28. Lindow, S. E., S. S. Hirano, W. R. Barchet, D. C. Arny, and C. D. Upper. 1982. Relationship between ice nucleation frequency of bacteria and frost injury. Plant Physiology 70:1090-1093.

29. Loomis, N. H. 1939. Note on grape foliation as affected by time of pruning. Proc. Amer. Soc. Hort. Sci. 37:653-654.

30. Magoon, C. A., and I. W. Dix. 1941. Relation of pruning to performance of grapes. Proc. Amer. Soc. Hort. Sci. 38:369-372.

31. Mckenzie, J. S., C. J. Weiser, and P. H. Li. 1974. Changes in water relations of *Cornus stolonifera* during cold acclimation. J. Amer. Soc. Hort. Sci. 99(3):223-228.

32. Nissen, O. and P. Wolberg. 1985. MSTAT: A microcomputer program for the design, management, and analysis of agronomic research experiments (version 3.0). Michigan State University, E. Lansing. 340 pp.

33. Olien, C. R. 1974. Energies of freezing and frost desiccation. Plant Physiology 53:764-767.

34. Olien, C. R. 1967. Freezing stresses and survival. Annu. Rev. Plant Physiology 18:387-408.

35. Perold, A. I. and B. A. Phil. 1927. A Treatise on Viticulture. Macmillan and Co., Limited. 335 pp.

36. Pierquet, P. and C. Stushnoff. 1980. Relationship of low temperature exotherms to cold injury in *Vitis riparia* Michx. Amer. J. Enol. Vitic. 31:1-6.

37. Proebsting, E. L. 1963. The role of air temperatures and bud development in determining hardiness of dormant Elberta peach fruit buds. J. Amer. Soc. Hort. Sci. 83:259-269.

38. Proebsting, E. L., P. K. Andrews, and D. Gross. 1982. Supercooling young developing fruit and floral buds in deciduous orchards. Hortscience 17(1):67-68.

39. Proebsting, E. L., M. Ahmedullah, and V. P. Brummund. 1980. Seasonal changes in low temperature resistance of grape buds. Amer. J. Enol. Vitic. 31:329-336.

40. Proebsting, E. L. and H. H. Mills. 1966. A standardized temperature-survival curve for dormant Elberta peach fruit buds. J. Amer. Soc. Hort. Sci. 89:85-90.

41. Quamme, H. A. 1986. Use of thermal analysis to measure freezing resistance of grape buds. Can. J. Plant Sci. 66:945-952.

42. Quamme, H. A. 1983. Relationship of air temperature to water content and supercooling of overwintering peach flower buds. J. Amer. Soc. Hort. Sci. 108(5):697-701.

43. Quamme, H. A. 1978. Mechanism of supercooling in overwintering peach flower buds. J. Amer. Soc. Hort. Sci. 103(1):57-61.

44. Schnabel, B. J. and R. L. Wample. 1987. Dormancy and cold hardiness in *Vitis vinifera* cv. White Riesling as influenced by photoperiod and temperature. Am. J. Enol. Vitic. 38(4):265-272.

45. Shaulis, N. 1971. Vine hardiness: A part of the problem of hardiness to cold in N. Y. vineyards. Proc. N.Y. St. Hort. Soc. 116:158-167.

46. SPSS Inc. 1986. SPSS^x User's Guide, second edition. McGraw-Hill Book Co., New York. 988 pp.

47. Stergios, B. G. and G. S. Howell. 1977. Effects of defoliation, trellis height, and cropping stress on the cold hardiness of concord grapevines. Amer. J. Enol. Vitic. 28:34-42.

48. Stergios, B. G. and G. S. Howell. 1973. Evaluation of viability tests for cold stressed plants. J. Amer. Soc. Hort. Sci. 98(4):325-330.

49. Thermocouple Reference Tables. 1979. N.B.S. Monograph 125. National Bureau of Standards, Washington, D.C.

50. Weaver, R. J. 1976. Grape Growing. John Wiley & Sons, Inc., New York. 371 pp.

51. Winkler, A. J., J. A. Cook, W. M. Kliewer, and L. A. Lider. 1974. General Viticulture. University of California Press, Berkley. 710 pp.

52. Wolf, T. K. 1986. Effects of rootstock and nitrogen fertilization on the growth, yield, tissue nitrogen concentrations, and dormant bud cold hardiness of "Chardonnay" (*Vitis vinifera* L.) grapevines. Ph.D. Thesis, Cornell University.

53. Wolf, T. K. and R. M. Pool. 1986. Microcomputer-based differential thermal analysis of grapevine dormant buds. Hortscience 21(6):1447-1448.

54. Wolfe, W. 1979. Minimizing winter damage: Site selection, vineyard establishment, and maintenance. Proc. Wash. St. Grape Soc. 9:67-75.

55. Wolpert, J. A. and G. S. Howell. 1985. Cold acclimation of Concord grapevines. II. Natural acclimation pattern and tissue moisture decline in canes and primary buds of bearing vines. Amer. J. Enol. Vitic. 36:189-194.

56. Wolpert, J. A. and G. S. Howell. 1984. Effects of cane length and dormant season pruning date on cold hardiness and water content of Concord grapevines. Amer. J. Enol. Vitic. 35:237-241.

57. Wolpert, J. A. 1983. Cold acclimation of Concord grapevines. Ph.D. Thesis, Michigan State University.

58. Yu, K. S., M. K. Rogoyski, and A. R. Renquist. 1986. Peach Orchard Management. Agric. Exper. Sta. Technical Report TR86-7, Colorado State University, Fort Collins.