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Michigan State University PH.D. 1983

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COLD ACCLIMATION OF 'CONCORD' GRAPEVINES

By

James A. Wolpert

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ABSTRACT

COLD ACCLIMATION OF 'CONCORD' GRAPEVINES

By

James A. Wolpert

Cold acclimation of 'Concord' grapevines (Vitis labruscana Bailey), investigated in Michigan during late summer and fall of three years, began in early August and continued to increase gradually through early November. Hardening occurred first in basal bud and shoot tissues (nodes 2-4), followed in order by middle (nodes 6-8) and apical (nodes 10-12) tissues. Increased hardiness to about -13°C in the early portion of the acclimation period (through late September) was related to loss of tissue water, but later hardiness increases were unrelated to water content. Increased hardiness was also positively related to shoot exposure to sunlight and advanced tissue maturation, the latter evidenced by a change in shoot color from green to brown. The relationship of cold hardiness, shoot maturation, and tissue water content is discussed.

Potted vines exposed to night-interrupted (NI) photoperiods had a greater percentage of shoots with active growing points and a greater number of nodes per shoot, when compared to plants exposed to naturally decreasing daylength in late summer and fall. Apical tissues of plants under NI photoperiods showed slightly inhibited

cold acclimation but NI treatment did not prevent full acclimation. Root resistance, measured as suction-induced water flow through detopped root systems, increased throughout the acclimation period, but was not influenced by NI photoperiod. Root resistance, measured at 500-590 torr, was significantly affected by premeasurement handling such as soil removal or low pressure pretreatment (5 min at 150 torr).

Differential thermal analysis (DTA) of freezing in acclimating shoot segments indicated that changes in tissue maturation were accompanied by changes in freezing pattern. During acclimation DTA profiles changed from that of a single bulk water exotherm to one showing 3 distinct exotherms.

This dissertation is dedicated to my parents

Louis and Vera Wolpert

for their love and unfailing support

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Finally, I wish to express a deep sense of gratitude to my advisor and friend, Stan Howell, for his guidance, counsel, and inspiration during my development as a scientist. About him I can say what Jacques Monod said of his mentor, Andre Lwoff--he is a man ". . . whose example I have done no more than try to follow, from whom I have learned that the sharpest criticism can also be generous and that the severest scientific exactitude, rather than forbidding actually authorizes and encourages enthusiasm for the boldest speculations."

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INTRODUCTION

In temperate climates, perennial plants must be adapted to winter temperatures if they are to survive and flourish. Inadequate cold hardiness results in cold injury and leads to the destruction of plant tissues which are important to crop production. The importance of winter survival can be seen from the fact that nearly 14% of all crop losses in the United States over the past 40 years were due to cold temperatures (6). Of interest to agriculturists is how survival is accomplished and how injury can be prevented.

The experiments comprising this dissertation constitute an effort to understand how plants become adapted to low temperature stress. Experiments are arranged in sections, each of which addresses an aspect of cold acclimation of grapevines. Each section has its own introduction, description of experiments, and discussion of results and it is intended that each will be submitted later to a journal for publication, after condensation and style modification.

The purpose of the literature review, which follows, is to present a detailed background in several areas of cold acclimation of woody plants. Not all of the topics discussed in the review will be addressed in this dissertation. Rather, it is hoped that the review will provide a general frame of reference against which the experiments and conclusions can be judged.

Reviewing literature and drawing general principles about cold acclimation from diverse studies is complicated by several considerations.

1. Plant species used in various studies differ dramatically in their abilities to acclimate to cold; herbaceous plants gain very limited resistance, while some woody plant tissues can acclimate to survive immersion in liquid nitrogen (-196°C). The mechanisms by which these plants avoid or tolerate freezing stresses may be quite different and unrelated.

2. The method of exposure of plants to low temperatures (either field or laboratory conditions, at rates which vary by 2 orders of magnitude) may influence the amount of injury.

3. Evaluating injury by different means, such as regrowth, tissue browning, solute leakage, etc., may preclude meaningful comparison of reuslts from study to study. Recognition of the above difference is crucial in avoiding erroneous conclusions.

LITERATURE REVIEW

Woody plants actively growing in summer have little innate cold hardiness. Current season's succulent growth may survive a freeze of only a few degrees below 0°C. In late summer and early fall these tissues undergo a dramatic increase in cold resistance so that by early winter, tissues are able to tolerate temperatures of -40°C and below.

This increase in hardiness, termed cold acclimation, has been studied in detail and will be discussed in several sections: (1) acclimation of plants under field conditions, (2) acclimation of plants under artificial conditions, (3) changes in tissues accompanying acclimation, and (4) ecological aspects of acclimation.

Acclimation of Plants under Field Conditions

Increases in cold hardiness which occur under natural field conditions have been monitored in a number of plants by use of frequent, artificial freezing tests. Hardiness begins to increase in late summer or early fall, in most cases well in advance of freezing temperatures. In one-year-old shoots of Cornus (127) and apple (42), the increase in hardiness occurs in two stages, separated by a plateau of several weeks. Hardiness in the first stage reaches

-15° to -25°C while in the second stage it is from -50° to below -90°. Increases in hardiness in the second stage occur very rapidly, as much as 60 to 70°C in a 2- or 3-week period (127), and coincide with the occurrence of the first freezing temperatures in the fall (42). Acclimation appears to occur in only one stage in living bark of black locust (108) and stems of Pinus contorta (97) and Hedera helix (112). Differences in the pattern of cold acclimation can be seen in ecotypes and races of several species and these will be discussed in a later section.

Acclimation under Artificial Conditions

Research to determine the physiological mechanisms underlying cold acclimation has concentrated on manipulating regimes of light, temperature, and water under controlled environment conditions. These factors will be discussed separately.

Photoperiod

The results of many experiments with altered photoperiod is a generally accepted hypothesis that the first stage of cold acclimation is induced by short days (SD) (28). The SD effect is best expressed if accompanied by warm temperatures (ca. 20°C) (28), implying that important metabolic changes are taking place. When plants growing under long days (LD) (16 hr) and warm temperatures are transferred to a SD regime, 4 to 5 weeks are needed to induce the SD effect (28, 132). Leaves are the site of reception (28, 48). Although the leaves do function as a simple trigger (48), there is

also a required photosynthetic function (114). Very short photoperiods (less than 6 hr) (75) or low light intensities (122, 125) inhibit full hardiness development. However, in Hedera helix, light-enhanced acclimation is not due to accumulated photosynthetic products (112).

Early speculations that cold acclimation was mediated by photochrome (48, 126) were subsequently borne out. In plants which respond to SD, acclimation is inhibited by interruption of of the long night by red light (76, 122, 125, 129). Indications that the amount of inhibition is related to the intensity of the interruption (75) suggests that the effect may be more complex than the more common "on-off" photochrome response.

Further information about mechanisms of hardening has been gained from studies involving plants modified by the following treatments: defoliation, girdling, grafting together genotypes which differ in their acclimation response, and by exposing portions of plants to different environments (split plants). Results from these studies indicate that the SD-leaf produces a hardiness promoter (27, 42, 49) which moves in the phloem (27) and which can induce hardiness in portions of a split plant under noninductive conditions (42). The LD-leaf is also the source of a hardiness inhibitor, as evidenced from experiments in which defoliation of the LD side of a SD/LD split plant gave a hardiness increase (42, 49).

Despite these demonstrations of SD promotion and LD inhibition, LD conditions have failed in several studies to prevent

eventual acclimation under field (76) and controlled environment conditions (42, 50, 124).

The critical daylength which triggers the SD response differs among species and ecotypes. In red-osier dogwood, a 12-hr photoperiod is SD for the clone from Dickenson, North Dakota, but is LD for the clone from Seattle, Washington (28). Growth cessation in ecotypes of Norway spruce from high latitudes is triggered when plants are shifted from continuous light (24 hr) to "short days" of 16-20 hr (35).

Temperature

Low temperatures (LT) are also effective in inducing cold hardiness (99). In plants which respond to SD, LT (15°/5°C, day/night) regimes confer an additional increase in cold hardiness, but only after completion of the SD response (127). The addition of 1 hr of freezing temperatures (-2 to -5°C) results in maximum hardiness (34). In those plants, LT during LD can give hardiness increases, but only to about -10° (127). However, in some plants, gradually decreasing temperatures can alone result in maximum hardiness (33, 113).

The LT effect is oxygen-requiring (34, 37) and is best elicited if it occurs in the dark rather than the light portion of the photoperiodic cycle (132). However, light *per se* is not required, provided that plants have sufficient carbohydrates available (49, 112). Unlike the photoperiodic response, the LT

effect cannot be translocated from the cold half to the warm half of a split plant (42).

Neither SD nor LT can induce cold hardiness when plants are undergoing active growth (127) and one aspect of the SD response is to cause shoot growth cessation by terminal bud set (129). Although it is often suggested that growth cessation is a prerequisite to cold acclimation (81, 128), there is no evidence to support the notion that the two are necessarily at odds at all times. In fact, Levitt (61) cites a French reference as concluding that the base of an actively growing shoot can, by lignification, become cold acclimated while the tips remain cold tender.

Prior to natural acclimation, buds enter physiological rest (110, 127), but entry into rest is not a prerequisite to acclimation (50, 122).

Water

Subjecting potted plants of red-osier dogwood to water shortage increases hardiness from 6° to 10°C over controls, regardless of photoperiod (19, 10), but after plants are returned to well-watered conditions for 7 days, the LD plants lose stress-induced hardiness while SD plants retain it (85). Although SD treatments do alter water relations of dogwood (77, 84) (discussed in a later section), the effects of SD and water stress are apparently independent and additive (18). Maximum hardiness can be achieved without soil water stress (28), and in fact, the wet, marshy natural habitat of dogwood does not apparently inhibit its hardening. On the

other hand, in Douglas-fir, even though water stress enhances growth cessation, it actually inhibits both photoperiodic- and temperature-induced hardiness increases (121). This suggests that a severe water stress may inhibit metabolic changes required for acclimation.

Changes in Tissue Accompanying Acclimation

During cold acclimation, many changes take place in plants and these have been investigated to determine which, if any, are causally related to cold hardiness increases. Cellular constituents which have been examined include: sugar (68, 83); starch (68) (including starch-sugar interconversions [25]); proteins (8, 33, 65, 68); free amino acids (68); organic phosphate (69); nucleic acids (9, 33, 34, 65, 66, 105), phospholipids (109, 131); fatty acyl chains (56); and glycolipids (130).

Although it is largely agreed that sugars do not offer much cold protection by colligative action, hardiness increases from infiltration of millimolal quantities of sugar (112) suggests a special protective role, which may include hydrogen bonding to sensitive proteins in membranes (103). Demonstration of this protection is lacking in woody plants which develop resistance to very low temperatures.

Studies on the living bark of black locust have led to conclusions that increases in soluble proteins are most closely related to acclimation (107). The source of the protein precursors may be senescing leaves (111), but demonstration of soluble protein

increases in leaves of evergreen boxwood reduces the likelihood that the changes are due to leaf senescence, unrelated to acclimation (33). Also seen are very high rates of protein synthesis, as evidenced by incorporation of ^{14}C-glycine (105) and the entire process is preceded by a sharp rise in several types of RNA (105). The net result of this protein synthesis is "augmentation" of cell protoplasm (105). Lack of any increase in DNA (33, 105) suggests that cell division is not responsible for the observed changes. However, it has not yet been determined that the agumentation of protoplasm is causally related to acclimation.

Membrane phospholipids have been the subject of investigation in acclimation, in part because of the attention paid to their role in chilling injury. Alterations in classes of phospholipids or in the relative unsaturation of their fatty acid moieties have been monitored because they may contribute to observed increases in membrane permeability (63, 64, 78). Evidence concerning these changes is contradictory. Full hardiness can be attained in black locust bark without changes in fatty acid unsaturation (109), but this is not a prima facie case against the theory because membrane permeability has not been shown to be a limiting factor in locust acclimation. Increases in fatty acid unsaturation may be due to increased activity of desaturase enzyme at low temperatures.

Changes in enzymes accompany acclimation (3, 24, 74, 98). In a very detailed comparison of ribulose bisphosphate carboxylase-oxygenase (RuBPCase) from hardened and unhardened wheat and rye,

Huner and Macdowall observed that RuBPCase from hardened plants was more stable in low temperature (-20°C) storage and had a lower Km for CO_2 at temperatures below 10°C (and a higher Km above 10°C) (43, 46). In the case of potato (47), RuBPCase from hardy and nonhardy species did not differ in KM for CO_2 but differed dramatically in Vmax of CO_2 fixation; at low temperature (5°C), the hardy plant had a higher Vmax than the nonhardy plant, but at a higher temperature (25°C) the former had a much lower Vmax. In both cases, differences in catalytic properties were related to conformational changes in enzyme subunits, as evidenced by differences in net charge and number of exposed SH groups (44, 45, 47).

Increases in the permeability of cells to water and salts during cold acclimation were one of the first well-documented changes (63). Recently, in dogwood, membrane permeability as measured by the efflux of tritiated water, increased during the SD phase of cold acclimation but did not change in response to low temperature treatment (78). Although Stout et al. (120) agree that permeability of the plasmalemma increases during acclimation, they report that it is a very small part (0.5%) of the total resistance to water movement, even when fast (31°C/min) cooling rates are used (119). Rather, membrane injury may occur as a result of cell contraction during freezing (117, 118). Also, injury to spruce needles may result from tonoplast damage, leading to loss of compartmentalization and cellular disruption (133).

With the advent of electron microscopy, researchers have attempted to relate ultrastructural changes to increases in cold

hardiness. One feature common to 2 studies is an increase in plasmalemma material, leading to membrane invaginations and a "pinching off" of membrane-bound vesicles (80, 90). This is consistent with reported increases in total membrane phospholipids (109) and may account for changes in membrane permeability, discussed previously. During acclimation of pine needles, changes in ultrastructure of chloroplasts includes a breakdown of the stroma thylakoids (72), and this may explain observed decreases in the efficiency of photosynthetic electron transport (73, 82, 104). More studies are needed before confidence can be placed on the relationship between physiological and ultrastructural changes.

During early acclimation, hardiness increases can, in part, be explained by tissue water loss (7, 33, 77, 78, 87). Plant water loss may occur as a result of decreased water uptake, increased transpiration, or tissue maturation. Root suberization may account for increased root resistance (77, 84), but fully suberized root systems are capable of absorbing large quantities of water (22). However, since high soil water levels do not affect tissue water content or cold acclimation (86), the role of roots in influencing cold acclimation, by regulating water uptake, remains a question.

Decreasing stomatal resistance seen in leaves of dogwood when exposed to SD conditions in growth chambers (77, 84) also may be responsible for declining tissue water. However, under field conditions, no difference in stomatal resistance was found among dogwood clones which differed in stem water content and cold hardiness (7). Nor were decreases in stomatal resistance in autumn found

in leaves of other hardwoods (29, 123) nor in hardened spruce and pine seedlings (21). The evidence seems to indicate that differences seen under growth chamber conditions may not reflect those in the field.

Tissue water content, when expressed on a fresh or dry wt basis, may change by virtue of a change in dry wt alone (59). When these changes are corrected for by use of water saturation deficit (WSD) (20, 59), a reduction in tissue water content is still seen. This decline is closely related to a SD-induced senescence and dehydration of pith cells (77). Examination of stem water by nuclear magnetic resonance (NMR) spectroscopy indicates that acclimation occurs from base to apex of a shoot (11, 76). SD and LD light treatments, which yielded the previously reported hardiness differences had NMR spectra which were identical at the third node from the apex, but different at the fifth node from the apex (76). This invites the suggestion that daylength may influence hardiness by lowering water content at different rates. Although water content can be credited with a great deal of importance in cold hardiness (14, 15, 51), hardiness fluctuations cannot always be equated with changes in water content (33). In spite of the importance placed by Li and Weiser (67) on their experiments which demonstrate that artificially decreasing water content of dogwood stem segments proportionately increased cold hardiness, they overlook instances in their data in which field water content decreased substantially with no increase in hardiness and a 50°C hardiness increase was not accompanied by any change in water content.

Even though it was thought for many years that supercooling of water provided only limited protection from freeze injury (62), recent evidence has shown that supercooling is crucial to survival of many fruit crops, such as peach (1, 92), grape (89), apple (94, 95), blueberry (4), and cranberry (23), and a variety of other woody plants (31, 32, 38, 101, 102). This may be responsible for differences in ecological distribution of woody plants (2, 30). Ability of tissues to supercool changes during acclimation (53, 54, 79, 88), and during tissue maturation (53, 54) in response to changes in water content (53, 55) and low temperature (37).

Ecological Aspects of Acclimation

Studying ecotypes of a single species has provided a convenient tool for examining cold hardiness, in general, and acclimation specifically. The most common procedure, called a provenance test, involves the collecting at one site individuals which represent the extreme conditions of a population's geographical distribution, including factors such as: latitude (and with it photoperiod), longitude, altitude, average annual minimum temperature, and length of growing season. In this way one can identify factors which may be related to the adaptation of an ecotype to its environment. In general, accessions from northern latitudes acclimate first (16, 26, 60, 71, 96, 110) and those from high elevations before those from low elevations (60, 97). However, in dogwood, clones from a similar latitude (Dickenson, North Dakota; and Seattle, Washington) acclimate quite differently. Their acclimation pattern (77) correlates

better with the length of growing season of their native range (110). Differences in hardiness among these populations are not necessarily present throughout acclimation; oak accessions differ most in hardiness in October (26), while pines differ more in September (97). Growth cessation and bud set correlate well with resistance to injury from the first fall frost (60, 96), but neither factor correlates well with resistance to freezing injury in early winter (96). Redfeldt (96) speculates that resistance to frost damage can be achieved if bud set is sufficiently early to complete shoot lignification and that both this process and resistance to later freezing injury are under genetic control, but are not necessarily related.

Climate of origin may not be the deciding factor in potential hardiness. Willow species, collected from tropical and subtropical climates and planted on 1 site in southwest Japan, were able do survive -15° and could be hardened (by storage at -3°C for 14 days) to survive -50°C (100). This may indicate that some species introduced to a warm climate may still develop a hardiness level which exceeds that required for survival (10).

Logic of the Dissertation Research

Research into mechanisms of perennial plant cold acclimation has been primarily done on noncrop plants, such as dogwood and black locust (12, 106, 128). In mature tree fruits the ripening crop may present an additional facet of complexity in the acclimation picture; ripening fruit are sources of hormones and sinks for

photosynthates and minerals. Indeed, in some plants acclimation and fruit ripening are occurring simultaneously and interactions of the two processes are poorly understood.

Although cold acclimation has been studied in a number of important horticultural fruit crops, such as blueberry (5), rasberry (52), cranberry (23), apple (57, 58), cherry (40), and peach (93), interpretation of the results is hindered by 2 aspects of the methods. Firstly, most studies did not include artificial freezing tests often enough to determine when and at what rate acclimation occurred. Differences in hardiness among treatments may vary during the course of acclimation and may influence subsequent mid-winter survival. Secondly, studies did not indicate from what portion of the plant sampling material was taken. Differences in cold resistance due to position-related tissue maturation (39) and due to the position of buds (17, 39) or shoot segments (70) on the growing shoot, suggest that caution must be exercised in order to collect uniform samples over the duration of a long study.

Grapevines are an interesting choice for cold hardiness research. Some cultivars have the capacity to develop high resistance to cold injury and are grown near the northern limit of perennial plant culture in many countries. When compared to other woody plants used in hardiness research, grapevines differ in growth habit, most notably in the production of very long shoots which show indeterminate growth (91). The lack of growth cessation by terminal bud set provides an interesting contrast to other plants.

In addition, it is known that mid-winter cold hardiness of grapevines can be affected by cultural practices in the previous growing season which influence vine growth and fruit yield and maturation; these include: growing site, method of training, cropping stress, exposure of leaves to sunlight and presence of adequate leaf area, and weed control (13, 39, 41, 115, 116). How these effects are manifested is not known. It may be enlightening to know more about the acclimation period in order to understand how summer practices affect winter hardiness.

Because very little is known about acclimation of grapevines, the objectives of this dissertation were to provide a sound background for further study of acclimation and, specifically, to answer the following questions:

1. What constitutes a rational set of sampling criteria for hardiness tests in acclimating grapevines?
2. What is the natural acclimation pattern of grapevines?
3. Is photoperiod important in regulating cold acclimation in grapevines and is root water uptake an important feature?
4. Can differential thermal analysis be used to assess and investigate cold acclimation of grapevines?

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SECTION I

NATURAL ACCLIMATION OF 'CONCORD' BUD AND CANE TISSUES

INTRODUCTION

Cold injury to grapevines is an important problem, especially at the northern limits of culture where extensive damage to buds can result in severe economic loss. Efforts to understand how injury occurs in grapevines have concentrated on the relationship between cultural practices during the growing season and subsequent mid-winter hardiness. Cold hardiness differences have been related to growing site (34), cropping stress (14), defoliation (21, 35), weed control (6), and training-induced exposure of shoots to sunlight during the growing season (12, 35). How these practices exert an effect on hardiness is not well understood.

In an effort to understand how hardiness is gained, much research attention has focused on cold acclimation, a period in late summer or early fall during which plant tissues undergo a dramatic increase in cold resistance. Increases in cold hardiness have been monitored in a number of plants by use of frequent artificial freezing tests. Hardiness begins to increase in late summer or early fall, in most cases well in advance of freezing temperatures. Cold acclimation occurs in 2 stages in stems of dogwood (36) and apple (15), separated by a plateau at about -15° to -20°C, but it appears to occur in only 1 stage in tissues of other plants, such as living bark of black locust (30) and stems of pine (29) and English ivy (32).

During early portions of the cold acclimation period, hardiness increases can be explained, in part, by tissue water loss (3, 10, 23, 24, 26). Research on dogwood has indicated that SD-induced acclimation involves an increase in root resistance and a decrease in stomatal resistance (23, 26) which may lead to stem water loss. Decline in stem water content occurs from base to apex of a shoot (4, 24) and apparently involves senescence and dehydration of pith cells (24). Whether death of pith cells alone can account for hardiness increases in the whole stem is not known.

However, most of the research into mechanisms of perennial plant cold acclimation has been done primarily on noncrop plants (5, 31, 37). In mature fruit trees the ripening crop may present an additional facet of complexity in understanding acclimation since ripening fruits are sources of hormones and sinks for photosynthates and minerals. In some plants the two processes of fruit maturation and cold acclimation are occurring simultaneously and their interactions are poorly understood.

Cold acclimation has been studied in a number of important horticultural fruit crops, such as blueberry (2), raspberry (16), cranberry (8), apple (18, 19), cherry (13), and peach (28), but interpretation of the results is hindered by 2 aspects of the methods. Firstly, most studies did not perform artificial freezing tests often enough to determine when and at what rate acclimation occurred. Differences in hardiness among treatments may vary during the course of acclimation and may influence subsequent mid-winter

survival. Secondly, studies did not indicate from what portion of the plant sampling material was taken. Differences in cold resistance due to position-related tissue maturation (12) and due to the position of buds (7, 12) or shoot segments (20) on the growing shoot, suggests that caution must be exercised in order to collect uniform samples over the duration of a long study.

Grapevines also provide an interesting contrast to other woody plants in that their growth habit is indeterminate (27); they do not produce a terminal bud. Since growth cessation is considered a prerequisite for acclimation (37), a test for the hypothesis may be possible.

With these considerations in mind, a study was undertaken with two objectives: (1) to identify sampling criteria which would minimize extraneous variability, and (2) to determine the onset and rate of acclimation of 'Concord' grapevines.

MATERIALS AND METHODS

Studies were conducted over a 3-year period in mature 'Concord' vineyards (Vitis labruscana Bailey) at 2 locations in Michigan. Vines growing in the Wm Cronenwett vineyard in Lawton were of medium vine size (0.4 - 1.4 kg of cane prunings per vine), yielding 5 - 7 tons/acre, and vines growing at the Horticulture Research Center of Michigan State University in East Lansing were of large vine size (1 - 3 kg of cane prunings per vine) yielding 5 - 6 tons/acre. Vines in both vineyards were trained to an upper-wire (1.7m) bi-lateral cordon (also called Hudson River Umbrella), and throughout the experiment were balance-pruned by means of a 30 + 10 formula (i.e., 30 buds retained for the first 0.4 kg of cane prunings and 10 buds for each additional 0.4 kg).

Sampling criteria varied within an individual study (as detailed in the Results), but in all cases, sampling material consisting of 1-year-old, 8 - 12 cm node-internode pieces. Samples gathered in the field were sealed in plastic, stored at ambient temperature for less than 24 hr before being randomly divided into 2 lots for separate determinations of cold hardiness and water content of buds and canes.

Cold Hardiness Assessment

The freezing technique was slightly modified from that of Howell and Weiser (15) and Stergios and Howell (34). Samples were inserted into vacuum flasks in contact with layered aluminum foil to facilitate heat removal and were placed into a Revco chest freezer. Moist cheesecloth in contact with the base of cane pieces served to inoculate the samples with ice, preventing supercooling (22). Freezer temperature was manually lowered to provide a freezing rate of 3 - 5°C/hr for samples; tissue temperature was monitored via a 24 gauge copper-constantan thermocouple inserted into the pith of a representative cane piece in each flask. Flasks were removed at each of several test temperatures and allowed to warm slowly overnight in a 2°C cooler. A temperature range was chosen such that the warmest temperature produced no injury and the coldest was lethal for all tissues. If this was not achieved, then it was assumed for the purposes of calculations that the next warmest or coldest temperature would have, respectively, produced complete survival or complete death. Thawed samples were placed into a humid chamber for 7 - 10 days, after which tissues were sectioned and rated as alive or dead by the method of tissue browning (33). Buds were rated as dead when primordia were brown and water-soaked; canes were rated as dead when the phloem-cambium layer was brown-colored.

Hardiness was expressed at T_{50} (the temperature at which 50% of tissues are theoretically killed), which was calculated by means of the Spearman-Karber equation (1). The formula used was as follows:

$$T_{50} = (b_{n+1} - b_n)\left(\frac{t_n + t_{n+1}}{2}\right)$$

where: b_n is the percent of dead tissues at one test temperature

b_{n+1} is the percent dead at the next lowest test temperature

t_n is the value of one test temperature in -°C and t_{n+1} is that for the next lowest test temperature

T_{50} values were statistically separated by a Chi^2 method (17) in which the numbers of live and dead tissues for each treatment, combined across selected test temperatures, were fitted into an R x 2 contingency table (25). The calculations were that of a standard Chi^2 test and results were compared to the tabular value of a 1-tailed Chi^2 distribution, $p = 0.05$. When the overall comparison of treatments indicated significance, individual treatment T_{50} values were separated in a pair-wise manner.

Tissue Water Measurement

Tissue water content of treatments was determined by placing 2 - 4 bud or cane pieces each into air-tight glass weighing vials fitted with ground-glass stoppers. Tissues were oven-dried for 36 hr at 70°C (vials open) and reweighed. Water content was calculated by difference after correction for vial wt and expressed as g water/g tissue dry wt.

RESULTS

Results are divided into 2 studies: Study 1 was designed to more clearly define a set of sampling criteria which would permit hardiness measurements over time with a minimum of extraneous variability and Study 2 traced the natural acclimation of bud and cane tissues for two years employing the sampling criteria from Study 1.

Study 1

Potential sources of variation in a grapevine canopy were identified along with levels of each factor to be investigated (Figure 1). Factors were studied individually and asterisks indicate at which level other factors were held constant when not being tested. For example, when 3 levels of vine size were being tested, all sampled material was from middle node positions, on exposed shoots, with 3 clusters.

Vine size (Table 2) had no significant effect on hardiness or water content of either primary bud or cane tissues. An increasing number of clusters per shoot from 0 to 3 (Table 3) decreased cane hardiness by 2°C in 1979, but had no effect on bud hardiness or on water content for either tissue. When the effect was investigated again in 1981 in a different vineyard, the presence of clusters had no effect on hardiness at any of 3 node positions (Table 3).

To test the effect of shoot exposure to sunlight on hardiness, material was sampled from the exterior (exposed) and interior (shaded) portions of a vine canopy. After collection, it was noticed that the material from shaded positions could be divided into 2 groups, based on whether or not the shoots had begun to develop brown coloration. These distinctions were retained throughout the hardiness evaluation and calculations. Bud and cane tissues from exposed positions (Table 4) were 2° to 6°C hardier than both groups of shaded tissues. In the shaded category, if tissues had begun to develop brown colorations, they were about 4°C hardier than shaded green shoots. Tissue water content was lowest in the hardiest treatment and greatest for the least hardy.

On 5 dates throughout the acclimation period, node position on the shoot was investigated for its influence on hardiness and water content of primary buds (Table 5) and canes (Table 6). Data for buds and canes are similar and will be discussed together. All tissues increased in hardiness and decreased in water content from August 25 through November 4. Water content increased on the last sampling date (November 9) but in comparison to other years, these data appear anomalous. The greatest hardiness effect due to node position was in August when basal tissues were about 5°C hardier than apical tissues. Differences in hardiness of 3°C were also present in September in buds and hardiness ranking was inversely related to water content. By October 26, hardiness and water content differences in both buds and canes were not found. In November, basal

bud and cane tissues were 2 - 3°C hardier than middle or apical tissues, but these differences were not reflected in changes in water content.

Study 2

Natural acclimation pattern was determined for bud and cane tissues for 1980 and 1981. Based on data from Tables 1 through 6, the sampling method was to take whole canes from positions well exposed to sunlight, but without respect to vine size or number of clusters per shoot. This would allow the monitoring of effects of node position (Tables 5 and 6). Although cane hardiness was affected by cluster number, that factor was not considered because of the difficulty of obtaining sufficient material of one category for the duration of the study.

Primary bud and cane hardiness and water content data for 1980 and 1981 are presented in Figures 1 through 4. In all cases increasing hardiness (decreasing T_{50}) was accompanied by decreasing water content through about the end of September. Water content decline was most rapid from early August (2 - 3 g H_2O/g dry wt) to mid-September (1 g/g dry wt). Acclimation increases seemed to be linear, except for primary buds in 1981, when a distinct plateau was seen between mid-September and mid-October. Hardiness increases after the end of September were not accompanied by changes in water content.

In both years, base bud and cane tissues acclimated before apical tissues and this was accompanied by earlier water loss in

those tissues (Figures 1 through 4). With a few exceptions, differences in early acclimation were related to water loss; when these water content differences disappeared in mid-September so did hardiness differences. However, further increases in hardiness not related to water loss may have been due in 1981 to low temperature in late October (Figure 5).

DISCUSSION

Results presented here support the conclusion that a study of acclimation requires specification of sampling criteria. Differences due to exposure of shoots to sunlight (Table 4) are in agreement with those of Howell and Shaulis (12) who reported that exposed shoots were 6°C hardier than shaded shoots in September and 8°C hardier in November. Position of the sampled material on current season's growth influence hardiness (Tables 5 and 6) and in this and other studies it is basal tissues which are hardier than apical tissues (4, 7, 12, 20, 38). Reasons for these positional effects are not known but Section II provides further data and presents an hypothesis to explain the effect.

Effect of fruit load on acclimation was minor (Table 2), but a more detailed study is needed. Vine cold hardiness is reduced when vines are stressed either by retaining excess fruit (12, 14) or by defoliation (35). In this study, use of vigorous vines pruned to provide a recommended crop load may have precluded the importance of different fruit loads per shoot; soluble solids translocation within a vine occurs quite readily in response to separation of fruit sinks from source leaves (21).

Acclimation of buds and canes (Figures 1 through 4) begins in early August and increases in a smooth, uninterrupted manner through

mid-November. Only primary bud tissues in 1981 (Figure 3) show a plateau (mid-September to mid-October) similar to that seen in acclimation of dogwood (36) and apple (15) stems. These latter studies have been interpreted in light of evidence that 2-stage acclimation is controlled first by short day photoperiod (9, 15) and then by low temperatures (11, 15). Presumably, observed plateaus are caused by a temporal separation of the environmental signals. One can speculate that if low temperatures were to come immediately after completion of the short day response, no plateau would be seen and acclimation would appear as a single, uninterrupted hardiness increase. If this is true, seasonal differences in temperature decline might account for differences in bud acclimation in 1980 (Figure 1) and 1981 (Figure 3). Experiments in controlled environment conditions are needed to determine if both photoperiod and temperature are required for grapevine acclimation.

Decline in water content in both buds and canes over 3 seasons (Tables 5 and 6, Figures 1 through 4) accompanies increase in hardiness suggesting that the two may be related early in the acclimation period. These results agree with those for dogwood stems (23) and leaves of evergreen boxwood (10) where tissue water decline correlates with early stages of hardiness increased, but not later stages. Water content may also explain differences in exposure (Table 4) and node position (Tables 5 and 6) in which lower hardiness values were usually accompanied by high water content. These factors are addressed in Section II which discusses interactions of hardiness, water content, and tissue maturation.

These studies can be used as a basis for further research into the mechanisms of cold acclimation of grapevines. More research will be needed to understand how cultural practices influence winter hardiness and to what extent these processes can be elucidated by studying cold acclimation. Sound management decisions can then be made in order to ameloriate winter injury and reduce economic loss.

Table 1. Potential factors affecting cold acclimation of 'Concord' grape buds and canes.[z]

Source of Variation	Factor	Level
Within vineyard	Vine size (kg of cane prunings)	0.4 ± 0.1 0.9 ± 0.1 1.4 ± 0.1*
Within vine	Exposure of shoot to sunlight	Exposed* Shaded
	Clusters per shoot	0 1 3*
Within shoot	Node position	Base (#2-4) Middle (#6-8)* Apex (#10-12)

[z]Factors were investigated individually. Asterisks indicate the level of each factor held constant when other factors were being tested.

Table 2. Effect of vine size on hardiness (T_{50})[z] and water content[y] of primary buds and canes of 'Concord' grapevines, September 16, 1979.[x]

Vine size (kg of cane prunings)	Primary Buds		Canes	
	T_{50}	H_2O	T_{50}	H_2O
.4 ± .1	-11.1a	1.47a	-11.1a	1.23a
.9 ± .1	- 9.4a	1.37a	-10.9a	1.32a
1.3 ± .1	- 9.9a	1.38a	- 9.9a	1.26a

[z] T_{50} calculated by means of Spearman-Karber equation, separations within columns by χ^2 test, $p = 0.05$.

[y] g H_2O/g dry wt, differences were not significant at $p = 0.05$ when separated within columns by Duncan's multiple range test.

[x] Sampled material was from middle node positions (#6-8) on shoots well-exposed to sunlight, each shoot possessing 3 clusters.

Table 3. Effect of cluster number per shoot on hardiness ($T50$)[z] and water content[y] of primary buds and canes of 'Concord' grapevines.

Clusters per shoot	Node positions	Primary Bud		Cane	
		T_{50}	H_2O	T_{50}	H_2O
September 15, 1979, Lawton, Michigan[x]					
0	6-8	- 9.8a	1.22a	-13.1a	1.02a
1	6-8	- 9.4a	1.16a	-12.2ab	1.10a
3	6-8	- 9.5a	1.24a	-11.1b	1.10a
October 22, 1981, East Lansing, Michigan[w]					
0	2-4	-14.0a	--	-14.0a	--
	6-8	-14.0a	--	-14.0a	--
	10-12	-13.7a	--	-14.0a	--
2-3	2-4	-13.7a	--	-14.0a	--
	6-8	-13.7a	--	-14.0a	--
	10-12	-13.7a	--	-14.0a	--

[z] T_{50} calculated by means of Spearman-Karber equation, separations within columns by χ^2 test, $p = 0.05$.

[y] g H_2O/g dry wt, value separated within columns by means of Duncan's multiple range test, $p = 0.05$.

[x] Material sampled from shoots well-exposed to sunlight on vines with 1.4 ± 0.1 kg of cane prunings.

[w] Material sampled from shoots well-exposed to sunlight without respect to vine size.

Table 4. Effect of shoot exposure to sunlight on hardiness (T_{50})[z] and water content[y] of primary buds and canes of 'Concord' grapevines, Lawton, Michigan, September 30, 1979.

Treatment[w]	Cane Color	Primary Bud		Cane	
		T_{50}	H_2O	T_{50}	H_2O
Exposed	Brown	-13.2a	0.90c	-13.5a	0.91c
Shaded	Lt. Brown	-10.5b	1.11b	-11.5b	1.24b
Shaded	Green	- 6.4c	1.69a	- 7.5c	2.71a

[z]T_{50} calculated by means of Spearman-Karber equation, separations within columns by χ^2 test, p = 0.05.

[y]g H_2O/g dry wt, mean separation within columns by Duncan's multiple range test, p = 0,05.

[x]Sampled material was from middle node positions (#6-8) on shoots with 3 clusters. Vines had 1.4 ± 0.1 kg cane prunings.

[w]Exposure status was visually determined.

Table 5. Effect of node position on cold hardiness (T_{50})[z] and water content[y] of primary buds of 'Concord' grapevines, 1979.[x]

Node position	Sampling dates									
	August 25		September 13		October 26		November 4		November 9	
	T_{50}	H_2O	T_{50}	H_2O	T_{50}	H_2O	T_{50}	H_2O	T_{50}	H_2O
Base (#2-4)	-8.3a	--	-10.7a	1.20c	-16.5a	.81b	-18.6a	.67a	-20.0a	.97a
Middle (#6-8)	-6.8b	--	- 9.7a	1.32b	-16.6a	.87a	-17.0b	.71a	-19.0b	.95a
Apex (#10-12)	-3.0c	--	- 7.2b	1.58a	-15.1a	.88a	-15.9b	.72a	-18.5b	.95a

[z] T_{50} calculated by means of Spearman-Karber equation, separations by χ^2 test, p = 0.05.

[y] g H_2O/g dry wt, mean separations by Duncan's multiple range test, p = 0.05.

[x] Sampled material was from shoots well-exposed to sunlight, each shoot possessing 3 clusters. Vines had 1.4 ± 0.1kg of cane prunings.

Table 6. Effect of node position on cold hardiness (T_{50})[z] and water content[y] of canes of 'Concord' grapevines, 1979.[x]

Node position	Sampling dates									
	August 25		September 13		October 26		November 4		November 9	
	T_{50}	H_2O	T_{50}	H_2O	T_{50}	H_2O	T_{50}	H_2O	T_{50}	H_2O
Base (#2-4)	-8.4a	--	-11.2a	1.09b	-16.8a	.86b	-20.9a	.70a	-20.5a	.95a
Middle (#6-8)	-7.3a	--	-12.2a	1.20a	-16.5a	.92a	-18.3b	.78a	-17.8b	.94a
Apex (#10-12)	-3.9b	--	-11.5a	1.22a	-15.2b	.85b	-17.1b	.76a	-16.7c	.90a

[z] T_{50} calculated by means of Spearman-Karber equation, separation within columns by χ^2 test, $p = 0.05$.

[y] g H_2O/g dry wt, mean separations within columns by Duncan's multiple range test, $p = 0.05$.

[x] Sampled material was from shoots well-exposed to sunlight, each shoot possessiong 3 clusters. Vines had 1.4 ± 0.1 kg of cane prunings.

Figure 1. Hardiness (T_{50}) and water content of primary buds of 'Concord' grapevines during cold acclimation, August to November, 1980, Lawton, Michigan.

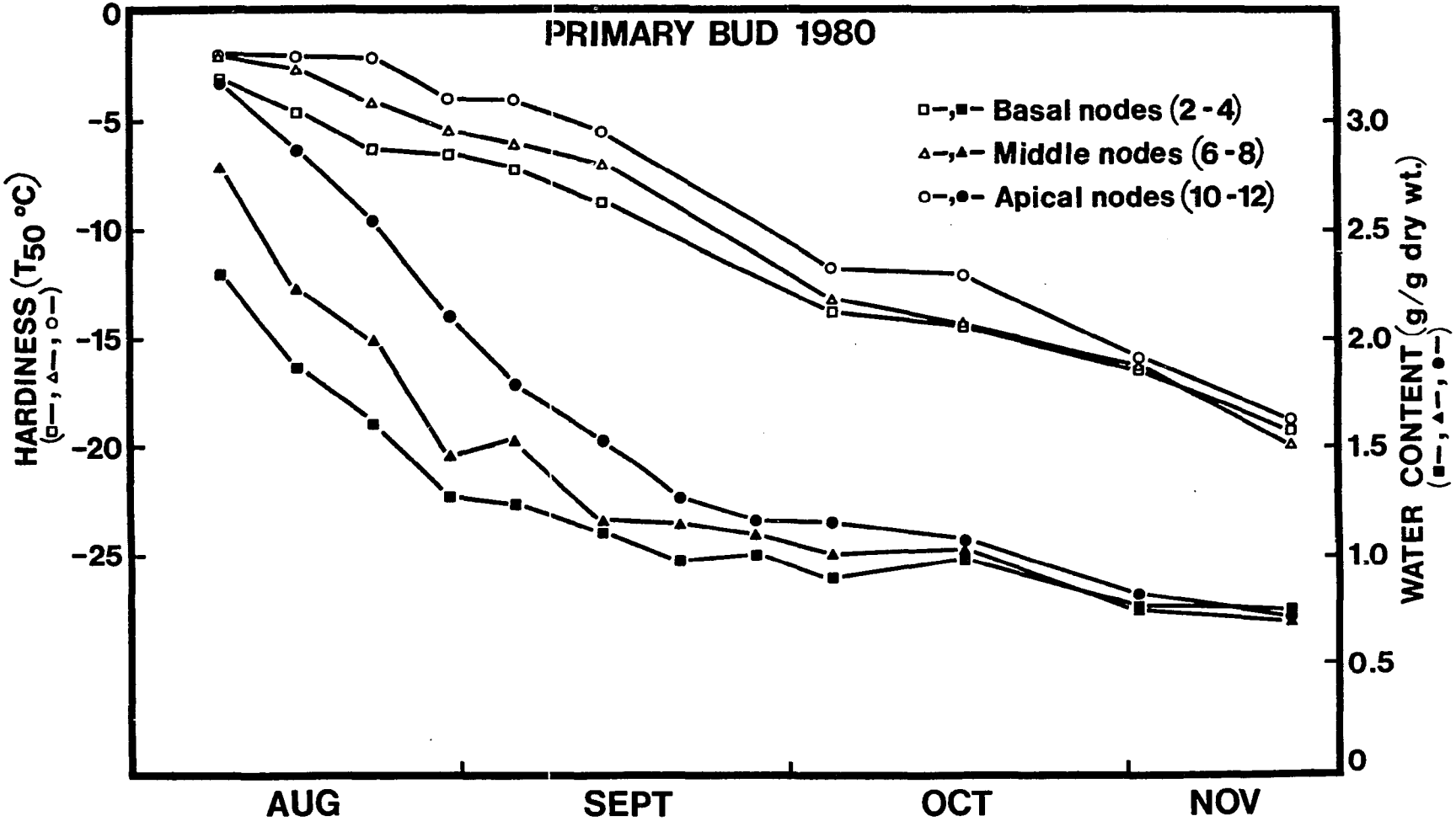

PRIMARY BUD 1980
□–,■– Basal nodes (2-4)
△–,▲– Middle nodes (6-8)
○–,●– Apical nodes (10-12)
HARDINESS (T50 °C)
(□–, △–, ○–)
WATER CONTENT (g/g dry wt.)
(■–, ▲–, ●–)
0
-5
-10
-15
-20
-25
3.0
2.5
2.0
1.5
1.0
0.5
0
AUG
SEPT
OCT
NOV

Figure 2. Hardiness (T_{50}) and water content of canes of 'Concord' grapevines during cold acclimation, August to November, 1980, Lawton, Michigan.

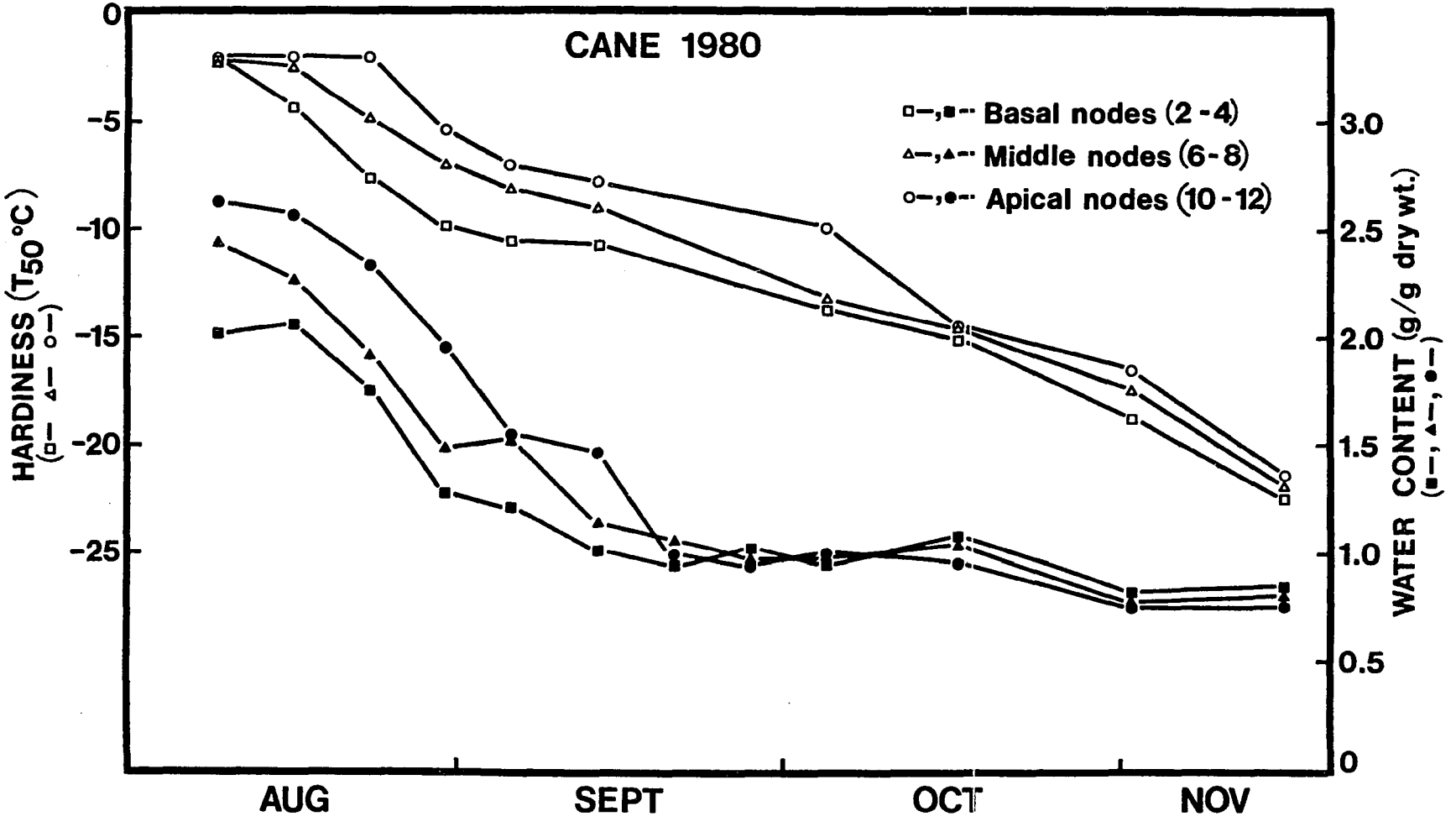

CANE 1980
Basal nodes (2-4)
Middle nodes (6-8)
Apical nodes (10-12)
HARDINESS (T_{50} °C)
WATER CONTENT (g/g dry wt.)
0
-5
-10
-15
-20
-25
3.0
2.5
2.0
1.5
1.0
0.5
0
AUG
SEPT
OCT
NOV

Figure 3. Hardiness (T_{50}) and water content of primary buds of 'Concord' grapevines during cold acclimation, August to November, 1981, Lawton, Michigan.

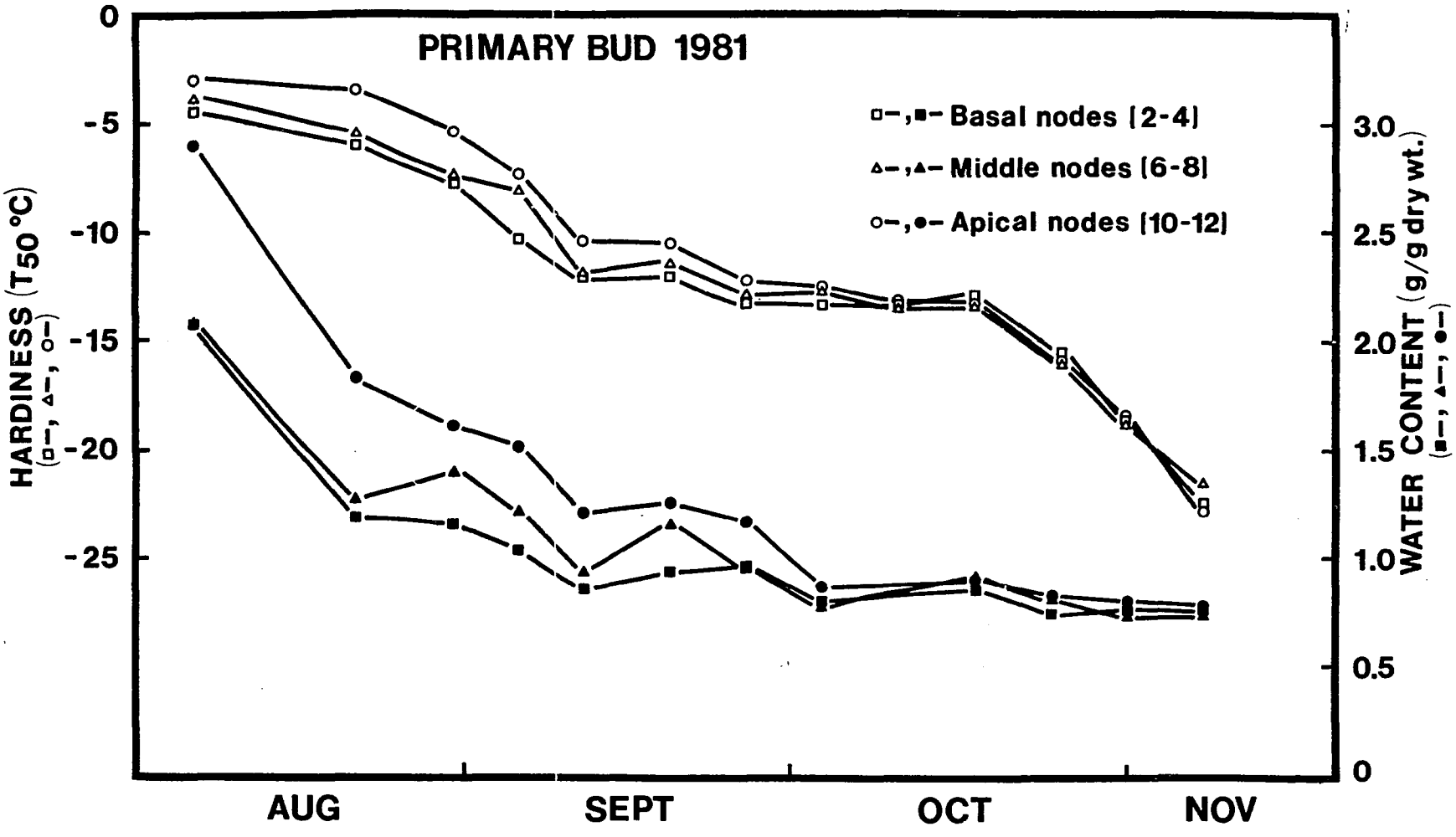
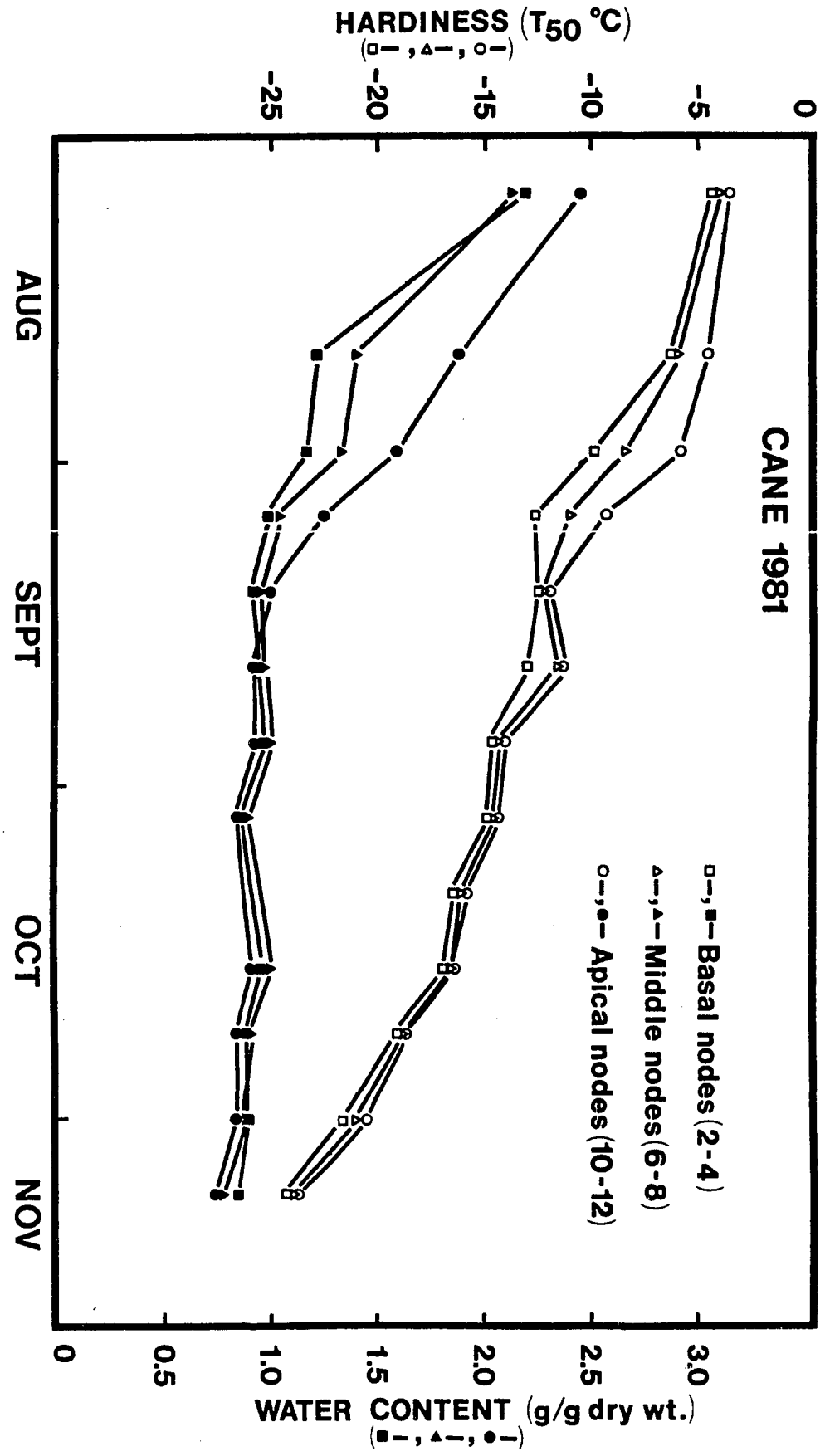

PRIMARY BUD 1981
□–,■– Basal nodes (2-4)
△–,▲– Middle nodes (6-8)
○–,●– Apical nodes (10-12)
HARDINESS (T50 °C)
(□–, △–, ○–)
WATER CONTENT (g/g dry wt.)
(■–, ▲–, ●–)
0
-5
-10
-15
-20
-25
3.0
2.5
2.0
1.5
1.0
0.5
0
AUG
SEPT
OCT
NOV

Figure 4. Hardiness (T_{50}) and water content of primary buds of 'Concord' grapevines during cold acclimation, August to November, 1981, Lawton, Michigan.


HARDINESS (T_{50} °C)
(□—, △—, ○—)
0
-5
-10
-15
-20
-25
CANE 1981
□—, ■— Basal nodes (2-4)
△—, ▲— Middle nodes (6-8)
○—, ●— Apical nodes (10-12)
AUG
SEPT
OCT
NOV
0
0.5
1.0
1.5
2.0
2.5
3.0
WATER CONTENT (g/g dry wt.)
(■—, ▲—, ●—)

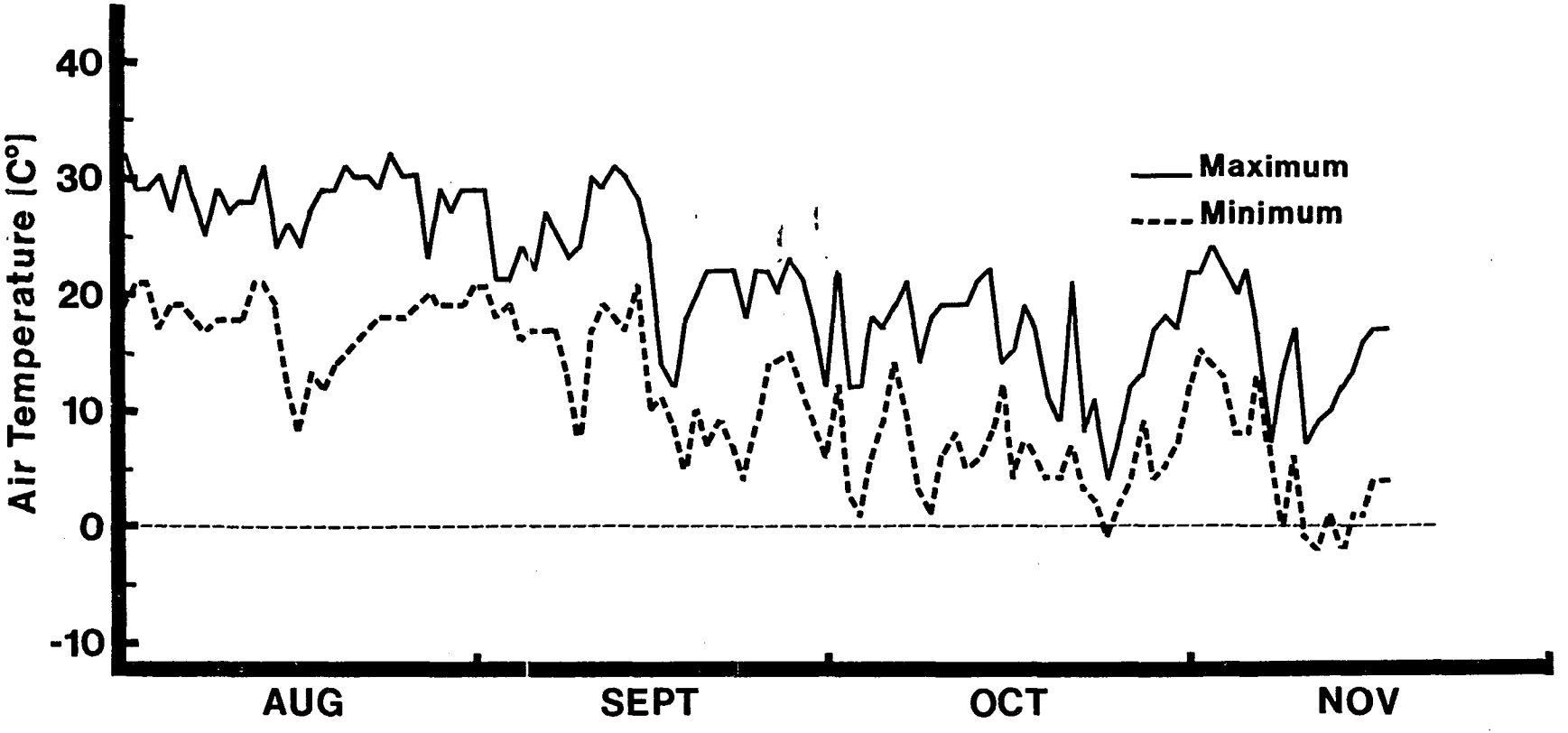


Figure 5. Maximum and minimum air temperatures (°C) during late summer and fall, 1981, at Lawton, Michigan.

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SECTION II

RELATIONSHIP OF COLD HARDINESS, WATER CONTENT, AND TISSUE MATURATION IN 'CONCORD' GRAPEVINES

INTRODUCTION

Cold resistance of a large plant often varies as a function of the position from which tissue samples are taken (9). One of the most widely reported hardiness differences is that found between the basal and apical bud and twig tissues. Greater levels of hardiness in basal tissues have been reported for ash buds (12), peach twigs (3, 4), raspberry canes (10), and sweetgum twigs (18).

In dogwood stems, nuclear magnatic resonance (NMR) spectroscopy has shown that line broadening from about 20 Hz to 250 Hz is associated with a dramatic increase in hardiness (from -5° to -196°C) and the change begins at the base of a stem and develops acropetally (2, 13). Burke et al. (2) report that basal stem tissues of acclimating and nonacclimating dogwood plants have similar wide NMR line widths, but authors do not comment on the relative hardiness of the basal tissues. If NMR line widths are indicative of hardiness, one can speculate that cold hardening may be occurring in actively growing "nonacclimating" plants, perhaps, as a result of advanced physiological age of base tissues. Unfortunately, when positional effects were studied in dogwood, numbering was done from the apex without any indication of relative distance from the base (13, 14).

In a detailed study, variation in cold hardiness of tissues within a grapevine canopy was found to be dependent on leaf exposure

to the sky, cane diameter, persistent lateral status, and presence and color of periderm tissue (9). Specifically, an intact 6-node cane segment was found to have hardier tissues at the basal end (-12° hardier canes and -15°C hardier buds) and was related closely to development of periderm. In Section I differences due to node position on the shoot were also seen and these were associated with water content differences.

In this section, additional data are presented which relate cold hardiness, water content, and tissue maturation. The decision to present these data in a one separate section was made after experimentation was finished. The data do not represent an attempt to thoroughly investigate the relationship among the 3 factors. Rather, these data will serve to raise questions about the interpretation of data from previous studies and the conduct of future studies in woody plant cold hardiness.

MATERIALS AND METHODS

Vines and sampling method used in this study were identical to those used in Section I.

Hardiness and water content was measured as detailed in Section I.

In addition, water saturation deficit (WSD) (11) was measured on canes in one case, using a formula and method as follows:

$$WSD = \frac{\text{saturation wt - fresh wt}}{\text{saturation wt - dry wt}} \times 100$$

Saturation weight was determined, after fresh weight was taken, by placing cane internode segments (2 - 3 cm long) into glass weighing vials (24 x 48 mm) with ground-glass stoppers, the cut ends in contact with ca. 2 ml water. In closed vials, cane pieces were allowed to take up water for 48 hr. Preliminary data indicated that consistent weight was achieved by this time. Pieces were removed, blotted dry of surface water, placed into a dry weighing vial, and handled as detailed in Section I.

Shoot color changes brought on primarily by the development of periderm (16) were assessed subjectively by eye. Relationship of shoot color to the presence or absence of periderm (9) is also subjective, but a gross relationship is known to exist (15, 16).

RESULTS

Data in Table 1 were taken on samples collected with respect to node position only; color assessments reflected the status of a representative twig. Increased hardiness was accompanied by a decrease in water content and a change in shoot color (Table 1). When shoots were green (August 9), water content was high (2.0 - 2.6 g/g dry wt) and hardiness was low (-2.0°C). Three weeks later (August 30), color had begun to develop at base nodes while apical nodes were still green. These brown nodes had the greatest hardiness and lowest water content. With all nodes showing brown color (September 13), base nodes were still the hardiest and lowest in water content. On the last date (October 4), a hardiness difference of almost 4°C between base and apical shoot segments was not associated with differences in water content or color.

Later in the acclimation period, when the color change was further toward the apex, samples were collected with respect to the node position where the color change was most pronounced green to brown (Tables 2 and 3). That node was counted as #4 on the 6-node segment and counting proceeded from that point. On both October 7 (Table 2) and October 13 (Table 3), the 6-node segment had dramatic hardiness differences (7 - 10°C for buds and 6 - 7°C for canes) and brown base tissues in all cases were greater in hardiness and lower in water content than green apical tissues.

A color change, yellow-green to brown was also seen on persistent lateral shoots (Table 4) and 3-node segments were collected in a manner which maximized the color difference along the segment. Again, brown nodes were hardier and lower in water content than yellow-green apical tissues. Also, hardiness and water content values were comparable to node positions of similar color on the main shoot (Table 2, nodes 3 - 5).

Hardiness and water content data presented in Table 5 are the same as in Section I, Figure 5, but here are compared to WSD values. WSD showed a general increase throughout the acclimation period except for a temporary decline on September 26. WSD values showed little relationship to hardiness; little change occurred in WSD when hardiness increased (August 30 to September 5), and a large change occurred when hardiness was constant (September 26 to October 3). WSD was greatest in apical tissues even when no hardiness or water content differences were apparent.

DISCUSSION

The stem color change which takes place in August and September (Table 1) is due to the development of periderm (16). Periderm in Vitis species forms in nonconducting primary phloem, by a redifferentiation of parenchymatic cells (5, 6, 7). The formation of suberized cells (phellerm) to the outside of the phellogen isolates the epidermis and cortex, resulting in the death of these tissues.

Anatomical aspects of periderm formation have been studied in grapevines (5, 6), but little is known about factors which influence its formation or development. Dark cane color, favored by exposure of shoots to sunlight, is associated with periderm formation (and increased hardiness) (9) but a detailed study of this has not been made.

The progression of periderm development along a shoot in grapevines has not been followed, but in other woody plants the development is acropetal (1), similar to the progression of stem color change (Tables 1-4). Tissue maturation in grapevines may be much more complex than just periderm formation; it may include pith cell senescence (14) and changes in cell walls (7).

In an intensive series of experiments on several woody species, Borger and Kozlowski determined that periderm formation is influenced

by photoperiod, light intensity, temperature, water stress, defoliation and growth regulators (see 1). Many of these factors also influence acclimation. If a close relationship exists between shoot maturation and cold acclimation as suggested by data here (Tables 1 to 4), a much more detailed study of periderm and factors which influence its formation, would be very useful.

These data raise questions about how to compare treatments in which tissues differ in shoot maturation if that maturation results in hardiness differences. For example, if stems from two plants are different in hardiness at the tips, but of equal hardiness at bases, is the difference important? Clearly, to answer the question one must know the plant species. For grapevines in which relatively short basal portions of canes are retained for fruiting, the two plants should be considered equal in terms of potential to bear a crop of fruit. However, for forest species used for lumber in which maintenance of uninjured terminal buds is crucial to development of a straight tree (and hence a saleable product), the hardiness difference at the tips may be of paramount importance.

Clearly, then the sampling method which is used should reflect the type of question one wishes to answer. Often, studies do not indicate from what part of a current season's growth samples are taken (8, 17) or do so by numbering nodes from the apex (13, 14), a method which cannot take into account the relative status of maturation which begins at the base. These studies are not unique and they point to the fact that full interpretation of the physiological

importance of studies cannot be done until sampling criteria are more clearly defined.

Section III provides further data on the relationship of cold hardiness and tissue maturation as affected by photoperiod and presents additional speculation about the interpretation of other studies.

Table 1. Relationship of cold hardiness (T_{50}), water content, and color of acclimating shoots of 'Concord' grapevines, 1980.

Twig Section[x]	Sampling Date			
	Aug 9	Aug 30	Sept 13	Oct 4
	T_{50}[z]			
Base	-2.0a	-9.8a	-10.7a	-13.7a
Middle	-2.0a	-7.0b	- 9.0ab	-13.3a
Apex	-2.0a	-5.3b	- 7.8b	-10.0b
	Water Content (g/g dry wt)[y]			
Base	2.02a	1.28a	1.01a	0.96a
Middle	2.44b	1.48a	1.13a	1.00a
Apex	2.63b	1.95b	1.47b	1.00a
	Shoot Color			
Base	Green	Brown	Brown	Brown
Middle	Green	Yellow/Brown	Brown	Brown
Apex	Green	Green	Brown	Brown

[z]T_{50} calculated by means of Spearman-Karber equation, means separated within columns by χ^2 test, p = 0.05.

[y]Mean separations of water content within columns by Duncan's multiple range test, p = 0.05.

[x]Base = indes #2-4, middle = nodes #6-8, and apex = nodes #10-12.

Table 2. Effect of cane color change on cold hardiness (T_{50})[z] and water content[y] of primary buds and canes of 'Concord' grapevines, October 7, 1979.

Node position on cane segment	Cane color	Primary Bud		Cane	
		T_{50}	H_2O	T_{50}	H_2O
1 (Basal)	Brown	-13.5a	1.22a	-13.7a	.94a
2	Brown	-13.4a	1.42bc	-13.5a	.99ab
3	Lt. brown	-11.5b	1.37b	-12.3ab	1.06b
4	Yellow	- 9.7c	1.62d	-11.5b	1.20c
5	Green	- 6.1d	1.60d	- 8.1c	1.41d
6 (Apical)	Green	- 3.1e	1.55cd	- 6.5d	1.40d

[z] T_{50} calculated by means of Spearman-Karber equation, separations by χ^2 test, $p = 0.05$.

[y] gH_2O/g dry wt, mean separations by Duncan's multiple range test, $p = 0.05$.

Table 3. Effect cane color change on cold hardiness (T_{50})[z] and water content[y] of primary buds and canes of 'Concord' grapevines, October 13, 1979.

Node position on cane segment	Cane color	Primary bud		Cane	
		T_{50}	H_2O	T_{50}	H_2O
1 (Basal)	Brown	-11.4a	1.01ab	-11.7b	.86a
2	Brown	- 8.7b	1.07bc	- 9.2bc	.90a
3	Lt. brown	- 6.7c	1.15cd	- 8.2c	.94ab
4	Yellow	- 4.1d	1.18cd	- 6.5c	1.00b
5	Green	- 4.8d	1.28de	- 6.1c	1.18c
6 (Apical)	Green	- 4.3d	1.33e	- 5.7c	1.20c
Well exposed	Dk. brown	-13.0a	.90a	-13.9a	.86a

[z] T_{50} calculated by means of Spearman-Karber equation; values separated within columns by χ^2 test, p = 0.05.

[y] g H_2O/g dry wt, means separated within columns by Duncan's multiple range test, p = 0.05.

Table 4. Effect of cane color change on cold hardiness[z] (T_{50}) and water content[y] of primary buds and canes of 'Concord' grapevines, September 25, 1980.

Node position on lateral cane segment	Cane color	Primary bud		Cane	
		T_{50}	H_2O	T_{50}	H_2O
1 (Basal)	Brown	-10.5a	1.56a	-12.0a	.95a
2	Lt. brown/ yellow	-8.6ab	1.66b	- 9.2	1.10b
3 (Apical)	Yellow/ green	-6.4b	1.93c	- 7.0b	1.38c

[z] T_{50} calculated by means of Spearman-Karber equation, separations by χ^2 test.

[y] g H_2O/g dry wt, values separated by means of Duncan's multiple range test, p = .05.

Table 5. Hardiness (T_{50}), water content, and water saturation deficit of canes of 'Concord' grapevines during fall, 1981.

Twig Section	Sampling Date						
	Aug 30	Sept 5	Sept 12	Sept 19	Sept 26	Oct 3	Oct 31
	T_{50}[z]						
Base	-9.8a	-12.7a	-12.3a	-13.0a	-14.5a	-14.8a	-21.6a
Middle	-8.5a	-11.0b	-12.0a	-11.7b	-14.5a	-14.5a	-21.0ab
Apex	-5.8b	- 9.3c	-12.0a	-11.5b	-14.0a	-14.5a	-20.6b
	Water Content (g/g dry wt)[y]						
Base	1.17a	0.99a	0.93a	0.94a	0.97ab	0.86a	0.89a
Middle	1.33ab	1.04ab	0.95a	0.96a	0.99a	0.90a	0.88a
Apex	1.59b	1.25b	0.99a	0.91a	0.94b	0.86a	0.84a
	Water Saturation Deficit[x]						
Base	11.0a	12.6a	13.2a	13.9a	12.3a	16.6a	19.9a
Middle	10.1a	12.1a	13.6a	14.0a	11.1a	18.1a	21.8b
Apex	11.6a	12.9a	17.0b	16.1b	14.9b	20.2b	22.5b

[z] T_{50} calculated by means of Spearman-Karber equation, means separated with columns by χ^2 test, p = 0.05.

[y] Mean separations of water content within columns by Duncan's multiple range test, p = 0.05.

[x] Water saturation deficit calculated as follows: $WSD = \frac{\text{saturation wt - fresh wt}}{\text{saturation wt - dry wt}} \times 100$

Mean separations by Duncan's multiple range test, p = 0.05.

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SECTION III

EFFECT OF NATURAL AND NIGHT-INTERRUPTED PHOTOPERIODS ON ACCLIMATION OF POTTED 'CONCORD' GRAPEVINES

INTRODUCTION

Acclimation in grapevines is not well understood. Although we have dealt with the natural acclimation pattern and within-canopy variation (see Sections I and II), little is known about how acclimation in grapevines is initiated.

Reports on dogwood and other woody plants have strongly suggested that the first stage of acclimation is initiated by short days (SD) (6, 20) and mediated by phytochrome (12, 23). Leaves are the site of reception (9) and must be present to facilitate full hardening (6). SD leaves produce a hardiness promoter (5) and long day (LD) leaves a hardiness inhibitor (11). Plants split between the inductive (SD) and noninductive (LD) photoperiods are intermediate in hardiness (8), suggesting an interaction of the two regulators rather than a single overriding control mechanisms.

An important aspect of cold acclimation appears to be the SD-induced decline in tissue water content (13), a portion of which results from pith senescence and dehydration (2, 13). McKenzie et al. (13) and Parsons (14) claim overall plant water decline may be facilitated, or perhaps controlled, by increased root resistance and decreased stomatal resistance (13, 14). If root suberization is the cause of increased resistance to water flow, it may account for observations that plants acclimate regardless of the amount of water

present in the root environment (15, 22). This is likely too simplistic an explanation, in view of reports that water stress can promote (3) and inhibit acclimation (19).

The purpose of this study was to answer the following questions: (1) Is short-day photoperiod a requirement for cold acclimation in grapevines? That is, will night interruption prevent acclimation? (2) If so, is it associated with growth cessation, tissue maturation, and/or decline in tissue water content? (3) Is an increase in root resistance related to acclimation of grapevines and is it related to photoperiod? (4) Will split plants simultaneously subjected to two photoperiods be intermediate in hardiness or will they be influenced more by one light regime than the other?

MATERIALS AND METHODS

Plants used in this study were purchased in 1980 from a commercial nursery as 1-year-old rooted cuttings and were potted into 3-gallon plastic pots, containing a sterilized medium of loam soil, sand, and peat (1:1:1 by volume). Plants were thinned to 2 shoots per pot, tied to bamboo stakes, and grown throughout the year without treatment. After a killing fall frost, plants were transferred to a protected lathhouse and mulched over winter. In spring of 1981, 110 uniform vines were randomly assigned to 2 blocks and equally spaced on a 5m x 15m flat concrete area. Plants were trained to 2 shoots, 1 on each of the 2 trunks which grew the previous year, tied to bamboo stakes, and trained when growth permitted to an overhead trellis (1.7m) constructed of a grid of wire and twine. Lateral shoots were removed on a regular basis.

Plants were watered as needed by means of a drip irrigation system. High pH water (7.8 - 8.0) necessitated the occasional adjustment of soil pH back to 6.0 - 6.5 optimum by use of iron sulfate. Fertilization consisted of 2 ca. 1-liter applications of water soluble (12-12-12) fertilizer (300 mg actual N per plot per application), the first in early June and the second in early July.

In the middle of each block a light barrier was erected (in a N-S orientation) which consisted of a black plastic wall (2m high)

on a wooden frame. Light treatments were the following: natural daylength (ND), in which plants were exposed to naturally decreasing daylengths (after June 22) and no artificial lighting; night-interrupted (NI) photoperiod, in which plants were exposed to natural daylength plus ½ hr of white incandescent light (2.4 ± .8 μEcm^{-2}) in the middle of the night period; and split plants (SP), which had 1 of the 2 shoots trained through the light barrier and exposed to natural daylengths (ND-SP) and the other shoot exposed to night-interrupted (NI-SP). Night interruption was begun on July 27 when daylengths were 14.5 hr and continued until October 30, even after leaves were killed by frost.

Measurements of shoot growth (total nodes), shoot maturation (extent of change in cane color, green to brown), and percent actively growing apices were taken at the beginning of night interruption and after 5 wks (September 3) to determine the influence of photoperiod on these parameters. Actively growing tips were those which had young leaves with a fresh green appearance.

On 4 sampling dates throughout the acclimation period, plants randomly selected from the three light treatments were assessed for hardiness, tissue water content (first 2 sampling dates only) and root resistance.

Techniques

Hardiness Assessments

Because of the importance of node position in studies in Section 1, nodes were separated into 3 groups: base, #2-4; middle, #6-8; and apex, #10-12 (numbered from the base).

Tissue Water Content Measurements

Water content of buds and canes was measured (as described in Section 1) on nodes from positions 1, 5, 9, and 13, numbering from the base.

Root Resistance

Root resistance was measured in the same manner as that described by McKenzie et al. (13) and Parsons (14). After collection of shoot growth for hardiness and water content measurements, vines were removed from pots and soil washed off by gentle agitation in a basin of water. Root systems were transferred to 7-liter buckets of fresh, room temperature tap water. Just prior to being attached to the root resistance apparatus, the stem was cut about 8 - 10 cm above soil line, loose bark was removed, and the outer surface of the stem was coated with vacuum grease to prevent entry of water or air.

The apparatus used in measuring root resistance is presented as a schematic diagram in Figure 1. The apparatus consisted of an upright 2 ml (2 x 0.01) glass pipet (held in place by small V-jaw clamps), vacuum tubing, and a glass "Y" tube. The bottom end of the glass "Y" was connected to the cut stem by vacuum tubing and secured

by a tightened hose clamp. One arm of the "Y" was connected to the pipet by vacuum tubing while the other arm was fitted with a short piece of tubing and a serum stopper. This formed a single unit which was connected to 5 other units by means of a manifold constructed of vacuum tubing and glass "T" tubes. The manifold was connected to a manometer at one end and to a vacuum pump at the other end. All connections were secured by hose clamps or were wrapped with parafilm and checked regularly for leaks.

Measurement of root resistance was done as follows. With roots in water, the vacuum tubing was attached, secured, and filled partially with water. The pressure was reduced to 150 torr for 5 min to clear the root system of air bubbles. The source of bubbles was not known, but the treatment was necessary to prevent air bubbles from lodging in the pipet leater in measurement. Water in the glass "Y" tube could be observed to ensure that bubbling had stopped. The vacuum was then released and a treatment at reduced pressure of 500 torr was applied. The water level at each position was individually adjusted to the lower end of the pipets by introducing water from a 50 ml syringe through the serum cap on the arm of the glass "Y". An initial reading of each pipet was taken followed by a final reading after 15 - 30 min.

After measurement of 3 - 6 replicates, root systems were oven dried and weighted (only the fibrous portion was weighed, excluding the stem portion of the original cutting). Water flow was expressed as water flow per hr per 100 g of dry roots.

RESULTS

The influence of light treatments on growth cessation, total shoot growth, and extent of shoot maturation is shown in Table 1. Plants (NI) and shoots (NI-SP) exposed to night-interrupted photoperiods had a greater percentage of actively growing shoots than plants exposed to natural daylengths. Data taken on total nodes per shoot, mature nodes per shoot, and percent mature nodes per shoot are presented as overall averages of plants in the treatment and as averages for actively growing shoots for comparison purposes (Table 1). Since not all shoots were actively growing, this will allow a comparison of these shoots versus the average shoot in the treatment.

NI plants had the greatest average number of nodes, about 2 or 3 more than other treatments. Those shoots still actively growing on September 3 ahd about 3 or 4 nodes more than the overall average. However, there were no differences among light treatments in numbers of mature nodes (i.e., nodes with brown coloration, indicating development of periderm). Actively growing shoots also had mature nodes, although perhaps an average of 1 node less per plant. This resulted in differences in % mature nodes per shoot, ND-treated plants, and shoots having the most, and actively growing shoots having the least.

Hardiness assessments were made on 4 dates throughout acclimation (Table 2). By the first sampling date, September 15, primary

bud and cane tissues had attained significant hardiness. In general, there were only small (2 to 4°C) hardiness increases for most tissues on the following 3 sampling dates.

Hardiness differences due to light treatments or node positions on the cane were sporadic (Table 2), but generalities can be made. Where differences exist as a function of light treatment, NI-treated plants were least hardy and ND-treated plants most hardy. Shoots of split plants showed no clear relationship to one light treatment or the other, although on several occasions, NI-SP shoots had the lower hardiness level of NI-treated plants.

Differences in hardiness due to node position were most prominent on the first and last sampling dates when apical nodes were less hardy than basal nodes.

Water content measurements (Table 3) were taken only on the first 2 sampling dates, but no consistent differences were seen due to light treatment. Differences due to node position were only seen on the second sampling date when node 13 was included in the study.

Light treatment had no effect on vine root resistance, as measured by suction-induced water flow (Table 4). However, root resistance increased over the course of the experiment as indicated by a decrease in water flow from 2.5 to 0.9 ml per hr per 100 g of dry roots.

DISCUSSION

Night interruption significantly delays cessation of shoot growth as evidenced by the greater number of nodes per shoot and the percent of shoots with actively growing tips (Table 1). Although cessation is not accompanied by the formation of a terminal bud in grapevines, as it is in other woody plants (18), the process does seem to be under photoperiodic control. Why only 17% of the NI-treated plants had actively growing tips, and not more, is unknown, but one might speculate that the night interruption was insufficiently intense. Although the intensity used here is comparable to that used in other studies (6, 8), the light intensity threshold for photoperiodic response in grapevines is not known. Cool night temperatures also may have played a role; several nights in August temperatures (Figure 3) were below 10°C and low temperature may be able to override a photoperiodic signal (8).

Shoot maturation (Table 1) was monitored as nodes developing brown cane color (7), which is associated with periderm development and death of epidermal and cortical tissues exterior to its origin (16). Periderm development in young seedlings of woody plants is affected by many environmental parameters (1), including photoperiod, but factors affecting periderm development in grapevines are unknown.

Neither night-interrupted photoperiod nor presence of an actively growing shoot tip affected shoot maturation (Table 1).

Although light treatments yielded minor (1 - 3°C) differences in hardiness, night-interrupted photoperiods did not prevent acclimation in this study (Table 2) and these results agree with those reported for Cornus (12), apple (8), and Viburnum (10). Consequently, without a clear distinction between hardiness of ND and NI plants, it is impossible to draw conclusions about split vines.

Since the data implicating short-day control and phytochrome involvement (12) are so conclusive in growth chamber studies, field results are puzzling. Several explanations may be proposed:

1. Plants are responding to a signal from an endogenous rhythm, which may bring about acclimation even under "noninductive" conditions (8, 21). It is well known that certain tropical tree species exhibit discontinuous shoot growth without any apparent environmental signal (24). Acclimation may be brought about by internally controlled shoot growth cessation without the SD trigger.

2. The most noticeable difference between the two sets of conflicting reports is the total use of artificial light in growth chamber studies (6, 20) versus the use of natural light in outdoor or greenhouse studies (8, 12). During the course of the studies using natural light, the sun changes in elevation. This causes changes in spectral distribution especially the ratio of red to far-red light at twilight (17) and these changes may have influenced or overriden night break or red light treatments.

3. Another possible explanation may lie in the reinterpretation of growth chamber studies. In these studies, plants are grown as rooted cuttings under warm temperatures and 16 hr photoperiods for 8 - 10 weeks (12) after which they are placed into short-day conditions or retained under long-day photoperiods. After a few weeks, SD plants set a terminal bud while LD plants presumably continue to grow. It is generally at this point where hardiness is assessed. Reinterpretation is required because no indication is given as to where the stem segment for hardiness was taken (6, 13). If the sample were taken from the tip, a hardiness difference would be expected because actively growing tissue on LD plants would be much more succulent than tissues from shoots which had set a terminal bud (13). Evidence indicates that both shoot maturation and acclimation proceed from the base of a shoot to the apex (2, Section I), but a cause and effect relationship has not been shown. One can speculate that the SD induction of terminal bud set allows acropetal maturation and acclimation to catch up with shoot growth, while in LD plants shoot growth continues. One of the unknowns in this speculation is the maturation status of long- and short-day shoots and whether SD photoperiod actually promotes maturation or if it is related to tissue age. The results might be to place shoot maturation between photoperiodic growth cessation and cold acclimation as an intermediate process.

In view of the lack of photoperiodic effect on acclimation, it is not surprising that root resistance (Figure 2) was not affected

by light treatment. The increase in root resistance over the course of the experiment (Figure 2) may reflect increased root suberization (13). McKenzie et al. (13) found increased root resistance in SD plants and used this observation as an explanation of water loss in dogwood stems. They imply that root suberization may restrict water absorption enough to cause the tissue water decline, but this argument has several inconsistencies: (1) suberized roots still have the capacity to absorb large quantities of water (4); (2) root-induced stem water stress should have increased stomatal resistance not decreased it; and (3) authors point to large increases in water flow (decreases in root resistance) when roots were killed by boiling. If suberization, a physical barrier, was the cause for increased resistance, killing protoplasts should have had no effect. It seems more likely that the decrease in root resistance may have been due to the death of a physiological barrier, perhaps the root cambium as proposed by Chung and Kramer (4) or due to a physical alteration of the suberin by boiling, i.e., an artifact.

In summary, plants under night-interrupted photoperiods had a greater number of nodes and a greater percentage of shoots with actively growing tips, but number of nodes with periderm was not affected. Cold acclimation was not prevented by night-interruption and in all treatments hardiness early in the acclimation period was greater at the basal node positions than at apical node positions. Root resistance increased throughout the acclimation period, but was not different among treatments.

Further research is needed to determine how environmental parameters affect shoot maturation and whether these affect hardiness. If photoperiod is not critical to acclimation, as suggested in this study, temperature would be an interesting parameter to investigate next. A study of low temperature or of temperature-photoperiod interactions may provide clues as to how cold acclimation in grapevine is controlled.

Table 1. Growth and maturation of shoots of potted 'Concord' grapevines under natural and night-interrupted photoperiods. Observations taken on September 3, 1981.

Light Treatment[z]	Actively growing shoots			Total nodes per shoot		Total mature nodes per shoot		Percent mature nodes per shoot	
	No. of actively growing shoots	Total shoots observed	Percent[y]	Overall[x] average	Avg. of shoots actively growing	Overall[x] average	Avg. of shoots actively growing	Overall[x] average	Avg. of shoots actively growing
ND	0	40	0a	20.1b	--	14.8a	--	73.9a	--
ND-SP	1	15	6.7ab	19.6b	23.0	14.3a	13.0	73.2a	56.5
NI-SP	3	15	20.0b	20.7b	24.3	13.6a	12.6	66.7b	50.8
NI	11	40	27.5b	22.8a	25.5	13.8a	13.0	61.8b	51.0

[z]Light treatment abbreviations: ND = natural daylength; NI = night interruption (½ hr incandescent light, 2.4 ± .8 μEcm^{-2}) in the middle of the dark period; SP = designation of split plant; 1 shoot trained into natural daylength (ND-SP) and 1 shoot trained into night-interrupted photoperiod (NI-SP).

[y]Mean separation within columm by χ^2 test, p = 0.05.

[x]Mean separation within column by Duncan's multiple range test, p = 0.05.

Table 2. Effect of night interruption on cold acclimation of bud and cane tissues of potted 'Concord' grapevines and the response of plants split between the two photoperiods.[z]

Date	Tissue	Twig Section	Light Treatment[y]			
			ND	ND-SP	NI-SP	NI
Sept 15	1° bud	Base	-12.4a a	-13.0a a	-14.2a a	-13.6a a
		Middle	-13.0a a	-13.0a a	-13.0a a	-14.5a a
		Apex	-11.2b a	-10.7b ab	- 8.3b b	- 9.5b b
	Cane	Base	-11.8a a	-13.6a a	-13.6a a	-13.6a a
		Middle	-11.8a a	-13.0ab a	-11.3a a	-13.0a a
		Apex	-11.2a a	-10.1b ab	- 8.3b b	- 8.3b b
Sept 24	1° bud	Base	-15.5a a	-14.5a bc	-15.0a ab	-13.9a c
		Middle	-15.0a a	-15.5a a	-13.2ab b	-13.2a b
		Apex	-14.5a a	-14.0a ab	-12.5b b	-12.5b b
	Cane	Base	-15.3a a	-15.5a a	-15.0a a	-14.0a b
		Middle	-15.5a a	-15.5a a	-15.0a a	-13.0ab b
		Apex	-13.0b a	-12.5b a	-12.0b a	-12.0b a

Table 2. Continued

Date	Tissue	Twig Section	Light Treatment[y]			
			ND	ND-SP	NI-SP	NI
Oct 10	1° bud	Base	-15.5a a	-15.2a a	-15.8a a	-15.4a a
		Middle	-14.9b a	-15.3a a	-14.5a a	-14.7a a
		Apex	-14.8b a	-15.3a a	-13.2b b	-13.2b b
	Cane	Base	-16.2a a	-16.6a a	-15.8a a	-15.8a a
		Middle	-15.8a a	-15.8a a	-15.8a a	-15.8a a
		Apex	-15.8a a	-15.8a a	-15.3a a	-14.9b a
Oct 29	1° bud	Base	-19.5a a	-19.5a a	-17.0a a	-17.4a a
		Middle	-18.5a a	-19.0a a	-17.5a a	-18.5a a
		Apex	-16.2b a	-17.9a a	16.0a a	-15.7b a
	Cane	Base	-21.3a a	-21.5a a	-20.0a ab	-19.3a b
		Middle	-21.3a a	-20.5ab a	-10.5a a	-19.8a a
		Apex	-19.0b a	-19.0b a	-17.5b a	-17.5b a

[z]T_{50} calculated by means of Spearman-Karber equation, mean separation by χ^2 test, $p = 0.05$. Letters following values indicate significance within columns for an individual date and tissue and letters below values indicate significance within rows. The same letter within a column or row indicates that respective values are not significantly different.

[y]Light treatment, abbreviations: ND = natural daylength, NI = night interruption, ND-SP = shoot of split-plant exposed to natural daylength, NI-SP = shoot of split-plant exposed to interrupted nights.

Table 3. Water content of bud and cane tissues of potted 'Concord' grapevines in response to natural daylength and night-interrupted photoperiods and response of plants split between two photoperiods.

Sampling Date	Tissue	Node[x] Number	Light Treatment[z]			
			ND	ND-SP	NI-SP	NI
			Water content (g H_2O/g dry wt)[y]			
Sept 10	1° bud	1	0.83	0.95	0.85	0.85
		5	0.81	0.97	0.88	0.95
		9	1.02	1.09	1.06	0.99
	Cane	1	0.86	0.85	0.87	0.88
		5	0.91	0.97	0.98	0.91
		9	0.91	0.95	1.00	0.91
Sept 24	1° bud	1	0.82a a	0.90a a	0.80a a	0.78a a
		5	0.84a a	0.90a a	0.89a a	0.88ab a
		9	0.90a a	0.99a a	0.93b a	0.96b a
		13	1.17b a	1.24b a	1.44c b	1.25c a
	Cane	1	0.89a b	0.79a a	0.83a ab	0.84a ab
		5	0.86ab a	0.84a a	0.84a a	0.87a a
		9	0.85ab a	0.85a a	0.85a a	0.85a a
		13	0.81b a	0.83a a	0.92b b	0.90a b

[z]Light treatment abbreviations: ND = natural daylength, NI = night interruption, ND-SP = shoot of split plant exposed to natural daylength, NI-SP = shoot of split plant exposed to interrupted night.

[y]Mean separattion by Duncan' multiple range test, p = 0,05. All values for sampling date, September 10, are not significant.

[x]Nodes numbered from the base of the shoot.

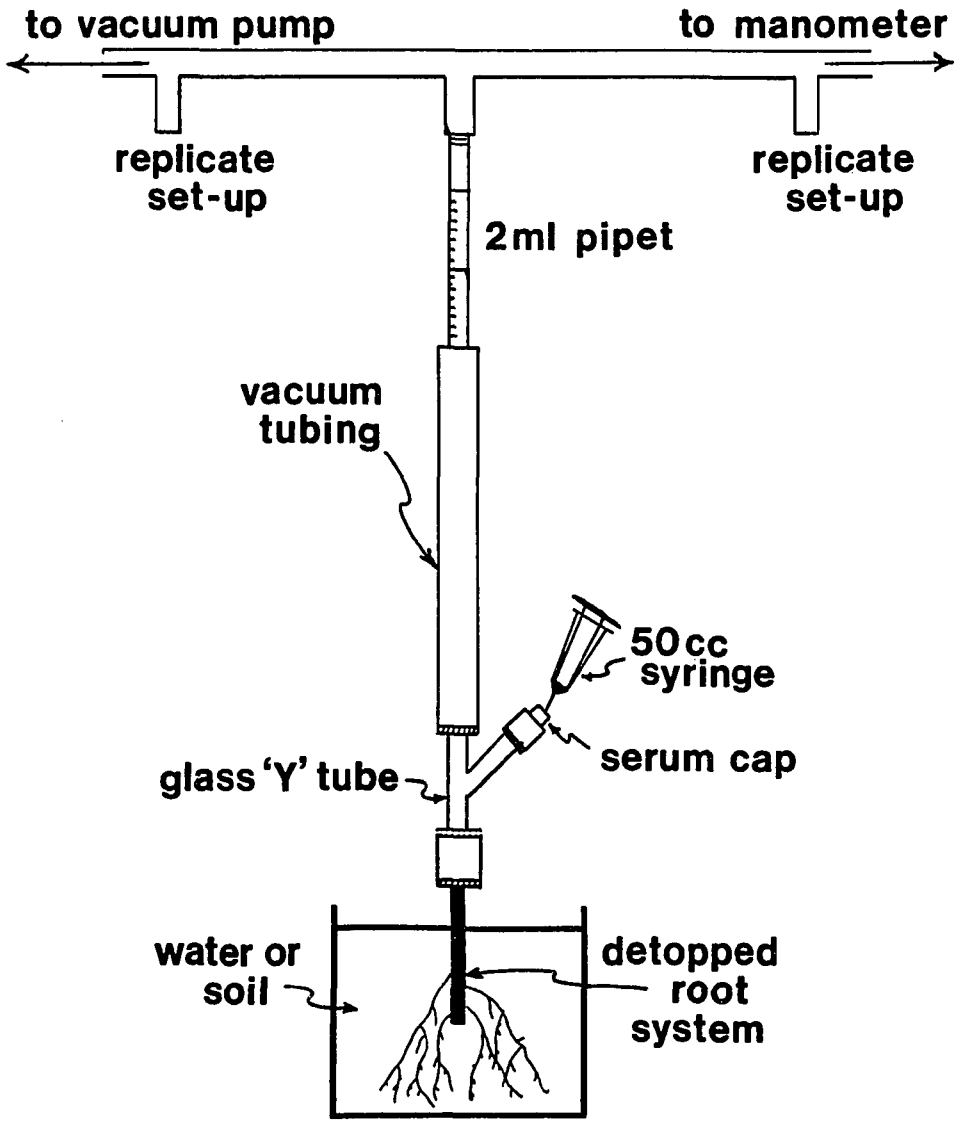

Figure 1. Schematic diagram of apparatus used to measure root resistance by suction-induced water flow through detopped root systems.

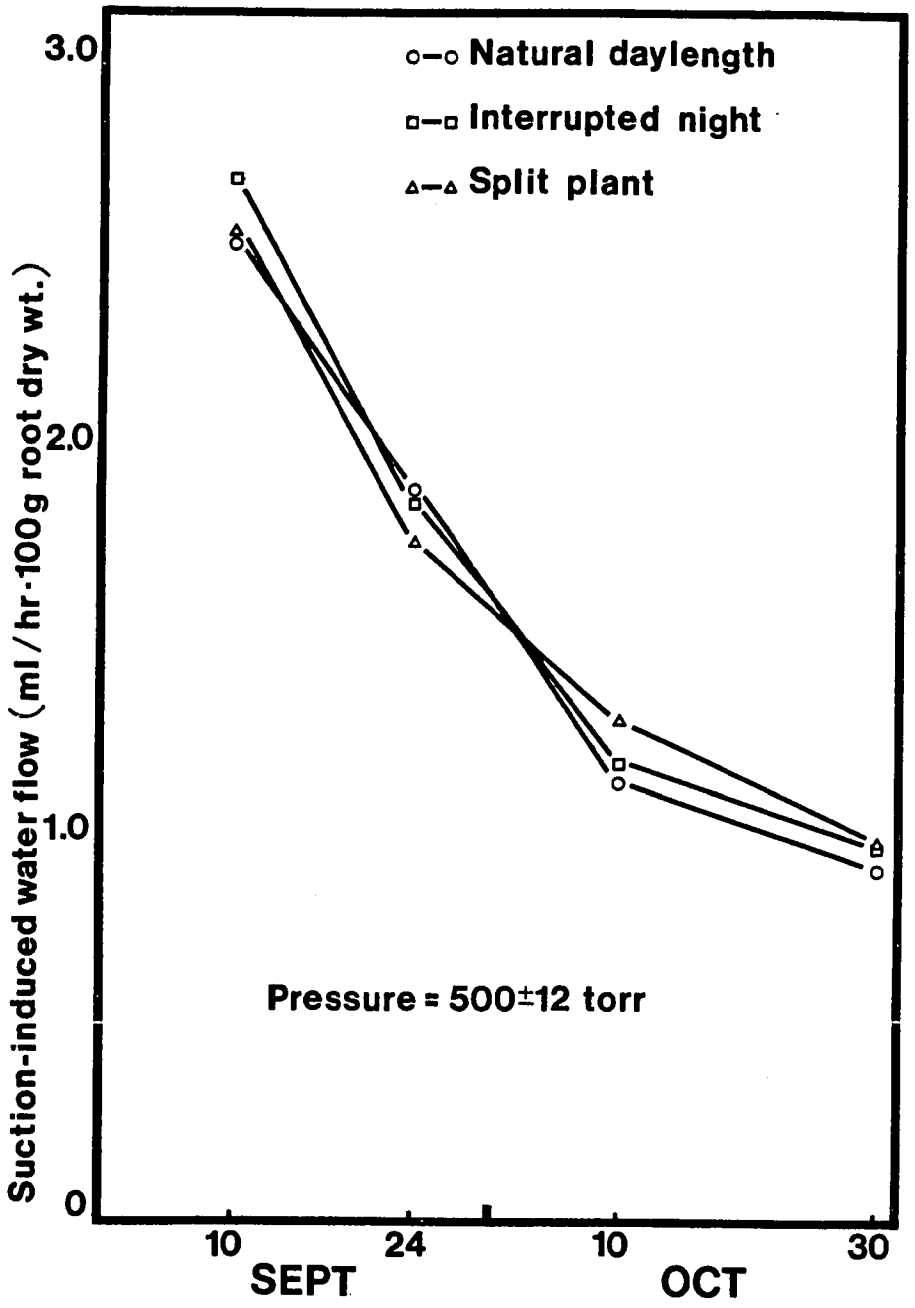

Figure 2. Root resistance of detopped potted 'Concord' grapevines in response to natural and night-interrupted photoperiods and the response of plants split between the two photoperiods (split plants). Root resistance measured as suction-induced water flow (pressure = 500 ± 12 torr).

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SECTION IV

PRELIMINARY STUDIES ON DIFFERENTIAL THERMAL ANALYSIS OF FREEZING IN CANES OF ACCLIMATING 'CONCORD' GRAPEVINES

INTRODUCTION

The freezing of water in plant tissues has attracted the attention of researchers for many years. The early reports by Weigand (41) that ice crystals were observed only in intercellular spaces of hardy woody plants and not within the cells, concentrated attention on extracellular freezing and resultant cellular dehydration. Analysis of the freezing process by Luyet and Gehenio (23) confirmed the presence of a double freezing point in living plant tissue and they proposed that the first point was due to freezing of extracellular water and the second to freezing of cellular water. Whether cellular water froze inside or outside the cell was not determined. This dispelled the then current notion that one of the two freezing points was an artifact of technique (see 23). Later suggestion that the second freezing point resulted from melting and then refreezing of extracellular water induced by outflow of cellular solutes (15) was not further confirmed.

In the above studies and in others (22, 25, 26, 38) initiation of the second freezing point rarely occurred below a temperature of -8 to -10°C. So, in spite of suggestions by Tumanov and coworkers (39, 40) that supercooled water occurred at much lower temperatures and was important in survival of hardy plants, it was still a commonly

held belief in the early 1970s that supercooling played no major role in freezing resistance of higher plants (21).

Since that time numerous reports have demonstrated that freezing avoidance by deep supercooling plays a crucial role in winter survival of plants or plant parts (5). These advances have been possible in large measure by development and refinement of 3 techniques: differential thermal analysis (DTA), differential scanning calorimetry (DSC), and nuclear magnetic resonance (NMR) spectroscopy. Emphasis here will be placed on the DTA studies. Along with technological advances, development of the theoretical foundation for the properties of supercooled water molecules (34, 35, 36) has provided a framework for interpretation of plant data.

Supercooling (also known as undercooling or subcooling) occurs when water is cooled below its equilibrium melting point (34). The water is said to be in a "metastable" state, that is, stable until either a more stable, solid (ice) phase is introduced or until the lower temperature limit is reached (see 6). This lower temperature limit, termed the homogeneous nucleation temperature (HNT), is the point at which ice forms spontaneously in water (34).

In practice, the HNT is determined by cooling, to very low temperatures, an emulsion composed of ultra-pure water finely dispersed in an organic solvent. This method effectively prevents the water from contacting foreign surfaces or suspended insoluble particles which catalyze ice nucleation. The HNT of pure water has been determined to be -38 ± 1° and is in agreement with theoretical

calculations (34). An endotherm near 0°C during a melting cycle of pure water provides evidence that supercooling is taking place and that freezing was not an artifact of the technique. Solute such as glucose, NaCl, or urea added to water lowers the HNT more than it lowers the melting point. This suggests that the effects of solutes at very low temperatures are more complex than the simple reduction in water activity which depresses the melting point.

Why water freezes at the HNT may be hypothesized as follows: supercooled water shows a tendency towards "icelikeness" through formation of clusters of water molecules; these clusters are subcritical in size, but favor a structure from which ice can easily nucleate (35). The authors proposed that this metastable, subcritical state becomes unstable at the HNT: (a) cluster distribution increases in mole fraction and in average and maximum cluster size with supercooling, such that some clusters reach critical size at the HNT, or (b) the critical cluster size capable of growth to macroscopic size is an exponential function of supercooling, decreasing as supercooling increases, so that below the HNT clusters smaller than those that would allow nuleation at -40°C assume critical character. More detailed discussion of behavior of supercooled water can be found (34, 35).

Application of this theoretical information to living cells (36) indicates that yeast cells do not survive indefinitely at temperatures above the HNT but, in fact, die by first order kinetics. The rate constant of death is a function of storage temperature.

Rasmussen et al. suggest that yeast (and perhaps higher plants) generate substrates on which ice can form, but corroboration has not yet been provided.

Studies on Higher Plants

Stem Studies

Early experiments on stems of deciduons agalea performed in 1967 by Graham and Mullin, published must later (10), demonstrate that an exotherm seen at -40.5°C is consistently associated with xylem injury. Later Quamme and coworkers (31, 32) reported that apple twigs have 4 exotherms--the first 3 occurring at relatively high temperatures, unassociated with injury of any tissue, while the 4th coincides with killing temperature of xylem ray parenchyma. This lowest exotherm is independent of bulk water freezing, cooling rate, and tissue viability. Presence of a melting endotherm at -5°C provides further evidence that the exotherm is, in fact, due to freezing of supercooled water.

Studies on infiltration of apple wood with various solutes led Krasavtsev (20) to conclude that supercooled water is prevented from flowing out of cells by a complex barrier composed of the cell wall, the plasmalemma, and the outer layer of protoplasm. The suggestion of a role for plasmalemma and protoplasm contradicts conclusions of Quamme et al. (32) by DTA and Burke et al., (4) by NMR that the living state of tissue is not responsible for the freezing pattern in xylem parenchyma. The potential for artifacts caused by

solute infiltration seems quite high and so might place a question on Krasavtsev's data (20).

In several woody plants species, xylem freezing occurs over a span of 20°C when single cells or groups of cells freeze in independent events; water or ice migration between frozen and unfrozen cells is slow, if present at all (11). Death of ray cells as assessed by staining and browning increases over the 20°C span in proportion to the % frozen water (13). Low temperature exotherms in apply can be lowered or raised by pretreatment with cold (-3°C) or warm (+5°C) temperatures respectively (12) even if treated for a very short period of time.

Bud Studies

Early experiments of Graham and Mullin (10) suggest that bud primordia of deciduous azalea survive low temperatures by supercooling as evidenced by the one-to-one association of the number of exotherms (between -17° and -27°C) and number of dead florets. A large exotherm seen at -8°C was attributed to freezing (without injury) in floral axes and bud scales. Later evidence that primorida supercooling is indeed taking place was provided by DTA and NMR studies (9) and further corroboration by DSC indicated that all of the the heat released was due to freezing water (7). That dead primordia also yield exotherms suggested to the authors that supercooling is due to structural features of primordia not to living state of protoplasm.

Studies on water relations of Rhododendron flower buds indicate that water in florets migrates to frozen bud scales in response to a low temperature (-5°C) treatment, resulting in increased floret supercooling (16). Time required for water migration from primordia to bud scales may explain the dependence of exotherm temperatures on cooling rate during floret supercooling (9, 19); at faster freezing rates, water cannot migrate fast enough and primordia with greater water content supercool to a lesser extent. Natural dehydration of florets during cold acclimation (18) coincides with a shift in DTA pattern from 1 large exotherm in autumn to 3 smaller sharp exotherms in winter (19).

After studying dye movement in Prunus buds, Quamme (30) proposed that, when bud scales freeze, water migrates from the bud axis to scales creating a dry zone which acts as a physical barrier to ice inoculation from stem xylem. Recently, Ashworth (1) provided evidence that it is viability of the bud axis, not the primordium, on which supercooling depends; if the axis does not freeze in a "normal" fashion, then supercooling does not take place.

The absence of xylem connections would also facilitate supercooling by isolating primordia from stem freezing. Subsequent xylem development in spring might serve as a mechanism for irreversible dehardening. In a study of Prunus buds (2), 11 of 14 species which had no xylem connections to bud primordia (as indicated by dye movement) demonstrated supercooling. The remaining 3 species had xylem connections and did not supercool, but authors did not specify how this related to primordia cold hardiness.

The two types of enhancement of bud supercooling, water migration from a floret (16) and physical isolation of a primordium by creation of a dry zone due to water movement in the bud axis (30) are not mutually exclusive and may to some extent be occurring simultaneously. This remains to be elucidated.

Water migration may also be important in shoot primordia in conifer buds. Primordia show exotherms at -30°C when left intact in an excised terminal bud, but exotherm at only -12°C when excised from the bud (37). Since artificially decreasing water content of excised shoot primordia decreases the exotherm and since bud scale removal has no effect, Sakai (37) suggested that "crown" tissue of the shoot primordium is the recipient of migrating water.

It is clear from these studies that while supercooling in both stems and reproductive primordia depends on structural integrity, the 2 processes differ in that the latter is dependent on cooling rates and presence of viable collateral tissues while the former is not.

The presence of low temperature exotherms has ecological and horticultural ramifications. Plants cannot be grown in climates which have a likelihood of frequently achieving the temperature at which exotherms occur and this can limit the natural distribution of woody plants (8, 3) and the location of temperate fruit tree production areas (29). Since presence of exotherms or the temperature at which they occur is not uniform for all species (33), introgressing desirable supercooling characteristics may improve hardiness of perennial crops.

Studies on freezing pattern of buds and stems of grapevines are very limited. Cane material of Vitis riparia (27) have 5 exotherms, 2 nonlethal, and 3 lethal. Of the lethal exotherms, one each is associated with injury to phloem ray parenchyma, to nonray phloem and to xylem tissue. Third and fourth exotherms cannot be seen if freezing rate is very slow, but authors did not indicate whether this affected the presence or absence of injury. The stem low temperature exotherm is about -45°C and rate dependent (28).

Unlike tissues previously cited, V. riparia buds have no bulk water exotherm. Exotherms are not present in fully acclimated buds (27) indicating that freezable water is either absent or in low, undectable amounts. If buds are thawed, 1 exotherm appears for each of the 3 bud primorida (28) leading authors to speculate that the degree of hydration is responsible for changes in seasonal pattern of bud hardiness.

This section of the dissertation began with 2 objectives in mind: (a) to assemble the equipment necessary to measure freezing events by DTA and (b) to study acclimation by DTA in hope that changes in the freezing pattern would shed light on important changes accompanying cold hardening. Because these efforts were only partially successful, the results presented here should be viewed as a preliminary investigation. Not all points raised in the introduction will be addressed by these studies, but they will provide a frame of reference for interpreting the results.

MATERIALS AND METHODS

Plant material used in these studies were collected from shoots of mature 'Concord' grapevines growing at the Horticulture Research Center of Michigan State University in 1980-82. Vines were trained to an upper-wire (1.8m) bilateral cordon (Hudson River Umbrella) and were large (1.2 - 2.8 kg of cane prunings) and yielded 5 - 6 tons/acre.

Shoots were selected from canopy positions well exposed to sunlight, but were chosen without respect to vine size or number of clusters per shoot. The node position on the shoot from which a sample was taken is indicated in each figure and nodes were numbered from the base of the shoot. Material consisted of stem cross-sections, 0.8 - 1.0 cm in length, taken from the middle portion of an internode.

Differential Thermal Analysis

The apparatus used for measuring freezing events by differential thermal analysis (DTA) was similar to that described by Quamme (30) and is shown in a schematic diagram (Figures 1A and 1B). The differential thermocouple was made in the laboratory by connecting 2 copper-constantan thermocouples (26 gauge) in series. The wire junctions were twisted together, soldered, and sharpened

slightly. One thermocouple was inserted into the pith of a freshly cut stem section and the other was inserted into an oven-dried sample, the latter being used as a reference. Dry biological or nonbiological material may be used as a reference, the main criterion being that the reference not exhibit a phase transition within the experimental range of temperatures.

The heat sink (Figure 1B) was made of a cylindrical aluminum block (2" diameter x 3" high) into which 2 holes had been bored (0.5" x 2.5" deep). One stem tissue was placed into each hole and held in place by a cork stopper. Mineral oil was introduced into both holes to provide thermal contact between the heat sink and the tissues, and it showed no phase transition to at least -50°C. A single copper-constantan thermocouple was also placed into the hole with the reference tissue in order to record the temperature at which freezing events were taking place.

Sample temperature and differential temperature were recorded simultaneously on a Leeds and Northrup Speedomax 250, 2-pen potentiometer. The differential signal was amplified through a Leeds and Northrup linear amplifier while the temperature signal was recorded directly. Temperature drop was achieved by placing the heat sink into a Revco chest freezer which was held at -65° to -85°C. The differential thermocouple generated a signal when the cooling rate of the sample differed from that of the reference. Data recorded on a time x temperature scale were transformed to a temperature x exothermic temperature scale.

Tissue Water Content

Tissue water content was measured and calculated as described in Section I.

RESULTS AND DISCUSSION

The shape of DTA patterns presented here (Figures 2 - 4) is a function of chart speed, rate of temperature drop, scale of differential response and sample dimensions. The rate of temperature drop is, in turn, dependent on freezer temperature and size of the heat sink. These factors were not consistent throughout the study as the technique was evolving and this complicates comparisons of groups of DTA patterns.

Two studies were made of the influence of node position in DTA pattern, one in mid-summer (July 25) and one in late summer (September 4). In the mid-summer study (Figure 2), 1 to 2 exoterms were seen depending on whether the shoot section was from basal or apical positions. The change from 2 to 1 exotherm occurred between the 11th and 14th node position (the 14th having a small shoulder) and corresponded closely to an increase in water content (Table 1), especially in the pith. Exotherm differences may be due to a lowering of the freezing point of water in a certain tissue or to structural inhibition of ice crystal growth (17, 38). Data were not taken on whether the freezing pattern had any relationship to killing temperature of these nodes. Hardiness data presented in Section 1 indicate that differences between basal and apical tissues are seen as early as the first week in August, but differ slightly from year to year.

Decline in stem water content due to death and dehydration of pith cells has been correlated with the first stage of cold acclimation in dogwood (24). However, whether the changes in freezing pattern (Figure 2) are causally related to pith water changes and the extent to which these are important in cold resistance needs further investigation.

In Section II (Tables 1 - 3) significant hardiness increases were associated with increase in tissue maturation and decline in water content. Figure 4 shows DTA patterns from portions of a 7-node shoot section which included mature (brown-colored) nodes at the basal end and less mature (green-colored) nodes at the apical end. Basal shoot sections (Figure 4a) showed 4 distinct and separate exotherms, while apical tissues (Figure 4c) showed 3 exotherms, poorly separated and at warmer temperatures (except for the bulk water exotherm). This strongly suggests that maturation-associated increases in hardiness (Section II, Tables 1 - 3) may be mediated through changes in the freezing process.

The DTA system used in this study (Figure 1) needs refinement, especially in control of the rate of temperature drop, in order to provide better information. Further sophistication would allow bud exotherm measurements. This would be advantageous because primary bud hardiness in 'Concord' grapevines is usually less than that of critical stem tissues (14).

Further research is needed to determine if a close relationship exists between hardiness and DTA profile. This has not been

done in grape stem tissues and is especially important because the critical tissue differs. In apple (31), Pyrus and Prunus species (38) and other woody species (1, 8), the most cold sensitive tissue in mid-winter is the xylem ray parenchyma, which yields a large, easily detectable exotherm at the killing temperature.

In grape stems, the critical tissue is the phloem-cambium layer, which is usually killed at a warmer temperature than the xylem, precluding the importance of the LTE (28). Phloem injury in wild grape (Vitis riparia) is associated with 2 exotherms, 1 coinciding with death of phloem ray parenchyma and 1 with nonray phloem tissue (27). These exotherms are smaller than the xylem exotherm and can even disappear during very slow rates of freezing. Although the authors do not indicate whether a change in injury was associated with exotherm disappearance, this does point up the need for further investigation into the relationship between DTA patterns and hardiness of critical tissues.

Table 1. Water content of stem tissues of 'Concord' grapevines on July 25, 1981.[z]

	Tissue			
Node number[x]	Phloem + cortex	Xylem + pith	Xylem only	Pith only
2	3.05a	2.01d	1.56b	5.34a
5	2.94a	2.02d	1.60b	4.53b
8	2.78b	1.96d	1.58b	5.68b
11	2.48c	2.18c	1.50b	4.95b
14	2.33d	2.47b	1.58b	7.10a
17	2.49c	3.07a	1.88a	7.52a

[z]Water content expressed as g H_2O/g dry wt. Mean separations within columns by Duncan's Multiple Range test, p = 0.05.

[x]Nodes numbered from the base of the shoot to the apex.

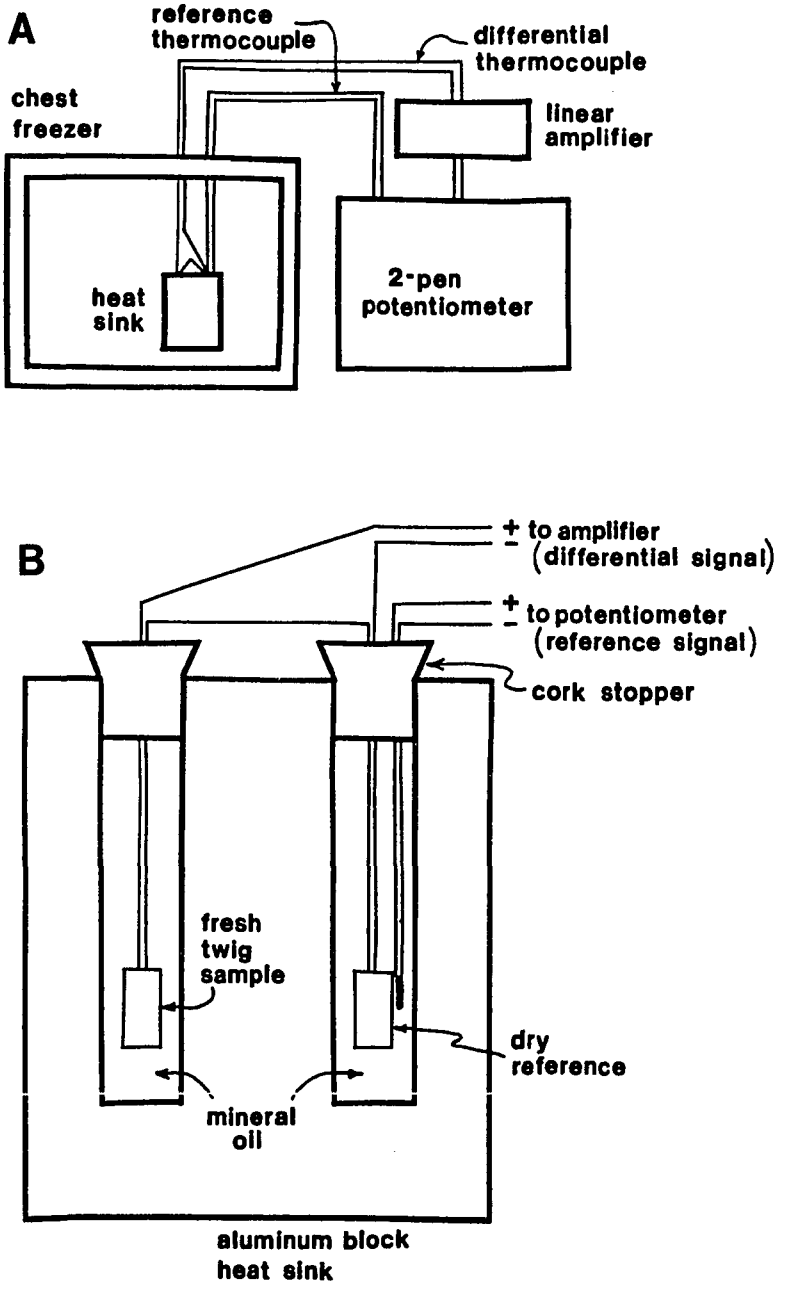
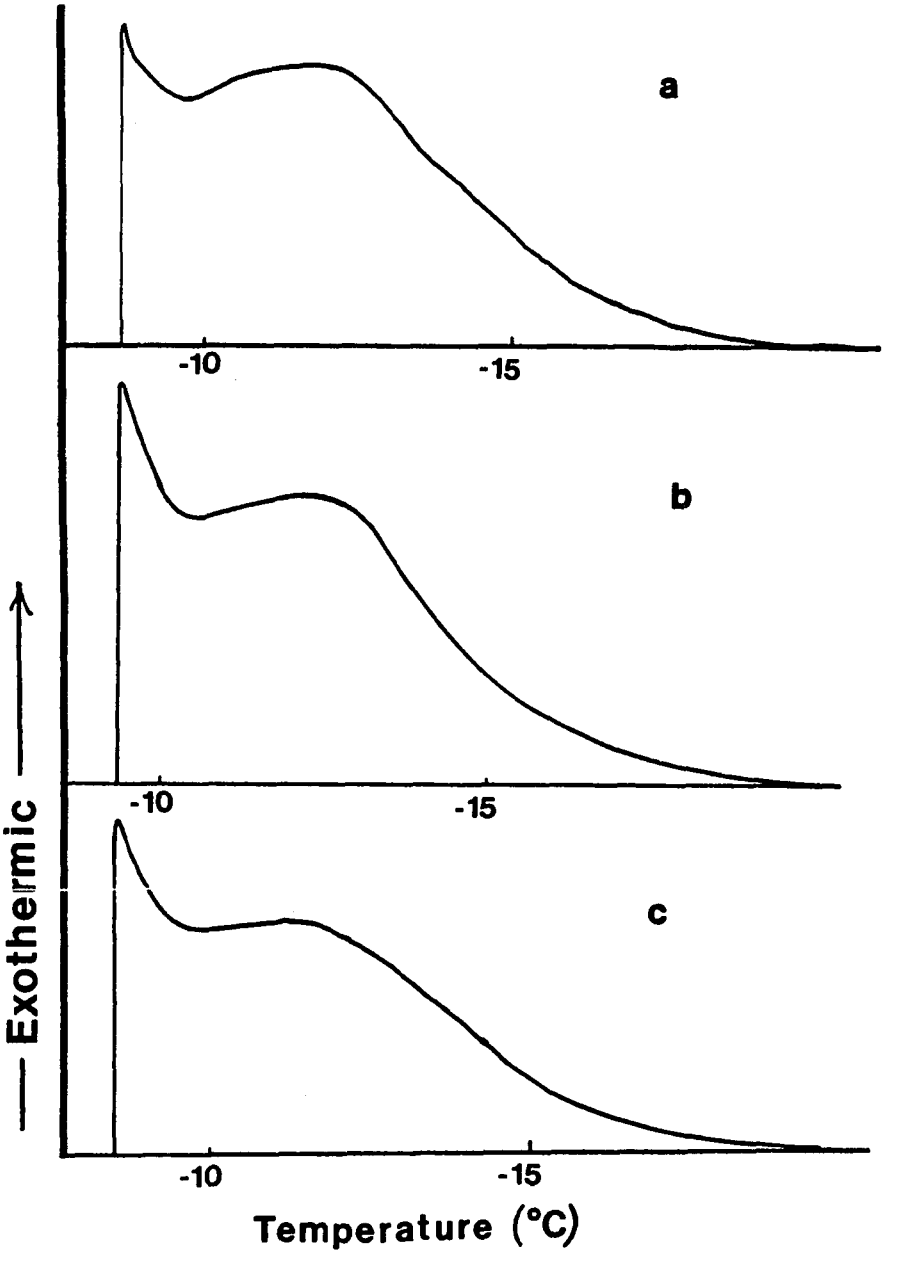


Figure 1. Schematic diagram of apparatus used in differential thermal analysis (A) and close-up of aluminum block heat sink and thermocouple placement (B).


a
-10
-15
b
-10
-15
c
-10
-15
Exothermic
Temperature (°C)

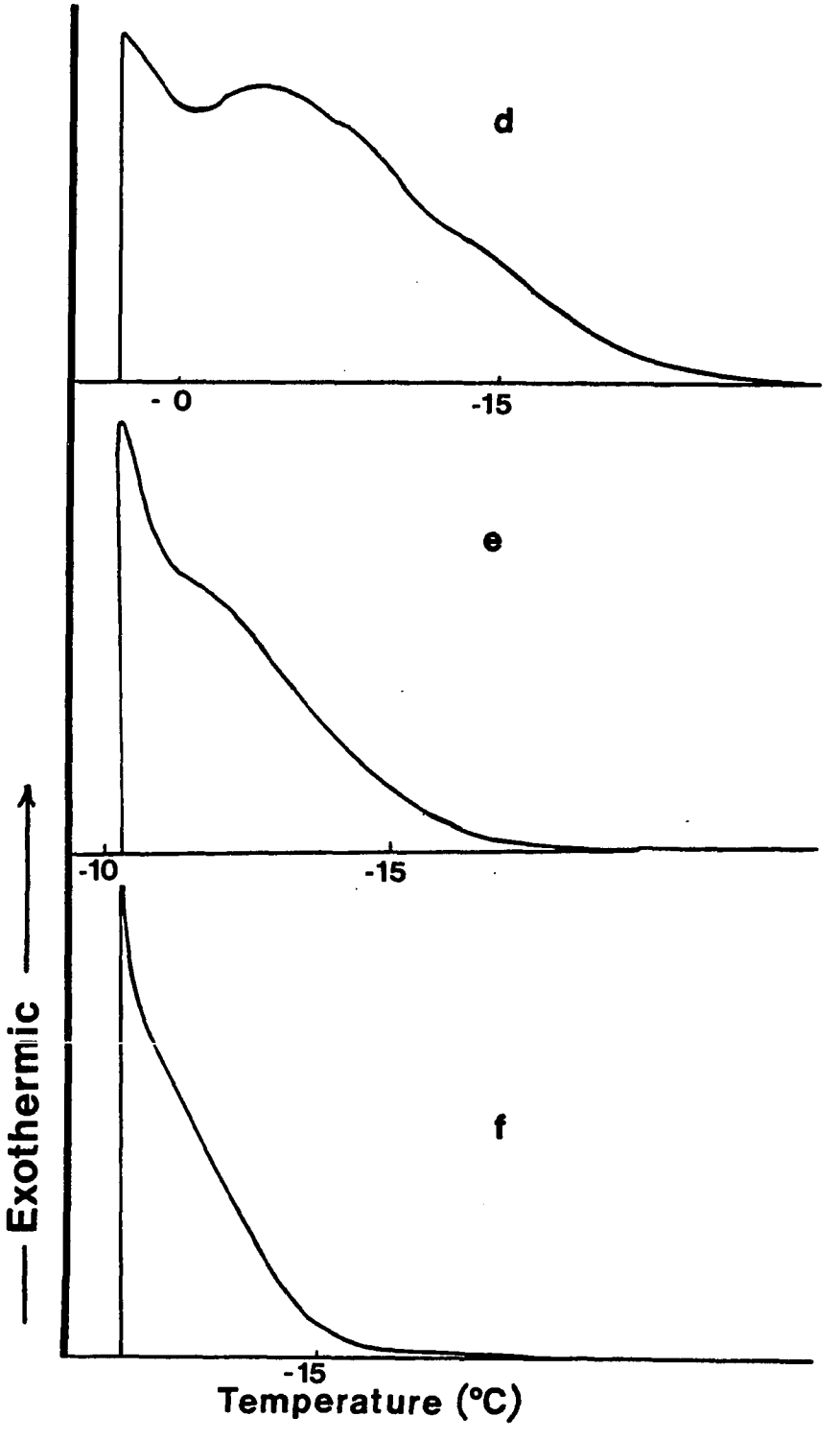
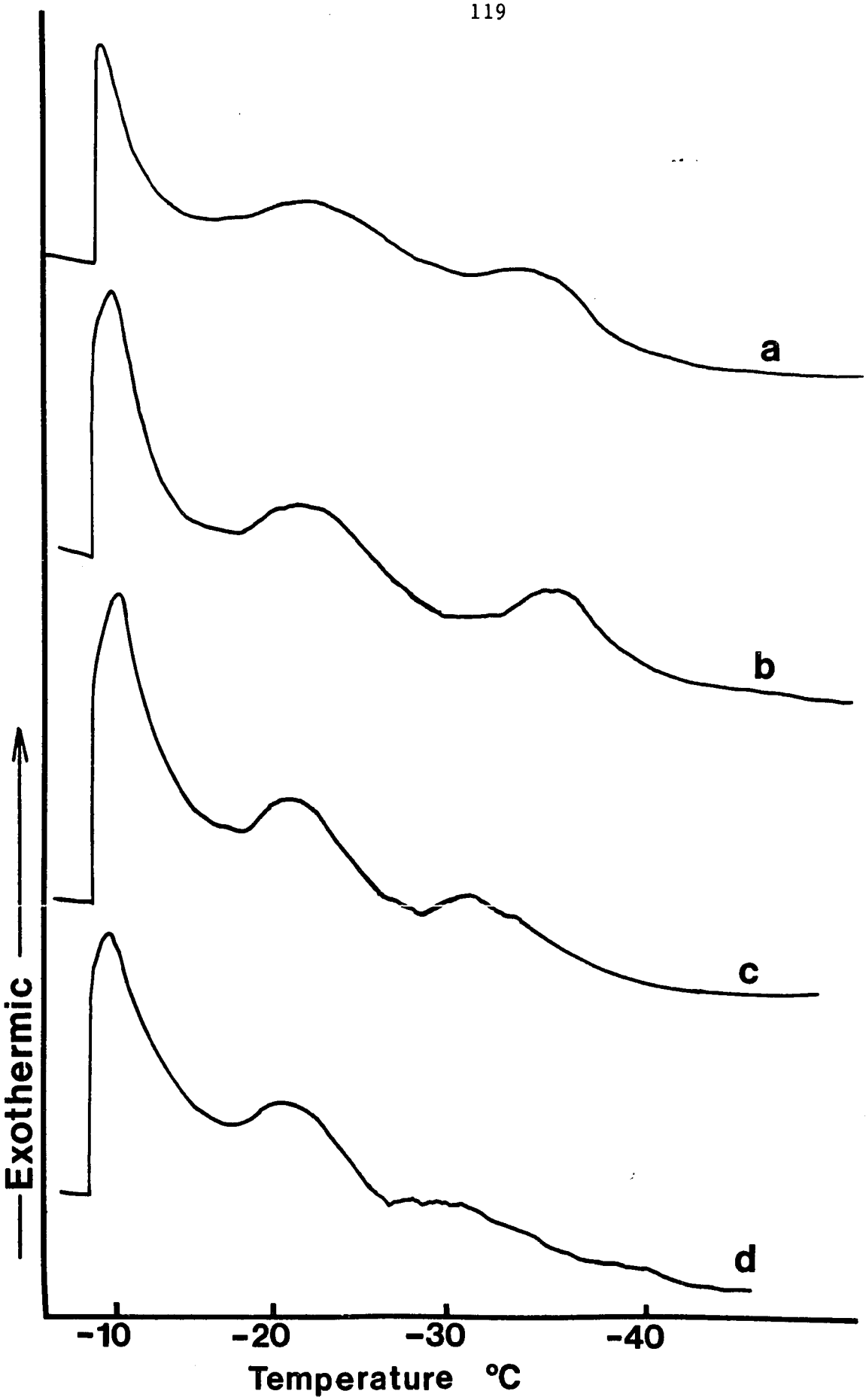


Figure 2. Differential thermal profiles of stem cross-sections of various internode positions from a single shoot of 'Concord' grapevine, July 27, 1981. Sections were collected from the middle of an internode apical to a numbered node and numbering commenced from the base. Profile a = node 2, b = node 5, c = node 8, d = node 11, e = node 14, and f = node 17.


a
b
c
d
Exothermic
-10
-20
-30
-40
Temperature °C

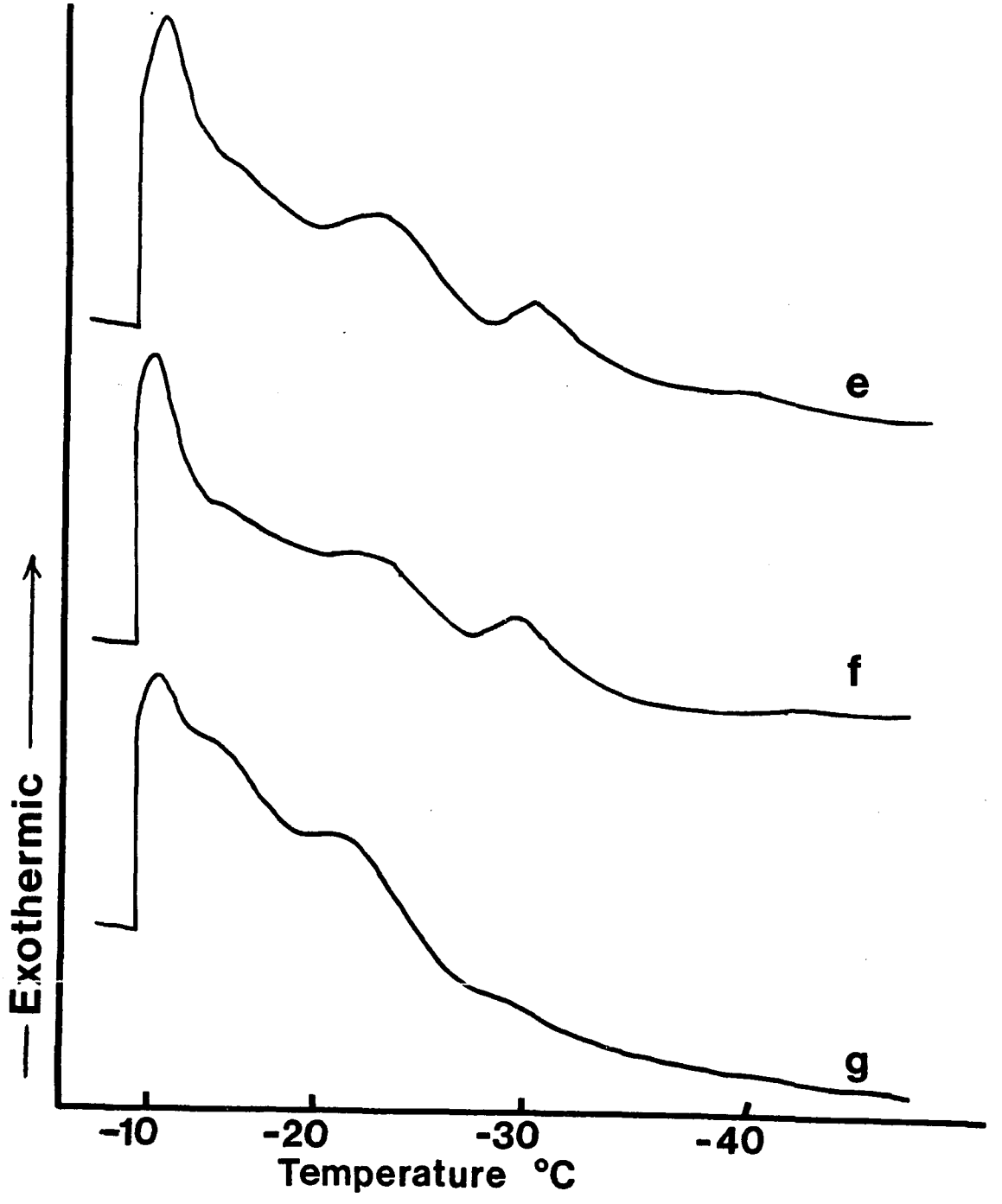


Figure 3. Differential thermal profiles of stem cross-sections of various internode positions from a single shoot of 'Concord' grapevine, September 4, 1982. Sections were collected from the middle of an internode apical to a numbered node and numbering commenced from the base. Profile a = node 2, b = node 5, c = node 8, d = note 11, e = node 14, f = node 17, and g = node 20.

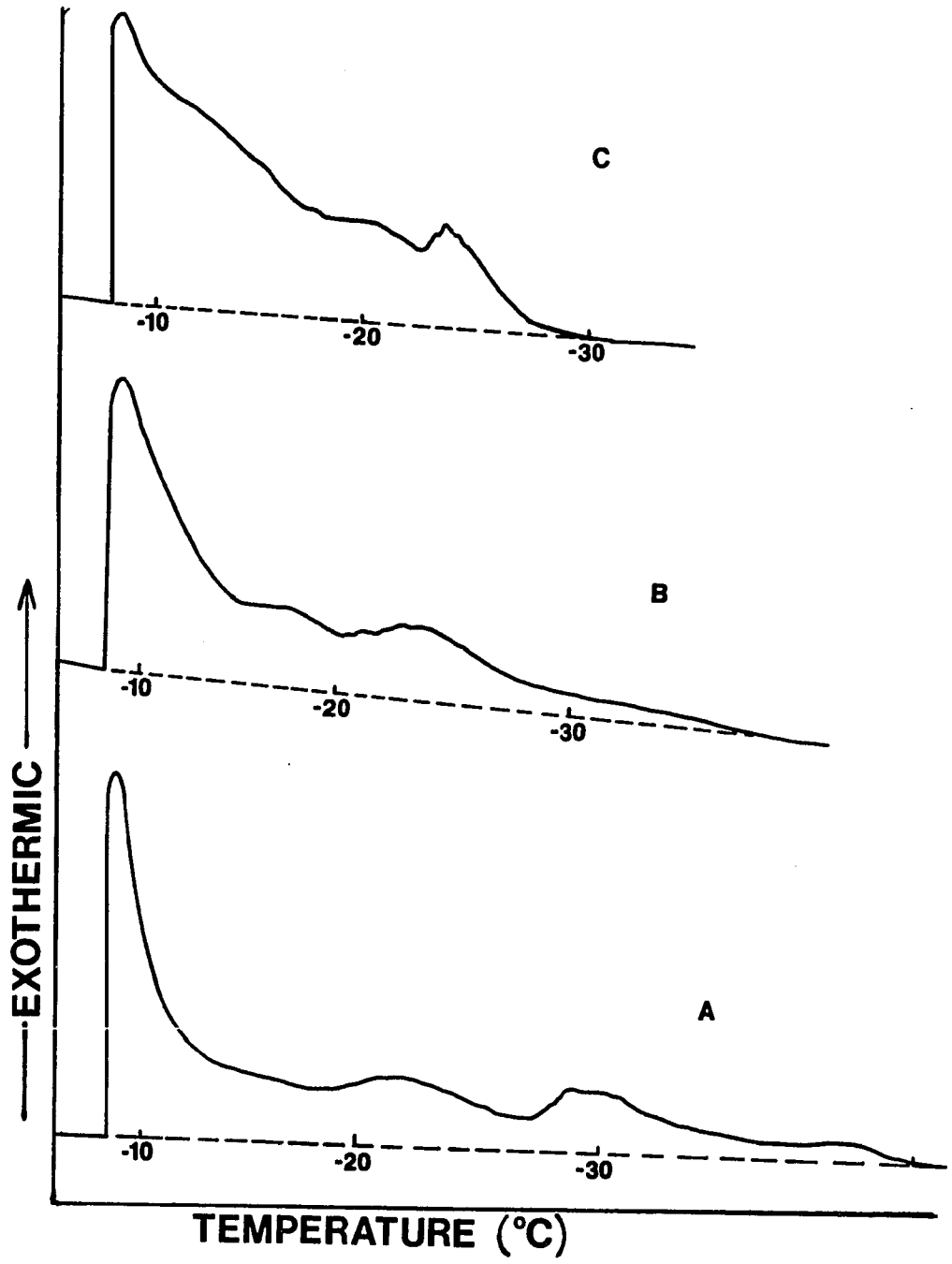


Figure 4. Differential thermal profile of a stem cross-sections of a seven-node shoot segment of 'Concord' grapevines. The segment was selected so that it was mature and brown-colored at the base (profile a), less mature and green-colored at the tip (profile c), and light brown-yellow in the intervening area between the tip and base (profile b).

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SECTION V

STUDIES ON ROOT RESISTANCE OF POTTED 'CONCORD' GRAPEVINES

INTRODUCTION

Water flow through detopped root systems in response to reduced pressure has been used as a measure of root resistance to water uptake (7, 8, 10, 1975). Water flow in response to different levels of applied suction was found to be linear or log-linear, depending on whether the suction was increasing or decreasing (6, 10).

Root resistance of dogwood increased as a result of exposure to short-day (SD) photoperiods (7, 8) and was correlated with increases in cold hardiness, leading the authors to claim that the SD influence on cold acclimation may be mediated in part by changes in root resistance via root suberization (7). However, when roots were killed by boiling, water flow increased 8 - 10x (7) which suggests that the resistance was due to cell protoplasm or membranes or that boiling disrupted the periderm layer. Grapevines under natural day and night-interrupted photoperiods had equivalent root resistance values (Section III, Figure 2).

Since this technique has not been previously reported to have been used on grapevines, additional studies were conducted to characterize water flow. Also, the technique used in Section III employed 2 pretreatments of soil removal and low pressure (to remove air bubbles) prior to flow measurement and the effect of these is not known. In order to address these areas and again background on root resistance in grapevines, several studies were conducted.

MATERIALS AND METHODS

Potted 'Concord' grapevines used in the following studies were grown identically to those used in Section II. Root resistance was measured as suction-induced water flow, employing either the system used in Section II (and shown again here in Figure 1) or a slightly modified system (Figure 2), detailed below. Handling of plants prior to resistance measurements differed among the studies.

All root systems (except those in Study 2) were prepared as follows: soil was removed by a light pressure water spray; roots were placed into buckets of water and subjected to low total pressure (150 mmHg) which removed air bubbles, preventing them from lodging in the pipet and interfering with subsequent flow measurements. In Study 2, these 2 factors--soil removal and low pressure pretreatment--were investigated for their effect on water flow as explained below.

Study 1

Root systems were subjected to different levels of pressure, either in an increasing or decreasing manner (Figure 3a and 3b, respectively).

Study 2

Pretreatment conditions, soil removal and low pressure removal of air bubbles, were investigated separately for their effects on

root resistance measurements. Soil was removed by a light pressure water spray, but not subjected to low pressure pretreatment.

Low pressure (150 mmHg) was investigated on an apparatus modified (Figure 2) to trap air bubbles away from the pipet and nullify interference. Low pressure was applied either once or twice to see if it effected flow and if the effect was cumulative.

Study 3

Flow through dead root systems was studied by killing roots either by submersion in boiling water for 2 min or by wrapping roots in plastic bags and placing them in a -70°C chest freezer for 12 hr.

RESULTS AND DISCUSSION

Water flow through root systems of 'Concord' vines varies with pressure, but the exact relationship differs, depending on whether the pressure is increasing or decreasing. When pressure is decreasing (i.e., increasing suction) water flow increases in a linear fashion (Figure 3a) while when pressure is increasing (i.e., decreasing suction) flow decreases in a log-linear fashion (Figure 3b). Shirazi et al. (10) reported log-linear pressure/flow relationships in tomato bean and cotton, but the response was seen for both increasing and decreasing pressure. On the other hand, Kuiper (6) showed results opposite to that found in this study, i.e., linear response during increasing pressure and log-linear response during decreasing pressure. The reason for differences from linearity are not known.

Removing air bubbles from root systems by pretreatment exposure to low pressure (150 mmHg) (Figure 4) affected the subsequent flow measurement at higher pressure (590 mmHg). This may be due to the unexplained effect of log-linear flow reduction when pressure is increased (Figure 3); part of the flow difference is an artifact of measurement. Because of the design of the modified system (Figure 2), air bubbles collect in a part of the tubing where they do not interfere with pipet readings. However, the volume of water they

displace at the collection site will nevertheless be erroneously reflected as water flow in the pipet. This may account for some of the difference. An additional treatment of low pressure for 10 min (Figure 4) did not significantly decrease flow. Air bubbles were a problem in measuring root resistance of dogwood, but unreported design features of the system negated the artifact (L. Parsons, personal communication).

Although soil removal from roots of dogwood did not affect root resistance (7), removal of soil from grapevines (Figure 4) greatly decreased water flow. Reason for this is not known, but may reflect loss of root surface by breakage of fine roots during handling.

Flow through root systems killed by boiling and freezing (Figure 5) presents differing results. In the first trial, boiling roots yielded a large decrease in root resistance, in agreement with dogwood results (7), but no differences were found in the second trial. The only known difference between the two trials is that in the first case the explant was pretreated with low pressure while in the second it was not. The relationship between boiling and low pressure treatment may be coincidental, but requires more study to be understood.

If killing the roots alone is sufficient to decrease root resistance, then freeze-killing should be effective, but it was not (Figure 5). Rather, freezing at -70°C for 12 hr had no effect in the first trial and slightly increased root resistance (decreased flow)

in the second. McKenzie et al. (7) suggest that root resistance is due at least in part to living root protoplasm which can be removed by killing it; however, at the same time they hypothesize that it is which root suberization plays the crucial role. These 2 statements are contradictory. If suberization imposes a physical barrier due to development of an impermeable waxy substance, then boiling should have no effect on it except as an artifact.

It is well known that suberized root surfaces are quite permeable (2) by virtue of lenticels, branch roots, and cracks in the periderm (1) and account for a large percentage of water and ion uptake in woody perennials (4, 5). Suberization may account for an increase in root resistance of 2x (2) but not the 8 - 10x seen in dogwood (McKenzie et al., 1974). This indicates that protoplasmic resistance is involved and may occur at the root cambium (2, 3). results here (Figure 4) indicate that handling may influence these conclusions, but living protoplasm cannot account for the bulk of root resistance in grapevines (Figure 5).

Root resistance also increases when root temperature is lowered (3, 10). This occurs in dead root systems and may be caused by increased water viscosity (3). The resistance increase is greater in live systems than dead again pointing to root protoplasm as a component of resistance.

Root resistance also changes diurnally (9, 10) and detopped roots maintain the cycling for up to 2 - 3 days (9). The regulation of the diurnal resistance flux seems to be due to the light

environment of shoots because when the shoot light/dark cycle was changed, root resistance changed with it (9). However, the mechanism for this continued cycling in resistance of detopped plants is unknown.

In this study neither root temperature nor time of day were standardized and these may have influenced variability in water flow from study to study or from plant to plant. Further research is needed to determine how these differences in techniques influence suction-induced water flow in order to elucidate the role of roots in regulating water uptake during cold acclimation.

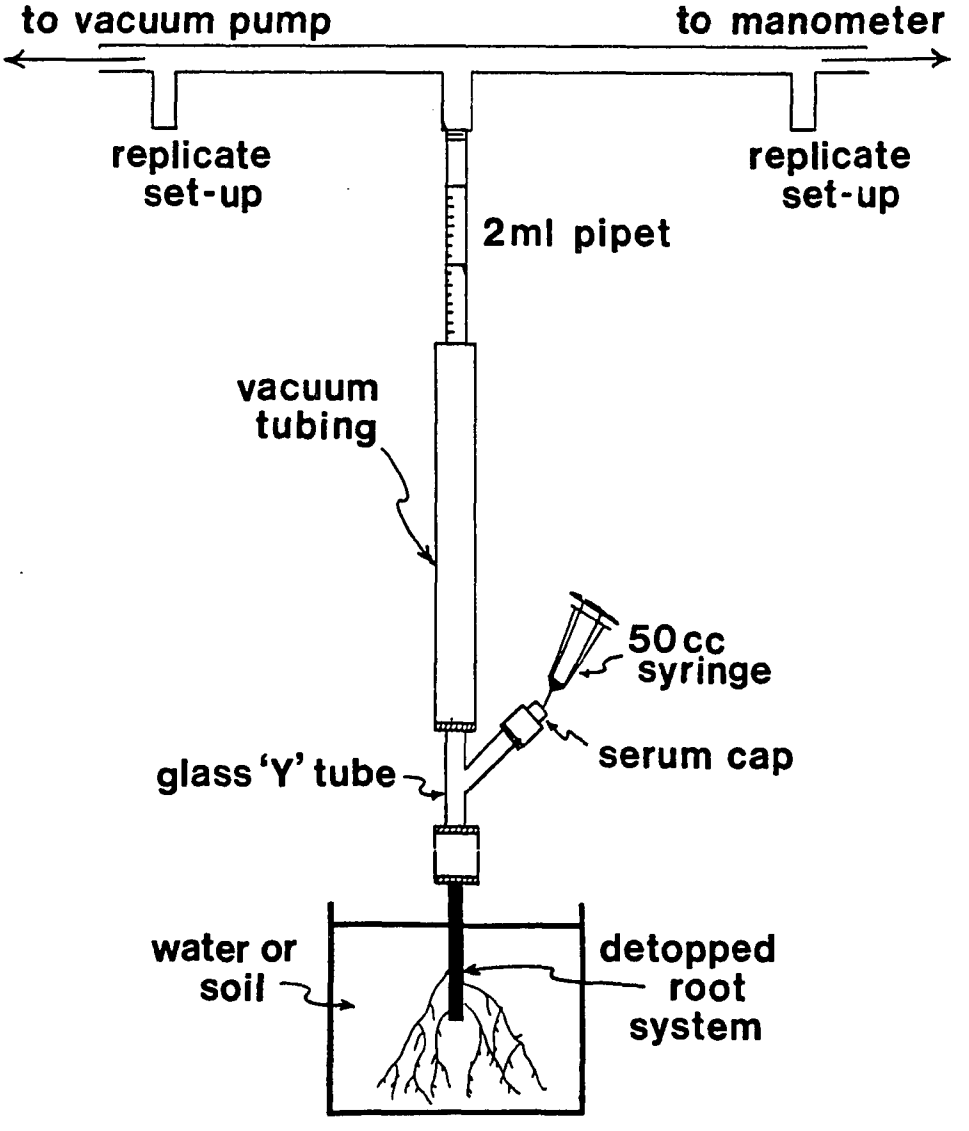

Figure 1. Schematic diagram of apparatus used to measure root resistance by suction-induced water flow through detopped root systems.

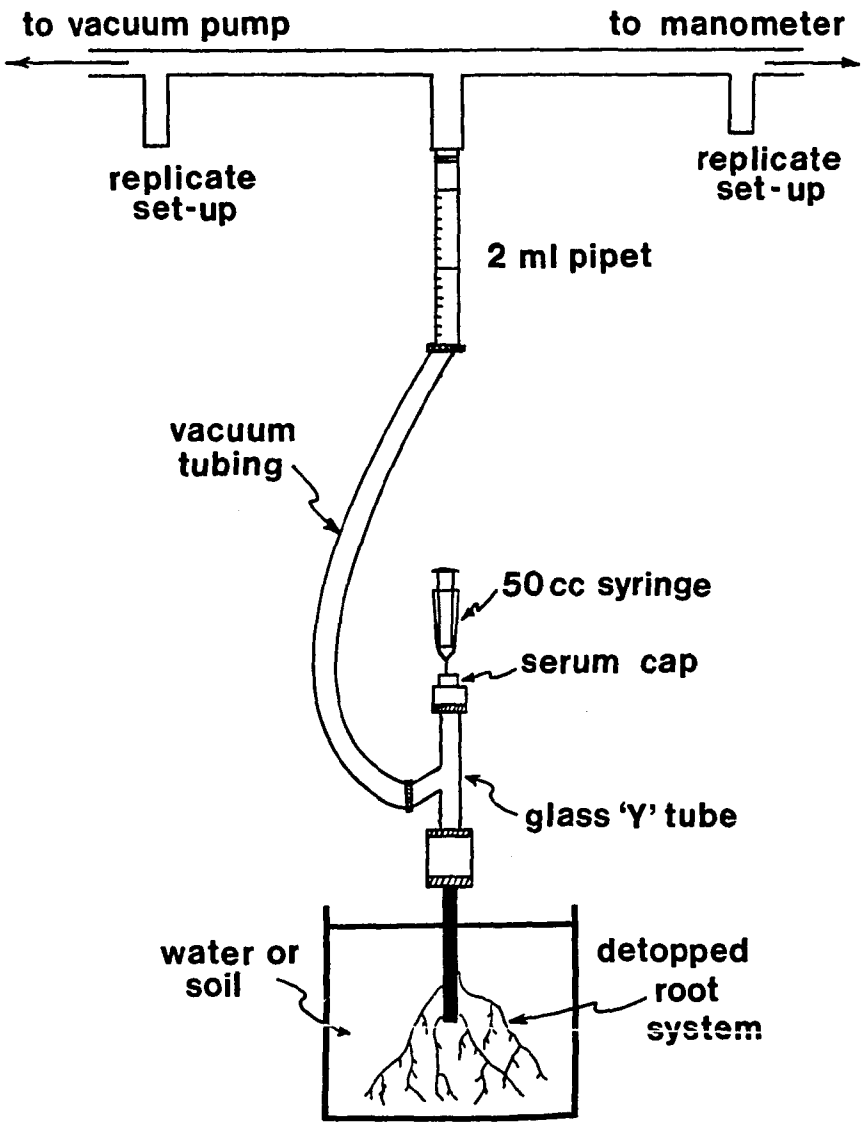

Figure 2. Schematic diagram of apparatus used to measure root resistance by suction-induced water flow, modified to prevent interference by air bubbles.

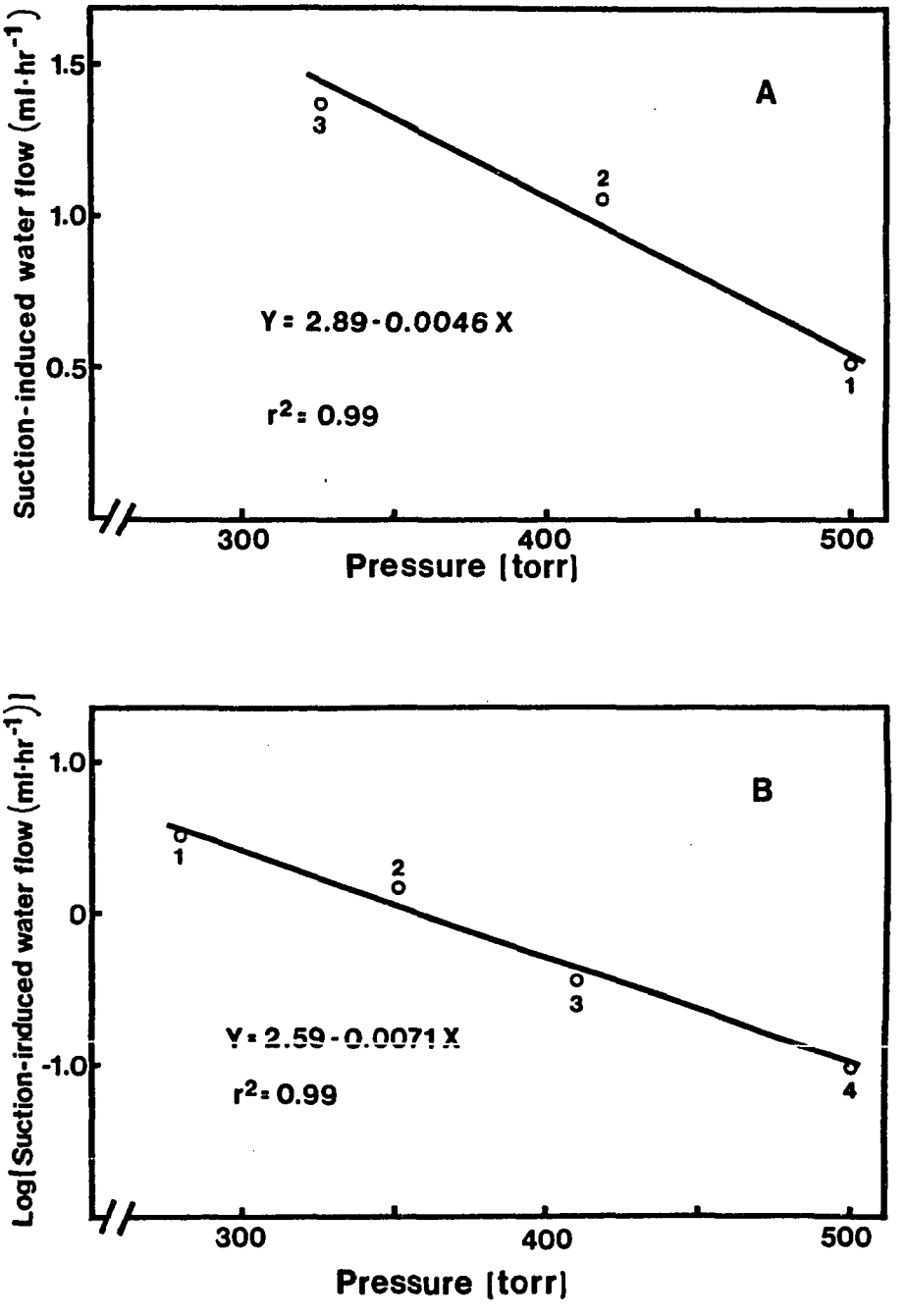

Figure 3. Relationship of suction-induced water flow to the total pressure applied to the stem end of detopped root systems of potted 'Concord' grapevines. Numbers near each point indicate the order in which measurements were taken:
A = decreasing pressure (increasing suction)
B = increasing pressure (decreasing suction).

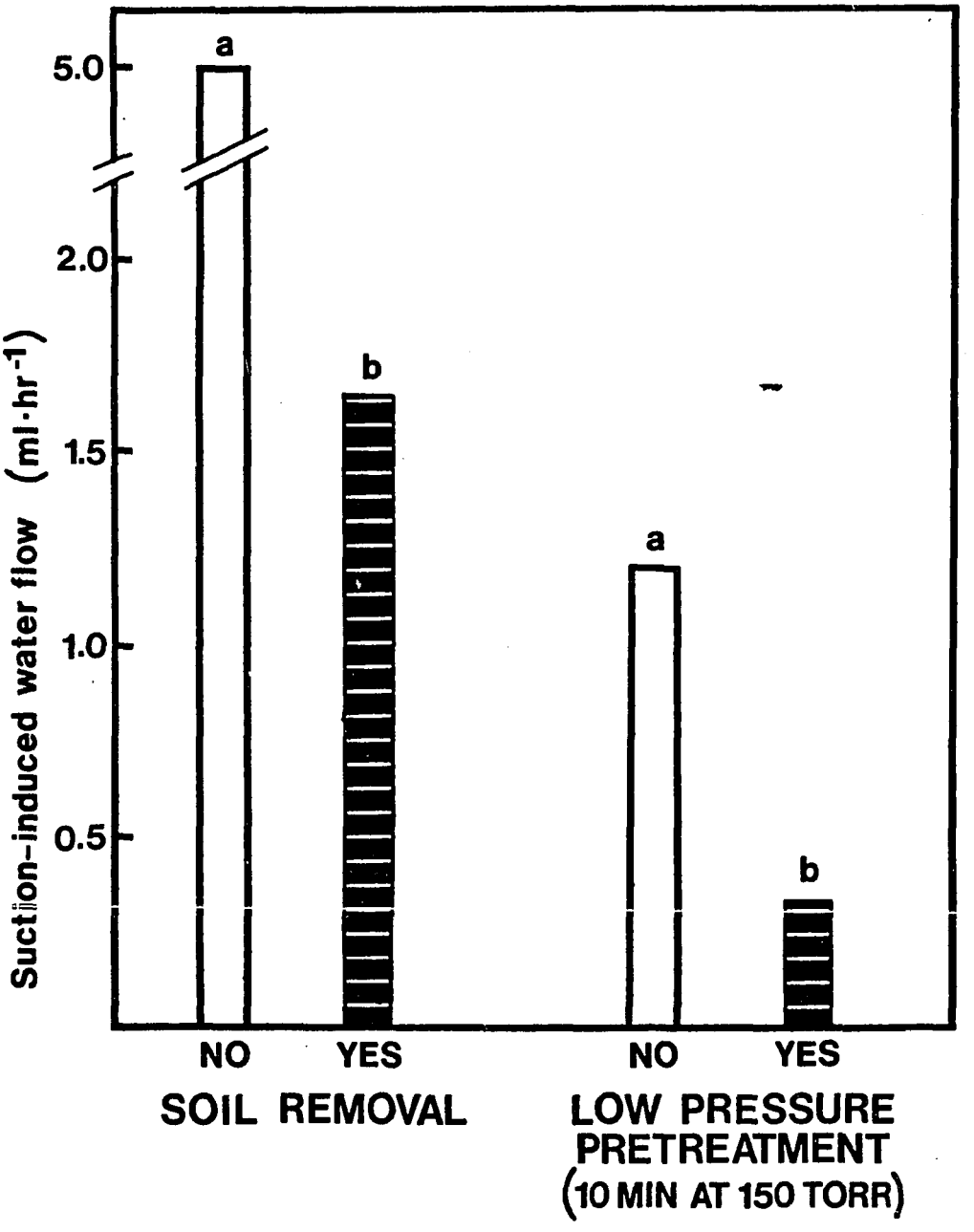

Figure 4. Effect of soil removal and low pressure pretreatment on suction-induced water flow through detopped root systems of potted 'Concord' grapevines.

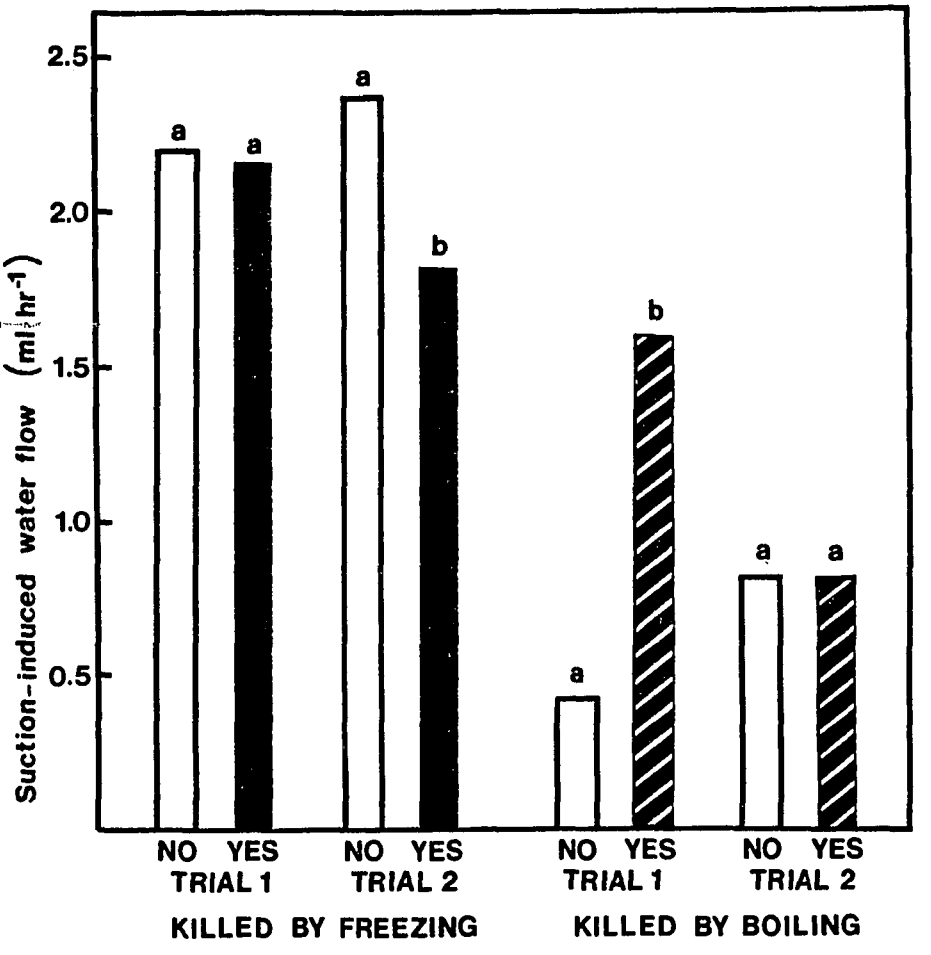

Figure 5. Suction-induced water flow through detopped root systems of potted 'Concord' grapevines, comparing living root systems with root systems killed by freezing and boiling.

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