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Cold acclimation of Concord grapevines III. Relationship between cold hardiness, tissue water content, and shoot maturation

by

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Frostabhärtung bei der Rebsorte Concord III. Beziehungen zwischen Frosthärte, Wassergehalt der Gewebe und Ausreifung der Triebe

Zusammenfassung: In Michigan setzt bei der Rebsorte Concord die Abhärtung im Basalteil der diesjährigen Triebe schon Ende August ein. Die Zunahme der Frosthärte steht in enger Beziehung zur Abnahme des Wassergehaltes in den ausreifenden Trieben. Die größten Unterschiede bei Frosthärte und Wassergehalt finden sich, sowohl bei Haupt- als auch bei Geiztrieben, in denjenigen Geweben, die sich im Grad ihrer Ausreifung am stärksten unterscheiden. Die Zunahme der Kälteresistenz steht in keiner Beziehung zum Wassersättigungsdefizit der Triebe.

Key words: cold, resistance, shoot, bud, lignification, hydration.

Introduction

Cold resistance of a large plant may vary with the position from which tissue samples are taken (HOWELL and SHAULIS 1980). One of the most widely reported differences is that of bud or shoot tissues in basal vs. apical regions of shoots. Greater levels of hardiness have been reported in basal bud or shoot tissues of ash (MAIR 1968), raspberry (JENNINGS and CARMICHAEL 1972), peach (CAIN and ANDERSEN 1976; CHAPLIN and SCHNEIDER 1974) and sweetgum (WILLIAMS and MCMILLAN 1971). The causes of these differences are not known.

The first stage of cold acclimation in dogwood has been associated with vegetative maturity of shoots (NISSILA and FUCHIGAMI 1978), but hardiness was treated as a whole, shoot condition and positional effects were not investigated. Acropetal tissue water loss accompanies acclimation in dogwood (MCKENZIE *et al.* 1974 a and b), but nodes were numbered from the apex without any indication of relative distance from the base of the shoot.

In grapevines, a detailed study (HOWELL and SHAULIS 1980) showed that variation in cold hardiness of tissues within a canopy was affected by leaf exposure, cane diameter, presence and size of persistent laterals, and presence and color of periderm tissue. Specifically, an intact 6-node cane section had hardier tissues at the basal end of the section (12 °C hardier wood and 15 ° hardier buds). Hardiness increased with periderm development.

Two previous papers in this series on cold acclimation of grapevines reported on the natural acclimation pattern of vines and variation within the canopy (WOLPERT and HOWELL 1985 a and b). In this paper we report the relationship of tissue maturation to cold acclimation and the associated loss of tissue water.

Materials and methods

Studies were conducted in mature Concord vineyards (*Vitis labruscana* BAILEY) at two locations in Michigan: The Wm. Cronenwett vineyard in Lawton and the Horticultural Research Center of Michigan State University in East Lansing. Vines in both plots were trained to an upper-wire (1.7 m) bilateral cordon ('Hudson River Umbrella'), were vigorous and high-yielding, and were balance-pruned (i. e., 30 buds retained for the first 0.4 kg of cane prunings and 10 buds for each additional 0.4 kg) throughout the experiment.

Sampling material consisted of 1-year-old node-internode pieces 8—12 cm long. Other sampling criteria such as sunlight exposure and node position are detailed in the 'Results' or in the tables. Samples were gathered in the field, sealed in plastic bags, and stored at outdoor temperature for not more than 24 h before being randomly divided into two lots for separate determinations of a) cold hardiness and b) moisture content of buds and canes.

The freezing technique was that of STERGIOS and HOWELL (1973), slightly modified as detailed previously (WOLPERT and HOWELL 1985 a). Representative samples from each treatment were frozen to several test temperatures, removed, and allowed to thaw

Table 1

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

Beziehungen zwischen Frosthärte (T_{50}), Wassergehalt und Färbung der sich akklimatisierenden Triebe der Rebsorte Concord; 1980

Twig section	Sampling date			
	Aug 9	Aug 30	Sept 13	Oct 4
	Cold hardiness (T_{50}) [1]			
Basal [2]	−2.0 a	−10.0 a	−10.5 a	−13.5 a
Middle [2]	−2.0 a	− 7.0 b	− 9.0 ab	−13.5 a
Apical [2]	−2.0 a	− 5.5 b	− 8.0 b	−10.0 b
	Water content (g/g dry wt) [3]			
Basal	2.02 a	1.28 a	1.01 a	0.96 a
Middle	2.44 b	1.48 a	1.13 a	1.00 a
Apical	2.63 b	1.95 b	1.47 b	1.00 a
	Shoot color			
Basal	Green	Brown	Brown	Brown
Middle	Green	Yellow/Brown	Brown	Brown
Apical	Green	Green	Brown	Brown

[1]) T_{50} calculated by means of Spearman-Karber equation. Means separated within columns by $\chi 2$ test, P = 0.05.

[2]) Basal = nodes 2—4; middle = nodes 6—8; apical = nodes 10—12.

[3]) Mean separation of water content within columns by Duncan's multiple range test, P = 0.05.

slowly overnight at 2 °C. Samples were incubated in humid chambers for 7—10 d, then sectioned and rated as alive or dead by the method of tissue browning (STERGIOS and HOWELL 1973). Buds were rated as dead when primordia were brown and water-soaked; canes were rated as dead when the phloem and cambium were brown. Hardiness was expressed as T_{50} (the temperature at which 50 % of tissues were killed), which was calculated by means of the Spearman-Karber equation as modified by BITTENBENDER and HOWELL (1974). Values were statistically separated by a χ^2 method as previously described (JOHNSON and HOWELL 1981; WOLPERT and HOWELL 1985 a).

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

Water saturation deficit (WSD) was calculated by the method of KRAMER (1969) as follows:

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

Saturation wt was determined, after fresh wt was recorded, by placing cane internode segments (2—3 cm long) in glass weighing vials (24 × 48 mm) with ground-glass stoppers, the cut ends in contact with about 2 ml of water. Cane pieces were allowed to take up water for 48 h, in closed vials, saturation wt being achieved by this time. Pieces were removed, blotted to remove surface water, placed into dry weighing vials, and handled as detailed above.

Shoot color changes brought on primarily by the development of periderm (HOWELL and SHAULIS 1980) were assessed subjectively by eye. Evaluation of the relationship of shoot color to the presence or absence of periderm (HOWELL and SHAULIS 1980) was also subjective, but a general relationship is known to exist (PEROLD 1927; PRATT 1974).

Results

Node position effect on acclimation

Increased hardiness of shoots was accompanied by a decrease in water content and a change in shoot coloration from green to brown (Table 1). When shoots were green (August 9), water content was high (2.0—2.6 g/g dry wt) and T_{50} was very high (−2.0 °C). 3 weeks later (August 30), when basal portions (nodes 2—4) had begun to turn brown, apical portions (nodes 10—12) were still green and differences in hardiness and water content were maximal. When all portions were brown (September 13), basal nodes were still the hardiest and lowest in water content. On the last date (October 4), basal sections were still almost 4 °C hardier than apical shoot sections but this difference was not associated with differences in water content or coloration (Table 1).

Association between color change and acclimation

On October 7, when more apical cane segments were turning brown, samples were collected at the point where the color change from green to brown was most pronounced, without respect to the absolute node position (Table 2). A 6-node segment was cut in which that node at the center of the zone of color change was counted as node 4. On October 7 (Table 2) and October 13 (data not shown), the difference in hardiness between basal and apical tissues was as much as 10 °C for buds and 7 °C for canes.

Table 2

Relationship between cane color and cold hardiness (T_{50}) and water content of primary buds and canes of Concord grapevines

Beziehungen zwischen Färbung der Tragruten und Frosthärte (T_{50}) sowie Wassergehalt bei Hauptknospen und Tragruten der Rebsorte Concord

Node position on cane segment	Cane color	Primary bud		Cane	
		T_{50}[1])	H_2O content[2])	T_{50}	H_2O content
		Primary cane (October 7, 1979)			
1 (Basal)	Brown	−13.5 a	1.22 a	−13.5 a	0.94 a
2	Brown	−13.5 a	1.42 bc	−13.5 a	0.99 ab
3	Lt. brown	−11.5 b	1.37 b	−12.5 ab	1.06 b
4	Yellow	− 9.5 c	1.62 d	−11.5 b	1.20 c
5	Green	− 6.0 d	1.60 d	− 8.0 c	1.41 d
6 (Apical)	Green	− 3.0 e	1.55 cd	− 6.5 d	1.40 d
		Lateral cane (September 25, 1980)			
1 (Basal)	Brown	−10.5 a	1.56 a	−12.0 a	0.95 a
2	Lt. brown/yellow	− 8.5 ab	1.66 b	− 9.0 ab	1.10 b
3 (Apical)	Yellow/green	− 6.5 b	1.93 c	− 7.0 b	1.38 c

[1]) T_{50} calculated by means of Spearman-Karber equation, separations by χ^2 test.
[2]) g H_2O/g dry wt, values separated by means of Duncan's multiple range test, P = 0.05.

Persistent lateral shoots exhibited similar color changes (Table 2) and 3-node segments were collected in a manner which maximized the color difference along the segment. Again, brown internodes were hardier and contained less water than yellow-green apical tissues. Relationship between hardiness and water content of persistent laterals was similar to that on primary shoots.

Hardiness and tissue water content of canes

In 1981, canes showed a negative relationship between hardiness and tissue water content during the acclimation period (Table 3), similar to that seen in 1980 (Table 1). For the first two sampling dates, the same negative relationship was seen among cane sections, with basal sections showing greater hardiness and less water. To illustrate a general relationship between hardiness and water content, data from Tables 1, 2 and 3, along with other data (WOLPERT and HOWELL 1985 a and b) are presented in the figure. Between T_{50} values of −2 °C, and −20 °C, both primary buds (Fig., A) and canes (Fig., B) showed a curvilinear relationship between hardiness and water content.

WSD showed a general increase throughout the acclimation period, except for a temporary decline on September 26 (Table 3). Little change in WSD occurred when hardiness increased (August 30 to September 5), and a large increase in WSD occurred when hardiness remained constant (September 26 to October 3). WSD was greatest in apical tissues even when no hardiness or water content differences were apparent.

Table 3

Cold hardiness (T_{50}), water content, and water saturation deficit of canes of Concord grapevines duing fall, 1981

Frosthärte (T_{50}), Wassergehalt und Wassersättigungsdefizit der Tragruten von Concord während des Herbstes; 1981

Node position	Sampling date						
	Aug 30	Sept 5	Sept 12	Sept 19	Sept 26	Oct 3	Oct 31
				T_{50} [1])			
Basal [4])	−10.0 a	−12.5 a	−12.5 a	−13.0 a	−14.5 a	−15.0 a	−21.5 a
Middle [4])	− 8.5 a	−11.0 b	−12.0 a	−11.5 b	−14.5 a	−14.5 a	−21.0 ab
Apical [4])	− 6.0 b	− 9.5 c	−12.0 a	−11.5 b	−14.0 a	−14.5 a	−20.5 b
				Water content (g/g dry wt) [2])			
Basal	1.17 a	0.99 a	0.93 a	0.94 a	0.97 ab	0.86 a	0.89 a
Middle	1.33 ab	1.04 ab	0.95 a	0.96 a	0.99 a	0.90 a	0.88 a
Apical	1.59 b	1.25 b	0.99 a	0.91 a	0.94 b	0.86 a	0.84 a
				Water saturation deficit [3])			
Basal	11.0 a	12.6 a	13.2 a	13.9 a	12.3 a	16.6 a	19.9 a
Middle	10.1 a	12.1 a	13.6 a	14.0 a	11.1 a	18.1 a	21.8 b
Apical	11.6 a	12.9 a	17.0 b	16.1 b	14.9 b	20.2 b	22.5 b

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

[2]) Mean separation of water content within columns by Duncan's multiple range test, P = 0.05.

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

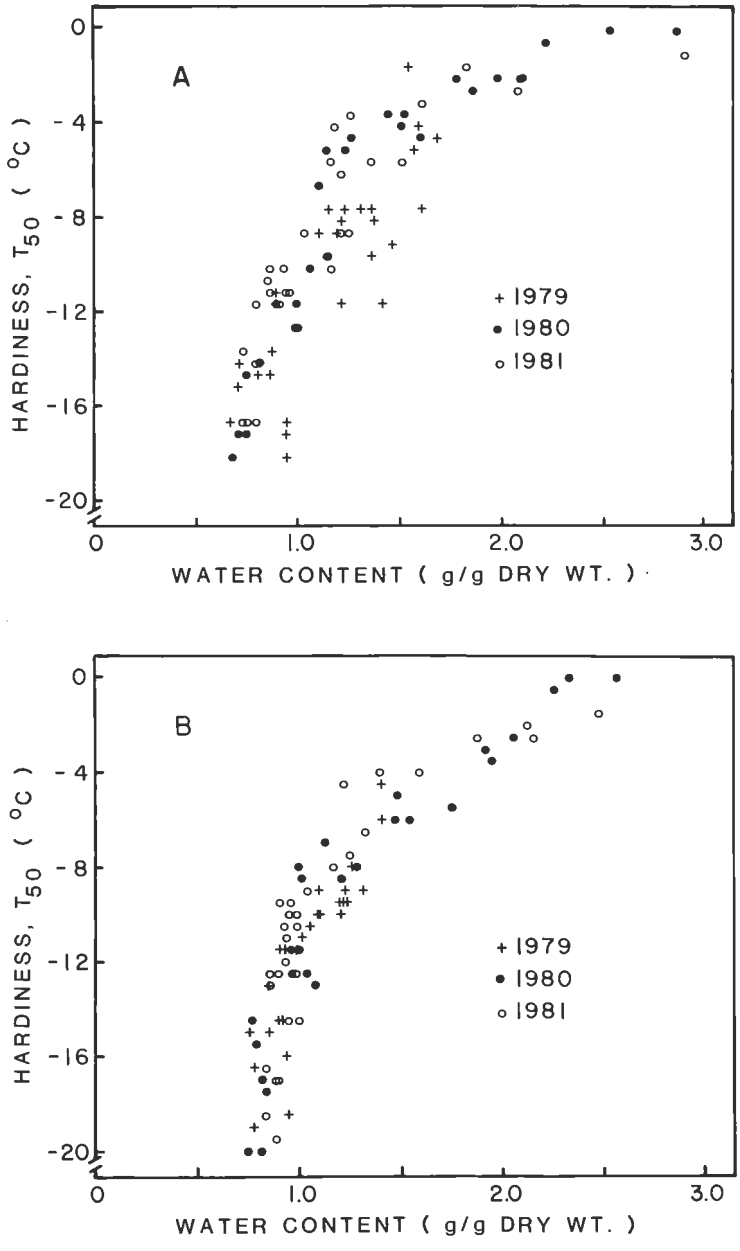
Mean separations by Duncan's multiple range test, P = 0.05.

[4]) Basal = nodes 2—4; middle = nodes 6—8; apical = nodes 10—12.

Discussion

The close inverse relationship between cold hardiness and tissue water content during acclimation seen here (Tables 1—3, Fig.) affirms observations in grapevines (WOLPERT and HOWELL 1985 a and b) and other woody plants (PELLETT and WHITE 1969; GUSTA and WEISER 1972; BURKE *et al.* 1974; MCKENZIE *et al.* 1974 a and b). The curvilinear relationship indicates that decreases in water are correlated with increases in hardiness through about −12 °C for both primary buds (Fig., A) and canes (Fig., B), the approximate level of hardiness associated with the short-day induced, first stage of hardiness (FUCHIGAMI and WEISER 1971). The data for primary buds are more variable (Fig., A) and this may indicate the involvement of another factor(s) or may be simply a reflection of the degree of difficulty in measuring water content of very small tissues.

Shoot maturation must also be considered when discussing changes associated with acclimation. Visible differences in shoot maturation are associated with large differences in hardiness (Tables 1 and 2). Although a relationship between vegetative



Relationship between cold hardiness (T_{50}) and water content in primary buds (A) and canes (B) of Concord grapevines for three acclimation periods (August—November), 1979—81. (Data from Tables 1, 2, and 3 herein and from WOLPERT and HOWELL 1985 a and b.)

Beziehungen zwischen der Frosthärte (T_{50}) und dem Wassergehalt bei den Hauptknospen (A) und den Tragruten (B) für drei Akklimatisierungsperioden (August—November); 1979—1981. (Daten aus den Tabellen 1—3 der vorliegenden Arbeit sowie aus WOLPERT und HOWELL 1985 a und b.)

maturity and acclimation has been suggested (NISSILA and FUCHIGAMI 1978), the association of progressive maturation with progressive cold acclimation has not been reported.

Development of the periderm (PRATT 1974) is responsible for the stem color change which takes place in August and September (Table 1). Periderm in *Vitis* species forms in non-conducting primary phloem by a differentiation of parenchyma cells. The formation of suberized cells (phellem) exterior to the phellogen isolates the epidermis and cortex, resulting in the death of these tissues. Anatomical aspects of periderm formation have been studied in grapevines (ESAU 1948; DAVIS and EVERT 1970), but little is known about its physiology. Dark cane color, favored by exposure of shoots to sunlight, is associated with periderm formation and increased hardiness (HOWELL and SHAULIS 1980) but a detailed study of the relationship between periderm formation and hardiness has not been made.

The progression of periderm development along a shoot has not been followed in grapevines, but in other woody plants the development is acropetal (BORGER 1973), similar to the progression of stem color changes (Tables 1 and 2). Shoot maturation includes pith senescence (MCKENZIE *et al.* 1974 b) and changes in cell walls (ESAU 1977).

In an extensive series of experiments, BORGER and KOZLOWSKI determined that periderm formation in several woody species is influenced by photoperiod, light intensity, temperature, water stress, defoliation, and growth regulators (BORGER 1973). Many of these factors also influence cold acclimation. If a close relationship exists between shoot maturation and cold acclimation (Tables 1 and 2), a much more detailed study of periderm and factors which influence its formation is warranted.

By implication, these data raise serious questions about the interpretation of data from many hardiness studies. They point to a need to accurately detail sampling procedures to ensure that correct conclusions are drawn (HOWELL and SHAULIS 1980; WOLPERT and HOWELL 1985 a). As a hypothetical example, if a treatment delays tissue maturation, is the reduction in hardiness observed an effect of position on the shoot or of maturity status? If extending the daylength prolongs shoot growth, the same question would arise. Assuming that tissue maturation proceeded acropetally and simultaneously in both plants, one would obtain conflicting results by evaluating the hardiness of bases vs. tips of shoots.

This may explain the data of BURKE *et al.* (1974) who reported that NMR spectra of 'hardy' and 'non-hardy' plants were very dissimilar at the tips (reflecting the hardiness status) but were very similar near the base (hardiness status not given). These considerations also make it difficult to interpret data on water content of different nodes of dogwood during acclimation because nodes were numbered from the apex (MCKENZIE *et al.* 1974 a and b). Differences in maturation, which presumably begins at the base, were not considered.

Since in grapevines the basal nodes are retained for fruiting, the hardiness of these nodes is of major interest, irrespective of the progress of acclimation at the shoot tip. Further, progression of acclimation and shoot maturation in basal nodes of potted grapevines is unaffected by growth activity at the shoot tips (WOLPERT and HOWELL, unpublished data). Thus, one tenet of woody plant acclimation, that growth cessation is necessary for acclimation, needs reinvestigation.

Summary

1. Cold acclimation of Concord grapevines in Michigan begins as early as late August in tissues at the base of current season's growth.

2. Increases in cold hardiness are closely related to decreases in tissue water content as stems achieve vegetative maturity.
3. Greatest differences in hardiness and water content are found in tissues which vary the most in extent of maturation on both primary shoots and summer laterals.
4. Increases in cold resistance are not related to water saturation deficit (WSD) of shoots.

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