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Harvest Date as a Factor in Carbohydrate Storage and Cold Hardiness of Cabernet Sauvignon Grapevines

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Abstract. **Cold-hardiness evaluations and soluble and insoluble-nonstructural carbohydrate analysis of dormant *Vitis vinifera* L. cv. Cabernet Sauvignon buds and cane tissue indicate a positive relationship between soluble carbohydrates and primary bud cold hardiness. Seasonal variations in soluble and insoluble carbohydrates appear to be related to changes in air temperatures and the dormancy status of the tissues. No differences were found in bud cold hardiness and only limited differences in carbohydrate levels of buds or stem tissues collected over 3 years from early harvest, normal harvest, or unharvested vines. These findings contrast with the widely held opinion that delayed harvest or failure to remove fruit results in reduced cold hardiness as a consequence of low storage carbohydrate content of the plants.**

Delayed harvest of perennial fruit crops maybe the result of poor management practices, such as heavy cropping and inappropriate fertilizer management. It may also be caused by factors outside of the grower's control, such as environmental stress, processor scheduling, or labor shortages. Regardless of the cause, delayed harvest has been linked to poor cold-hardiness development in various perennials (Flore and Howell, 1987; Shaulis et al., 1968; Stergois and Howell, 1977). However, these reports provide little documentation of this cause-and-effect relationship. Our understanding of this relationship is further complicated by killing frosts that occur before or immediately after harvest. Most reports, such as that of Potter (1938), are the result of observations rather than scientific experimentation. He reported that apple *(Malus domestics* Borkh.) trees grown in Montreal, Que., and harvested early survived the winter of 1933–34 better than those that were picked late. He also cited a similar experience for a different cultivar of apples grown in Rhode Island. Similar observations for grapes have been reported (Clore and Brummond, 1965; Denby and Vielvoye, 1979; Forsline, 1984; Gladwin, 1917; Miller et al., 1988; Shaulis, 1971; Stergios and Howell, 1977). These reports make clear that other factors, such as heavy croploads, heavy rainfall, late-season irrigation, an extended period of low light intensities, or insufficient heat unit accumulation may have contributed to the low-temperature injury observed. The lack of a clear understanding of the relationship of harvest date and bud cold hardiness and observations associated with an early frost in Sept. 1984 in eastern Washington prompted the following study.

The study was undertaken to examine the influence of early and no crop removal on the cold hardiness and carbohydrate reserves of 'Cabernet Sauvignon' grape.

Materials and Methods

A 0.1-ha 'Cabernet Sauvignon' grape research plot located at the Washington State Univ.–Irrigated Agriculture Research and Extension Center, Presser, was used for this study. The own-rooted vines were planted in 1974 on a 2.44 × 3.05 m spacing and trained to a bilateral cordon. The vines were spur-pruned leaving 24 nodes for each kilogram of 1-year-old wood removed, with a maximum of 60 nodes per vine. Irrigation, fertilizer, and pesticide applications were uniform across the vineyard throughout the 3-year study. The vineyard is located on a uniformly deep (>2 m) Warden fine sandy loam and is furrow irrigated.

The three treatments were: 1) early harvest, fruit removed at 18% soluble solids concentration (SSC); 2) normal harvest, fruit removed at 22% SSC; 3) no harvest, fruit left on the vine until pruning the following spring. Each treatment consisted of five vines and was replicated five times. Vines were selected for uniformity of age and development. Replicates were assigned randomly across the vineyard to account for variations that might exist within the vineyard.

Samples for cold hardiness and carbohydrate analysis were collected monthly from Nov. 1985–Mar. 1986 and at weekly intervals during the 1986–87 and 1987–88 winters. Samples consisted of 1-year-old cane pieces collected from the fourth to the eighth nodes. All samples were taken from the upper 180° of the cordon and showed uniformly good periderm formation. Internodes ranged from 6 to 13 cm long and 0.6 to 1.4 cm in diameter. Cold hardiness was determined by the procedure described in Schnabel and Wample (1987) during the first year of the study. Ten single-node cuttings per replicate were wrapped in aluminum foil with a thermocouple inserted in the center of the bundle. The bundles were placed in a Scientemp freezer (Adrian, Mich.) and allowed to come to temperature equilibrium at 0C for 2 h. The temperature was lowered at 4C/h and samples were removed at 2C intervals. Samples were placed at 4C overnight and then removed and kept for 48 h at room temperature. Freezing injury was determined by observing discoloration of both bud and cane tissues. The Spearman–Karber method (Bittenbender and Howell, 1974) was used to calculate the temperature required to kill 50% (T50) of the buds. Cold hardiness during 1986-87 and 1987–88 was determined by low-temperature exotherm analysis (Wample et al. 1990). Buds were col-

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lected from the fourth to the eighth nodes from the base of 1-year-old canes. Buds from vines representing the five replicates of each treatment were pooled and subsamples taken to prepare three thermoelectric modules (TEM) per treatment with 10 buds per plate. The T50 was estimated from the average of the temperatures at which the median low temperature exotherm occurred on each of the three TEMs.

Samples of buds and canes (1 year old) for carbohydrate analysis from the five replicates were frozen, freeze-dried, and ground to pass through a 60-mesh screen. Subsamples (10 mg) were extracted three times with 2 ml hot, 80% aqueous ethanol and once with hot water. The ethanol and water extracts were combined, dried, and redissolved in 1 ml acetate buffer (0.2 M, pH 4.8). Sucrose in the sample was converted to glucose and fructose by adding invertase (BDH Biochemical, Poole, England) and incubating at 25C for 30 min. Reducing sugars were quantified by the Shaffer–Somogyi method as modified by Nelson (1944).

Qualitative analysis of soluble carbohydrates followed the procedures of Roper et al. (1988). Hot ethanolic extracts from 10-mg samples were dried, and the residue was resuspended in 1 ml pyridine containing 30 mg hydroxylamine monohydrochloride and (ß-phenyl-D-glucoside/ml as an internal standard. The resultant oximes were derivatized to trimethysilyl ethers (Sweeley et al. 1963) and analyzed by gas liquid chromatography.

Starch determinations were made on the residue following soluble carbohydrate extraction using the procedure of Loescher and Nevins (1972). The sample was suspended in 1 ml of 20 mM phosphate buffer and heated in a water bath (95C) for 15 min to gelatinize the starch. Samples were cooled and 0.1 ml of pancreatic α-amylase (Type I-A, Sigma, St. Louis) was added and incubated at 37C for 16 h. Five milliliters of distilled water was added to terminate the reaction. The results are reported as equivalents of maltose, which is the primary product of the amylase reaction.

SAS/STAT (SAS Institute Inc., Cary, N. C.) was used to perform an analysis of variance (ANOVA) on the data; *t* test (LSD $\alpha = 0.05$) was used to compare means when justified by ANOVA.

Results and Discussion

The average calculated yields for the normal harvest vines were 13.0, 11.5, and 9.0 Mg·ha^{-1} for 1985, 1986, and 1987, respectively. The 1985 and 1986 yields are slightly higher than the Washington average (10 Mg·ha^{-1}). These yields reflect the initial vigor of the vines, which resulted in higher pruning weights and more fruiting nodes. As the higher yields reduced the vigor, pruning weights declined and fewer nodes were left at pruning. By 1987, the yields were consistent with the industry average.

The maximum and minimum temperatures for the sampling periods for the 3 years indicate that the coldest episode in the 3 years occurred in late Nov. 1985 when the minimum reached – 20C (Fig. 1). In both of the following years, the minima occurred from mid-December through January and did not go below – 10C.

Soluble carbohydrates in bud samples (Fig. 2) show an increase during the winter for all 3 years studied. This increase is followed by a decrease from late February through budbreak. The increase in early Dec. 1985 was associated with and preceded by the cold period. A peak also occurred in Feb. 1987 and can be associated with a preceding cold period. During 1987–88, temperatures were not as extreme and their changes were more gradual, which is reflected in the gradual increase

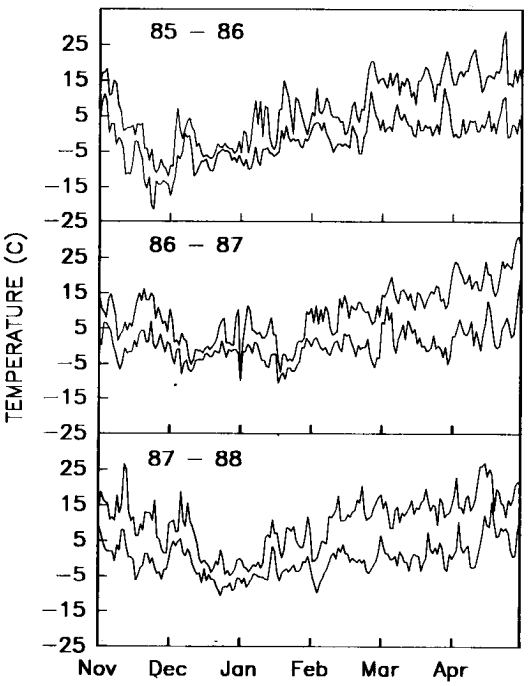

Fig. 1. Maximum and minimum temperatures at the Irrigated Agriculture Research and Extension Center, Presser, Wash., from November through April for 1985-86, 1986-87, and 1987-88.

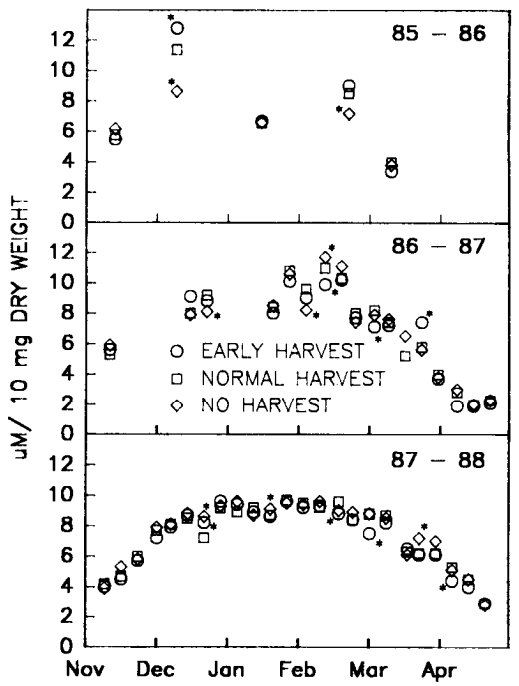

Fig. 2. Soluble carbohydrate concentration in buds of 'Cabernet Sauvignon' grapevines subjected to early harvest (18% SSC), normal harvest (22% SSC), or no harvest. Samples were collected from the fourth to the eighth nodes of l-year-old canes. Data represent the mean of five replicates of five vines each taken monthly in 1985-86, and weekly in 1986–87 and 1987-88. Data are presented as micromoles of reducing sugar following treatment of extracts with invertase. *Indicates significant difference ($P = 0.05$) from normal harvest.

and lower maximum soluble carbohydrate level. Significant differences in soluble carbohydrate levels in buds did occur in each of the 3 years. However, there was no consistent pattern asso-

ciated with harvest treatment, and in several cases, buds from unharvested vines contained significantly more soluble carbohydrate than those from normal harvested vines.

Soluble carbohydrates in current-season stem pieces (Fig. 3) followed the same general pattern as in buds (no data were collected in 1985–86). However, the maximum level was only ≈ 60% of that for buds. Once again, there was no consistent significant influence of harvest treatment on soluble carbohydrate levels and there were no differences at all on most sample dates.

Both sets of soluble carbohydrate analyses (Figs. 2 and 3) are in general agreement with the seasonal changes reported by Winkler and Williams (1945). "However, they do not support the widely held perception that carbohydrate levels in unharvested vines would be significantly reduced. Fruit removal ≈2 weeks ahead of normal harvest did not result in a significant increase in soluble carbohydrate levels in either bud or cane tissues.

Qualitative gas chromatographic (GC) analysis of the soluble carbohydrates from selected bud and stem samples collected during 1987-88 supported the calorimetric analysis. GC analysis showed that glucose and fructose made up most of the soluble carbohydrates in late fall and early winter (Fig. 4). By mid-February, sucrose was the predominant sugar present as both glucose and fructose declined. There was a peak that co-chromatographed with raffinose that appeared in mid-February in all samples (data not presented). The levels were very low and with no apparent difference among treatments.. This study provides no indication of the metabolic pathways responsible for these changes. With the exception of the mid-December stem samples, there were no consistent differences between harvested and unharvested samples.

Starch analysis of bud and stem sample for 1986-87 and 1987–88 (Figs. 5 and 6) shows an inverse relationship to the soluble carbohydrates. Bud and stem samples had similar starch contents within each year, while minor differences existed between years. There were occasional significant differences between early, normal, and unharvested vines, but there was no consistent treatment effect. Comparison of the temperature and starch profiles shows a close, positive relationship for both data sets. Other researchers (Grozova, 1978; Pickett and Cowart, 1941; Richey and Bowers, 1924; Schrader, 1924; Winkler and Williams, 1945) have reported such changes in starch in grape-

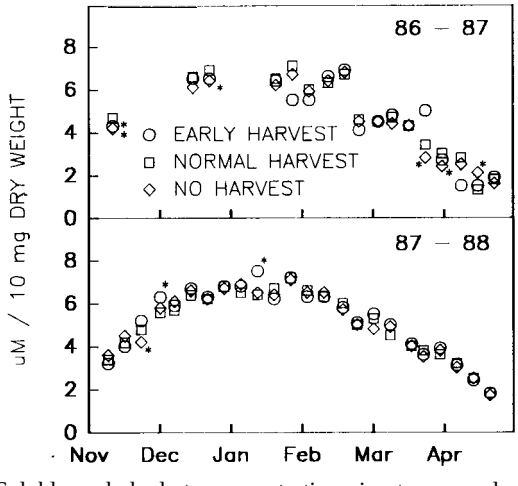

Fig. 3. Soluble carbohydrate concentrations in stem samples of 'Cabernet Sauvignon' grapevines subjected to early harvest (18% SSC), normal harvest (22% SSC), or no harvest. Samples were collected from the fourth to the eighth nodes of l-year-old canes. Data represent the mean of five replicates of five vines each collected weekly. Data are presented as micromoles of reducing sugars following treatment of extracts with invertase. *Indicates significant difference (P = 0.05) from normal harvest.

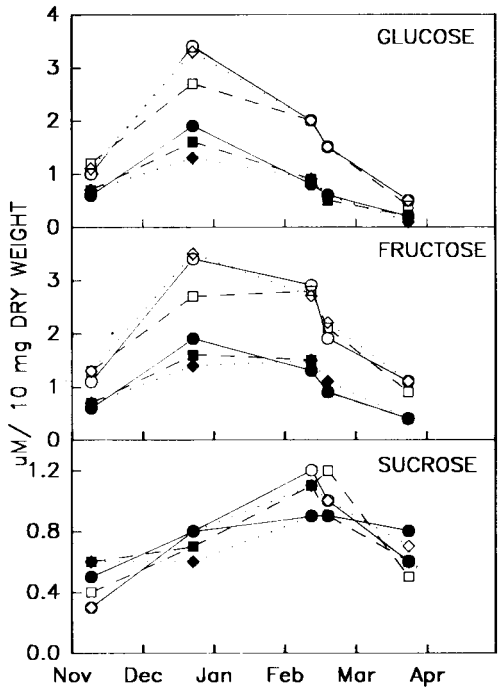

Fig. 4. Soluble carbohydrate concentration in buds and stems of 'Cabernet Sauvignon' grapevines subjected to early harvest (18% SSC), normal harvest (22% SSC solids), or no harvest. Samples represent selected dates from Nov. 1987–Apr. 1988. Data represent the mean of three gas chromatographic analyses of a composite subsample of the extracts from the five replicates before treatment with invertase. Open symbols, buds; closed symbols, stems; o, early harvest; q , normal harvest; ◇ , no harvest.

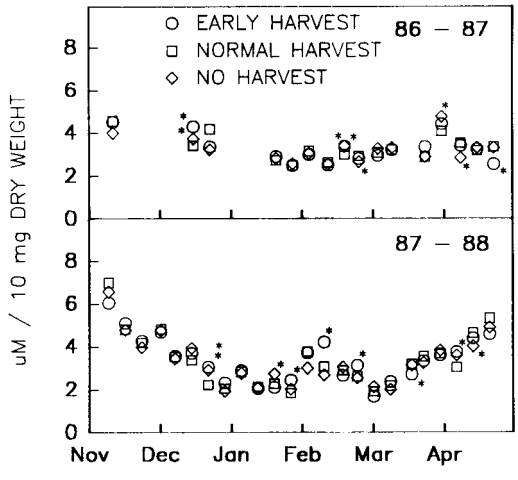

Fig. 5. Concentration of nonstructural, insoluble carbohydrates (primarily starch) in buds of 'Cabernet Sauvignon' grapevines subjected to early harvest (18% SSC), normal harvest (22% SSC), or no harvest. Buds were collected from the fourth to the eighth node positions of l-year-old canes about weekly from November through April during 1986-87 and 1987-88 and represent the mean of five replicates. Data presented as micromoles of maltose equivalents per 10 mg dry weight following treatment of the samples with a -amylase. *Indicates significant difference (P = 0.05) from normal harvest.

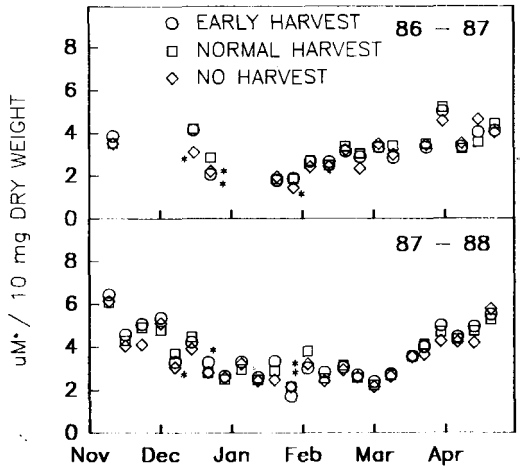

Fig.6. Concentration of nonstructural, insoluble carbohydrates (primarily starch) in stems of 'Cabernet Sauvignon' grapevines subjected to early harvest (18% SSC), normal harvest (22% SSC), or no harvest. Stems were collected from the fourth to the eighth node positions of l-year-old canes weekly from November through April during 1986-87 and 1987-88 and represent the mean of five replicates. Data presented as micro moles of maltose equivalents per 10 mg dry weight following treatment of the samples with α -amylase. *Indicates significant difference ($P = 0.05$) from normal harvest.

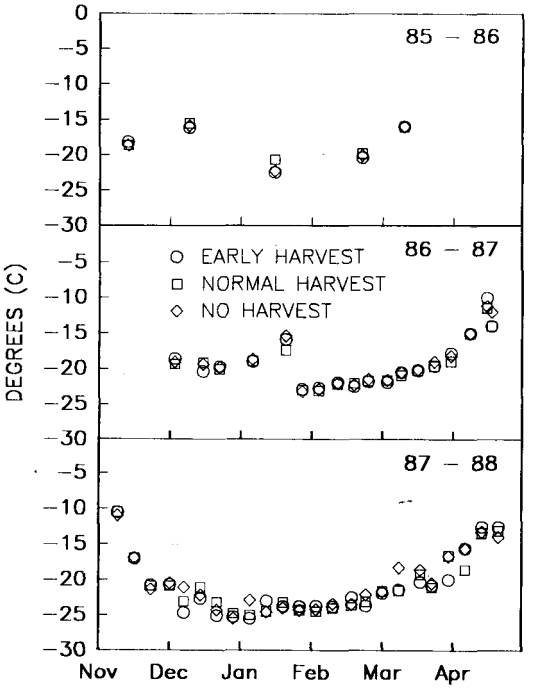

Fig. 7. Cold hardiness evaluation of the buds of 'Cabeme Sauvignon' grapevines subjected to early harvest (18% SSC), normal harvest (22% SSC) or no harvest. Buds were collected from the fourth to the eighth node of l-year-old canes. Samples were taken monthly from November through April in 1985-86 and weekly in 1986-87 and 1987-88. Values in 1985-86 are calculated by the Spearman-Karber method as modified by Bittenbender and Howell (1974) and represent the mean of five replicates of 10 buds each. Values in 1986-87 and 1987-88 represent the mean of three subsamples of 10 buds each using low-temperature exotherm analysis (Wample et al., 1990).

vine stems, but not in buds; nor have they demonstrated the close relationship with temperature.

Bud cold hardiness (Fig. 7) showed few, if any, differences between the early, normal, and unharvested vines during this 3-year study. Increases in bud hardiness closely follow decreases in the minimum temperature. The only exception is in Dec. 1985 which may reexplained by the extremely low temperatures and rapid temperature drop experienced just before this sample date. About 10% of the primary and secondary buds of control samples were killed (data not presented). Although data analysis accounted for this injury, exposure to near lethal temperatures possibly made the buds temporarily more sensitive to freezing. Bud cold hardiness subsequently recovered and reached its maximum in response to the sustained cold weather of late Dec. 1985 and Jan. 1986. The gradual warming trend that began in early February and continued through March was accompanied by a gradual loss of bud cold hardiness. Bud cold hardiness responded similarly in the next two seasons. The data for 1987–88 show that, regardless of the harvest treatment, there was a sharp increase in bud cold hardiness from early November to early December. Although results for the initial samples in 1986-87 were lost due to an equipment failure, the pattern would appear to have been the same. This result agrees with the demonstrated synergistic rather than additive effect of photoperiod and temperature on cold acclimation of grapevines (Schnabel and Wample, 1987; Wolpert and Howell, 1986).

Historically, increased cold hardiness has been linked to higher levels of soluble carbohydrates (Levitt, 1980). This relationship is supported by the data presented in Figs. 2 and 7 and in Table 1. Despite these data, there are examples of large changes in soluble carbohydrates with only small changes in cold hardiness. For instance, during late November each year, hardiness levels of – 18 to – 20C were accompanied by sugar levels of ≈ 6 μM/10 mg dry weight in buds, while 1 month later sugar levels had increased to >10 μM/10 mg dry weight, yet bud hardiness had increased by only 2 to 3C. It is also apparent from the data in Table 1 that harvest date had little influence on the relationship of bud cold hardiness to soluble carbohydrate level. All of this supports our current understanding, suggested >30 years ago (Vasil'yev, 1961), that although accumulation of soluble carbohydrates is associated with cold hardiness development, their role is limited and that there are other contributing factors.

Table 1. Statistical analysis for the regression of LTE_{50} vs. reducing sugars for 1986-87 and 1987–88 'Cabernet Sauvignon' bud samples for early, normal, and no harvest treatments.

	Harvest		
Statistic	Early	Normal	None
	1986–87		
n	13	13	13
df	1	1	1
F	22.4	41.6	21.7
R^2	0.65	0.78	0.64
	1987–88		
n	23	23	23
df	1	1	1
F	111.5	77.4	75.0
R^2	0.84	0.78	0.77

Despite the general expectation, neither early harvest nor leaving fruit on the vine throughout the winter significantly affected the soluble carbohydrate or starch reserves in bud or cane tissues of 'Cabernet Sauvignon' grapevines grown in Washington. Further, the dynamic nature of carbohydrate metabolism

also appeared to be the same in all harvest treatments. Finally, early or no harvest did not reduce bud cold hardiness compared with harvest at the normal date.

The implication that delayed harvest or the failure to remove fruit from grapevines would cause reduced cane and bud carbohydrate reserves and bud cold hardiness is not supported by this work. It is also clear that, under Washington growing conditions, there is sufficient photosynthesis to provide for fruit development and storage reserves. This conclusion assumes that cropload is not excessive and that good management practices are followed and may not be warranted in grape-growing areas where low light intensity and poor heat unit accumulation may reduce photosynthesis and delay. fruit ripening.

Literature Cited

Bittenbender, H.C. and G.S. Howell, Jr. 1974. Adaptation of the Spearman-Karber method for estimating the T_{50} of cold stressed flower buds. J. Amer. Soc. Hort. Sci. 99:187-189.

Clore, W.J. and V.P. Brummond. 1965. Cold injury to grapes in eastern Washington. Proc. Washington State Hort. Assn. 61:143-144.

Denby, L.G. and J. Vielvoye. 1979. Injury to grapes following the winter of 1978-1979. British Columbia Grower Newsletter 1-4.

Flore, J.A. and G.S. Howell, Jr. 1987. Environmental and physiological factors that influence cold hardiness and frost resistance in perennial crops, p. 139-150. In: F. Prodi, F. Rossi, and G. Cristoferi (eds.). International conference on agrometeorology. Fondazione Cesena Agricultural Publ., Cesena, Italy.

Forsline, P. 1984. Winter cold acclimation and deacclimation. Eastern Grape Grower and Winery News April/May:16-19.

Gladwin, F.E. 1917. Winter injury of grapes. New York Agr. Expt. Sta. Bul. 433.

Grozova, V.M. 1978. Carbohydrate metabolism of introduced grapevine cultivars during the period of winter hardening (in Russian). Referativyni Zhurnal 10:418. (Abstr.)

Levitt, J. 1980. Responses of plants to environmental stresses. vol. 1 (2nd cd.). Academic, New York.

Loescher, W. and D.J. Nevins. 1972. Auxin-induced changes in Avena coleoptile cell wall composition. Plant Physiol. 50:556-563.

Miller, D. P., G.S. Howell, and R.K. Striegler. 1988. Cane and bud hardiness of own-rooted White Riesling and scions of White Riesling and Chardonnay grafted to selected rootstock. Amer. J. Enol. Vitic. 39:60-66.

Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. Biochem. J. 153:275-279.

Pickett, T.A. and F.F. Cowart. 1941. Carbohydrate changes in Muscadine grape shoots during the growing season. Proc. Amer. Soc. Hort. Sci. 31:393-394.

Potter, G.F. 1938. Low temperature effects on Woody plants. Proc. Amer. Soc. Hort. Sci. 36:185-195.

Richey, H.W. and H.A. Bowers. 1924. Correlation of root and top growth of the Concord grape and translocation of elaborated plant food during the dormant season. Proc. Amer. Soc. Hort. Sci. 21:33-39.

Roper, T. R., J.D. Keller, W.H. Loescher, and C.S. Rem. 1988. Photosynthesis and carbohydrate partitioning in sweet cherry: Fruiting effects. Physiol. Plant 72:42-47.

Schnabel, B.J. and R.L. Wample. 1987. Dormancy and cold hardiness in *Vitis vinifera* L. cv. White Riesling as influenced by photoperiod and temperature. Amer. J. Enol. Vitic. 38:265-270.

Schrader, A.L. 1924. Seasonal changes in the chemical composition of the Concord grape vine. Proc. Amer. Soc. Hort. Sci. 21:39-44.

Shaulis, N. 1971. Vine hardiness a part of the problem of hardiness to cold in N.Y. vineyards. Proc. New York State Hort. Soc. 116:158-167.

Shaulis, N., J. Einset, and A.B. Pack. 1968. Growing cold-tender grape varieties in New York. New York State Agr. Expt. Sta. Bul. 821.

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

Sweeley, C.C., R. Bentley, M. Makita, and W.W. Wells. 1963. Gas-Iiquid chromatography of tumethylsilyl derivatives of sugars and related substances. J. Amer. Chem. Soc. 85:2497-2507.

Vasil'yev. I.M. 1961. Wintering of plants. [Translated from Russian by Royer and Roger, Inc. Washington, D. C.] AIBS, Washington, D.C. p. 125-129.

Wample; R. L., G. Reisenauer, and A. Bary. 1990. Microcomputer-controlled freezing, data acquisition, and analysis system for cold hardiness evaluation. HortScience 25:973-976.

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

Wolpert, J.A. and G.S. Howell. 1986. Effect of night interruption on cold acclimation of potted 'Concord' grapevines. J. Amer. Soc. Hort. Sci. 111:16-20.