



The relationship of osmotic potential and carbohydrate during acclimation in crowns of two winter wheats differing in winterhardiness
by Kelly John Thomas

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Agronomy
Montana State University
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Abstract:

Winterhardiness is important for winter wheat (*Triticum aestivum* L.) production in Montana. It has been shown that osmotic potential and carbohydrate content of winter wheat crowns are good indicators of winterhardiness.

This research used controlled environment acclimation to study the relationship of osmotic potential and carbohydrate in the crowns of two varieties of winter wheat, 'Froid' and 'Brawny', with high and low winterhardiness, respectively.

Measurements of osmotic potential, percent crown moisture, and concentrations of fructan, sucrose, glucose, fructose and total nonstructural carbohydrate (TNC) were taken each week beginning with the third and ending with the ninth week of acclimation following emergence.

Significant differences between varieties were detected for osmotic potential, percent crown moisture, fructan concentration and TNC concentration. Froid, the more winterhardy variety, maintained lower osmotic potentials, lower percent crown moisture and higher concentrations of fructan and TNC than the less winterhardy variety, Brawny.

In both varieties fructan, TNC and GFS (Glucose + Fructose + Sucrose) concentration increased while osmotic potential and percent crown moisture decreased during acclimation.

Multiple regression equations expressing osmotic potential as a function of several traits showed that fructan and GFS concentration accounted for 69% of the variation while TNC alone accounted for 66% of the variation. A single carbohydrate component, sucrose, accounted for 59% of the variation; however, much of the sucrose increase resulted from the breakdown of fructan.

Fructan was a major contributor to the total nonstructural carbohydrate pool accounting for up to 84% of TNC for the fourth and fifth weeks of acclimation after emergence.

The breakdown of fructan to shorter chain fructans and eventually to sucrose, glucose and fructose appears to be a major controlling force responsible for increased crown osmotic potential and therefore, increased winterhardiness in wheat.

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APPROVAL

of a thesis submitted by
Kelly John Thomas

This thesis has been read by each member of the author's graduate committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

Winterhardiness is important for winter wheat (Triticum aestivum L.) production in Montana. It has been shown that osmotic potential and carbohydrate content of winter wheat crowns are good indicators of winterhardiness.

This research used controlled environment acclimation to study the relationship of osmotic potential and carbohydrate in the crowns of two varieties of winter wheat, 'Froid' and 'Brawny', with high and low winterhardiness, respectively.

Measurements of osmotic potential, percent crown moisture, and concentrations of fructan, sucrose, glucose, fructose and total nonstructural carbohydrate (TNC) were taken each week beginning with the third and ending with the ninth week of acclimation following emergence.

Significant differences between varieties were detected for osmotic potential, percent crown moisture, fructan concentration and TNC concentration. Froid, the more winterhardy variety, maintained lower osmotic potentials, lower percent crown moisture and higher concentrations of fructan and TNC than the less winterhardy variety, Brawny. In both varieties fructan, TNC and GFS (Glucose + Fructose + Sucrose) concentration increased while osmotic potential and percent crown moisture decreased during acclimation.

Multiple regression equations expressing osmotic potential as a function of several traits showed that fructan and GFS concentration accounted for 69% of the variation while TNC alone accounted for 66% of the variation. A single carbohydrate component, sucrose, accounted for 59% of the variation; however, much of the sucrose increase resulted from the breakdown of fructan.

Fructan was a major contributor to the total nonstructural carbohydrate pool accounting for up to 84% of TNC for the fourth and fifth weeks of acclimation after emergence.

The breakdown of fructan to shorter chain fructans and eventually to sucrose, glucose and fructose appears to be a major controlling force responsible for increased crown osmotic potential and therefore, increased winterhardiness in wheat.

INTRODUCTION

Winterhardy varieties are very important for winter wheat (Triticum aestivum L.) production in northern latitudes. Much research is aimed at understanding the mechanisms which allow the wheat plant to escape winter injury (Pomeroy and Andrews, 1975; Gusta and Fowler, 1977; Fowler and Gusta, 1977; Roberts, 1979; Andrews et al., 1984; Roberts, 1985; Limin and Fowler, 1985).

Breeding winterhardy wheats is a difficult and time-consuming process. The historical approach is to field plant and evaluate winter injury. Due to variable weather, non-differential winterkill--all plants live or all plants die--is a common occurrence which makes it difficult to determine winterhardiness of wheat cultivars.

Winterhardiness screening techniques should provide a quick and precise indication of cultivar differences. These techniques often involve cold stressing plants at controlled temperatures and evaluating regrowth (Roberts and Grant, 1968; Metcalf et al., 1970; Pomeroy and Fowler, 1973). Although these techniques work well, the greenhouse space necessary to evaluate thousands of lines in a plant breeding program is prohibitive.

Research completed at Montana State University suggested that winter wheat crown osmotic potential (DeNoma, 1989) and carbohydrate concentration (Wille, 1985) are good indicators of a cultivars winterhardiness. This research was initiated to determine the relationship between crown osmotic potential and carbohydrate concentration in acclimating winter wheat.

LITERATURE REVIEW

Temperature greatly influences cold hardening in winter wheat (Triticum aestivum L.). Winter cereals grown at temperatures warmer than 15 C are no more frost tolerant than spring cereals (Gusta et al., 1983). Generally, temperatures of less than 10 C are necessary to stimulate cold hardening in wheat (Olien, 1967; Gusta and Fowler, 1979); however, maximum cold hardening is not achieved unless the plants experience temperatures near 0 C (Olien, 1967; Pomeroy et al., 1975; Gusta et al., 1982). Although low temperature is the initiator of the hardening process in winter wheat, many physiological changes within the plant must occur to result in winterhardiness.

Crown moisture content of cereals has been linked to the ability to withstand low temperatures (Metcalf et al., 1970; Gusta and Fowler, 1976). Metcalf et al. (1970) found that slight increases in percent crown moisture of barley (Hordeum vulgare L.) and wheat diminished their ability to survive killing temperatures. Taylor and Olsen (1985 and 1986) found that low temperature was not necessarily the major factor contributing to winter injury in wheat. Well hardened winter wheat withstood crown temperatures as low as -20 C without appreciable injury. In field and controlled

environment studies, they found that desiccation was a major cause of winter injury to wheat in Montana. Water relationships within the plant may play a large role in winterhardiness of winter cereals.

It has been suggested that frost tolerance in plants can be increased by artificially increasing the sugar content of the plant tissue. By infiltrating cabbage leaves (Brassica oleracea L.) with dextrose or fructose Levitt (1959) was able to increase frost tolerance by approximately 2 C. The actual results agreed very well with calculated values for increased frost hardiness on the basis of a purely osmotic effect (the decrease in osmotic potential caused a decrease in freezing point).

Russian researchers discovered that increases in winterhardiness of wheat placed in hardening conditions of 4 C TO -2 C closely paralleled substantial increases in oligosaccharide (short chain sugar) concentration (Bebenko and Gevorkyan, 1967). They concluded that oligosaccharides play a large role in protecting winter grains from low temperature injury.

Green (1972) increased cold hardiness in winter wheat by growing wheat seedlings in nutrient solution plus glucose, sucrose or mannitol. Measurements for frost tolerance, sugar accumulation and osmotic potential were recorded. Green found that although significant increases in frost tolerance occurred, he could not explain the increase due to

only an osmotic effect. All of the treated plants obtained similar osmotic potentials (leaf osmotic potentials of plants grown in glucose, sucrose and mannitol were -11.5, -14.9 and -12.1 atmospheres, respectively), but the mannitol treated plants were not nearly as cold tolerant as the glucose or sucrose treated plants. Green deduced that some other factor, possibly an oligosaccharide that accumulated in the sugar treatments, but not the mannitol treatment had imparted additional cold tolerance to the wheat plants by other than an osmotic effect.

If osmotic effect can't completely explain the role of sugars in winterhardiness of wheat, then what is the role of these oligosaccharides? Olien (1965) isolated polysaccharides (sugar polymers) from rye (Secale cereale L.) crowns that appeared to interact with the ice crystal interface and interfered with the freezing of water. He concluded that these polysaccharides helped protect the rye plant from low temperature injury not by reducing the freezing point of the solution, as if the effect was purely osmotic, but instead by interfering with the freezing process causing irregular ice crystal formation.

Several ways sugars in the crown may affect winter survival of wheat are by increased concentration of sugar within the cellular fluid reducing the freezing point of the crown tissue, by higher concentrations of sugar helping the plant resist water loss due to dessication, and by oligo-

and polysaccharides interfering with ice crystal formation within the crown tissue.

The oligo- and polysaccharides are polymers of fructose. These fructans, as they are called, have the formula G-F-(F)_n where G-F denotes a sucrosyl group and (F)_n denotes one or more fructose units completing the polymer (Edelman and Jefford, 1968; Pollock, 1986). Molecules with a DP (degree of polymerization) of three or more are classified as fructans. Fructan is the major storage carbohydrate found in wheat and many other temperate season grasses (Wagner et al., 1983).

Most of the early work to determine the mechanism of fructan synthesis was done on Jerusalem artichoke (Helianthus tuberosus L.) due mainly to the large amounts of fructan containing tissue that can be obtained from relatively few plants (Edelman and Jefford, 1964; Edelman and Dickerson, 1966; Edelman and Jefford, 1968). More recently, work with asparagus (Asparagus officinalis L.) has helped to further explain enzyme characteristics in fructan accumulating plants (Shiomi and Izawa, 1980; Shiomi, 1981; Shiomi, 1982).

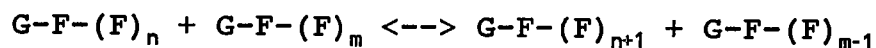
Edelman and Jefford (1968) outlined a general mechanism for control of fructan metabolism. They found that synthesis of fructans is controlled by two enzymes, SST and FFT, and depolymerization of the fructan molecule back to sucrose, glucose and fructose by another enzyme, FFH.

Sucrose:sucrose 1-fructosyltransferase (SST) was found to catalyze the following reaction creating a trisaccharide (DP = 3) from sucrose.



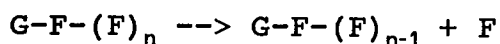
This enzyme uses sucrose as its only substrate and is inactive on the trisaccharide.

Fructan:fructan 1-fructosyltransferase (FFT) is highly specific for the transfer of fructosyl units (F) from one fructan to another fructan. It catalyzes the following reaction.



This enzyme works to increase the chain length or DP of fructans and can utilize any fructan as a substrate.

The breakdown or hydrolysis of fructan is accomplished by fructan 1-fructanohydrolase (FFH). This enzyme is also specific to fructan and catalyzes this reaction.



Although the major thrust of the work on fructan metabolism has been done on species such as Jerusalem artichoke, it is worth noting that SST activity has been found in barley (Wagner et al., 1986) (Wagner et al. 1983) and winter wheat (Housley and Daughtry, 1987).

Labeling patterns of fructans in winter wheat stems indicate that fructan synthesis in wheat is very similar to fructan synthesis in Jerusalem artichoke (Hogan and Hendrix, 1986).

Pollock and Ruggles (1976) found that in the temperate grass Dactylis glomerata L. fructan accumulation occurred in response to cool temperature (5 C) but not at warmer temperatures (20/15 C day/night). Several other reports of low temperature induction of fructan accumulation are available for barley (Sicher and Kremer, 1986), winter wheat (Wagner et al., 1983) and Agropyron ssp. (Chatterton et al., 1986). Fructan synthesis has been reported to occur at temperatures as low as 0 C (Pollock, 1986). In fact, SST remains very active at low temperatures. Wagner et al. (1983) found that SST isolated from wheat had an optimum activity at 28 C; however, half of the enzyme's activity was retained at 8 C. These results further emphasize that fructans may be involved in protecting plants from cold injury.

Wille (1985) in a study using eight varieties of winter wheat varying in winterhardiness, found a very high correlation ($r = .707$ to $.915$) between winterhardiness score and fructan concentration. The more winterhardy wheats tended to accumulate a higher amount of fructans than did less hardy wheats. Wille also determined that higher total nonstructural carbohydrate was associated with increased winterhardiness.

In contrast to the above study, Green and Ratzlaff (1975) found that concentrations of sucrose, glucose, fructose and raffinose were inversely related to

winterhardiness in winter wheat seedlings placed in fall hardening conditions.

Wagner et al. (1983) induced barley and wheat to accumulate fructans by removing their photosynthate sink and subjecting the plants to cold stress (5 C at night). Protoplasts of the barley leaves were then isolated and separated into their vacuolar and cytoplasmic components. They discovered that virtually all of the nonstructural carbohydrates (fructose, glucose, sucrose and fructan), along with SST and invertase activity, were associated with the vacuoles. Sucrose was the only sugar that approached equal concentration in both the cytoplasm and the vacuole. A similar situation was observed in the tubers of Jerusalem artichoke (Frehner et al., 1984). Wagner et al., (1983) concluded that storing fructans and other sugars in vacuoles is a good way of overcoming osmotic constraints of high accumulation of low molecular weight sugars. It is also ideally suited for osmoregulation of the plant during temperature or water stress. With an enzyme system such as discussed by Edelman and Jefford (1968), fructans can easily be depolymerized (converted to shorter chains, sucrose, glucose and fructose) decreasing the osmotic potential.

Osmoregulation is a very important factor in winterhardiness. DeNoma (1989) used crown osmotic potential as a criteria for ranking winter wheats according to winterhardiness score. He found high correlations between

crown osmotic potential and winterhardiness score of wheats
field sampled in December of 1983 and 1984 ($r = .88$ to $.91$).

MATERIALS AND METHODS

General Methods

Osmotic potential, several carbohydrates and percent moisture were measured in crown tissue of two acclimating varieties of winter wheat (Triticum aestivum L.), 'Froid' and 'Brawny', which vary in winterhardiness (Froid = 81% survival, Brawny = 39% survival, at 6 locations with differential winter injury). The experiment was replicated twice using a randomized complete block design for the first 4 weeks and a split-split plot design with two temperature treatments as the main plots, varieties as the subplots and sampling time (weeks of acclimation after emergence) as the sub-subplot for the remaining five weeks of the experiment.

Two seeds of each variety were planted at a 3.0 to 3.5 cm depth in 65 cubic centimeter containers using 1:1:1 (sand:soil:peat) greenhouse mix. Two racks (400 cones) of each variety were placed in an EGC M-13 customized growth chamber fitted with high intensity lamps and increased refrigeration capacity to simulate early fall field acclimating conditions. Thinning after emergence left one seedling per cone. Temperature conditions were split into three periods outlined in Tables 1 and 2. Period I which was carried out in the growth chamber for the first 4 weeks

of acclimation after emergence, simulated average fall acclimating conditions at Bozeman, Montana (Table 1). The growth chamber was unable to maintain temperatures low enough for the remaining acclimation periods; therefore, the plants were moved to modified chest freezers for periods II and III of acclimation. Temperature treatments were implemented during period II (fifth, sixth and seventh weeks of acclimation after emergence) by moving one rack of each variety from the growth chamber to the modified chest freezers at either +2 C or -2 C (Table 2). For Period III (eighth and ninth weeks of acclimation after emergence) the temperature in the freezers was lowered to -6 C (Table 2). Weekly random samples were taken beginning with the third week of acclimation after emergence for each variety and temperature.

Table 1. Weekly air temperature and photoperiod during period I in the plant growth chamber.

Weeks of Acclimation	Temperature (C)		Photoperiod (hrs)
	Day	Night	
1-2	20	7	13
3	15	-0.5	13
4	8	-3	13

Modifications on the freezers included replacing the lids with a clear cryolite top and suspending fluorescent lamps above to supply light for the plants. To assure no

Table 2. Weekly air temperature and photoperiod during plant growth periods II and III for the low and high temperature regimes in modified chest freezers.

Period	Weeks of Acclimation	Low (C)		High (C)		Photoperiod (hrs)
		Day	Night	Day	Night	
II	5,6,7	-2	-2	+2	+2	13
III	8-9	-6	-6	-6	-6	13

drought or nutrient stress occurred, the plants were watered and fertilized constantly while at above freezing temperatures by submerging the lower 2 cm of the cone in full strength Hoagland's solution. Photoperiod was set at 13 hours for the entire experiment with light levels of $1100 \text{ umol/m}^2 \text{ sec}$ for the first 4 weeks of acclimation after emergence (in the growth chamber) and reduced to $40 \text{ umol/m}^2 \text{ sec}$ for the remaining five weeks in the freezers.

Nine plants were sampled from each variety on a weekly basis beginning with the third week of acclimation. A section of crown tissue was excised from each plant extending from the base of the crown to one centimeter above. The crowns were placed in microcentrifuge tubes and heated in boiling water for 4 minutes to stop enzyme

activity. One crown was transferred immediately from the microcentrifuge tube to the thermocouple psychrometer sample chamber and the thermocouple junction inserted into the chamber. The chamber was sealed and placed on dry ice to freeze and disrupt the plant cell membranes. Readings for osmotic potential were taken using a microvoltmeter. The remaining crowns were frozen, vacuum dried and stored at -40 C for carbohydrate analysis later. Fresh and dry weights were taken on the crowns used for carbohydrate analysis to determine moisture percentage of the tissue.

Osmotic Potential Determination

Samples were prepared and measurements taken according to DeNoma (1989) with the following exceptions. Osmotic potentials were determined using J. R. D. Merrill model 84-2VC in situ thermocouple psychrometers and a Wescor HR-33T microvoltmeter. Temperature was controlled by submerging the psychrometers in water heated to 30 C in an AO Scientific Instruments shaking waterbath. The psychrometers were allowed to equilibrate in the water bath for a minimum of one hour prior to making measurements. One calibration curve was developed for each psychrometer using NaCl solutions as outlined by Weibe et al. (1971).

Carbohydrate Extraction

Dried crown tissue samples were ground with a Pitchford Manufacturing Corporation ball mill grinder. Fifty milligrams of ground plant material were extracted with 1.5 ml 80% v/v ethanol while agitating in a 60 C water bath, centrifuged at 1000 g for 5 minutes and the supernatant decanted and saved. The procedure was repeated on the pellet once more with 80% ethanol and twice with water. The supernatants were combined in a 10 ml beaker and placed in a 60 C constant temperature cabinet to drive off the alcohol. The remaining solution (approximately 1 ml) was frozen and thawed to help precipitate noncarbohydrate components in the solution and centrifuged to pellet out any undissolved material. The concentrated supernatant was washed with 25 ml of water through Dowex 50 and Dowex 1 cation and anion exchange resins, respectively, to remove charged molecules or particles. The solution was concentrated at 60 C to approximately 2.5 ml, divided into two equal portions, frozen and lyophilized. Acid hydrolysis of part of the lyophilized concentrate, as described below, resulted in the breakdown of all the carbohydrates to glucose and fructose. This information was used to determine the average degree of polymerization (AVGDP) of the fructan chains. The sample was dissolved in 250 ul of 0.1 N HCL and the solution heated in a waterbath at 85 C for 30 minutes. The solution was

neutralized with 125 μ l of 0.2 N NaOH, washed with 20 ml of water through Dowex 50 and Dowex 1 exchange resins to remove the sodium and chloride ions and then concentrated to about one ml at 60 C, frozen and lyophilized.

Carbohydrate Analysis

Relative amounts of carbohydrate in the extracts were determined by high performance liquid chromatography (HPLC). Separation of the carbohydrate components (fructose, glucose, sucrose and fructans DP 3 to 6+) was accomplished using a Bio-Rad Aminex Carbohydrate HPX-42C Column heated to 85 C. The mobile phase was degassed double distilled deionized water at a flow rate of 0.5 ml/min. Carbohydrates were detected by a Waters R-401 Refractive Index Detector. Chromatographs corresponding to the carbohydrate were plotted and peak areas determined by a Shimadzu C-R3A Chromatopac integrator. Chromatograms and peak areas were obtained for both the hydrolyzed and extracted portions of the sample. The dry sample portions were dissolved in 0.5 ml of double distilled water, filtered with a 0.45 μ m syringe tip filter to remove small particles and 100 μ l's of the sample injected into the sample loop by an ISCO ISIS HPLC Autoinjector.

Average degree of polymerization (AVGDP) of the fructan chains was calculated by first subtracting the fructose and glucose in the extracted plant sample from the glucose and

fructose in the hydrolyzed sample, computing the fructose to glucose ratio, and adding one to the ratio to account for the glucose unit in each chain.

Osmotic potential in wheat crowns is dependent on the total concentration of molecules in the cell sap (molality). Therefore, all concentrations of sugars were expressed as molality or as an estimation of molality.

Peak areas for sucrose, glucose and fructose were converted to concentration (molality) using standard curves and corrected for the mg of water per dry sample weight by the formula: $\text{molality} \times (500 \text{ mg sample soln}) / ((\text{mg fresh wt.} - \text{mg dry wt.}) / \text{mg dry wt.}) \times 25 \text{ mg dry sample wt.}) = \text{corrected molality}$.

Since no standards are available for fructan, an estimate of the number of molecules of fructan in the sample was determined by dividing fructan peak area by the average degree of polymerization (AVGDP) of the fructan chains. This estimate of moles of fructan was corrected for the amount of water per dry sample weight with the formula: $(\text{Peak area} / \text{AVGDP}) / ((\text{mg fresh wt.} - \text{mg dry wt.}) / \text{mg dry wt.}) \times 25 \text{ mg dry sample wt.}) = \text{estimated fructan molecules} / \text{mg H}_2\text{O per sample dry wt.} = \text{Fructan Index (FI)}$. Estimates of concentration for total nonstructural carbohydrates (TNC), fructan, sucrose, glucose and fructose were calculated using the formula: $\text{Peak area} / ((\text{mg fresh wt.} - \text{mg dry wt.}) / \text{mg dry$

wt.) x 25 mg dry wt. per sample) = peak area(mV sec)/mg H₂O per sample.

Thin Layer Chromatography

Thin layer chromatography (TLC) was used to qualify the retention times of the fructan peaks from the HPLC analysis. Precoated 20 x 20 cm silica gel glass plates (Keisilgel 60 F 254) were used to separate the fructans into individual DP's. The sample, and sucrose, glucose and fructose standards, were applied 1.5 cm from the bottom of the plate. One hundred ml of the mobile phase (25 ml water : 50 ml 1-butanol : 25 ml glacial acetic acid) was placed in the chromatography tank along with the TLC plate. Six hours were required for the solvent front to migrate to within 1 cm of the plate top. The plate was removed from the tank and allowed to air dry. The standards and a portion of the carbohydrate sample were developed by spraying with aniline-diphenylamine Sigma spray reagent and heating at 85 C for 10 minutes. Three distinct bands corresponding to fructan degrees of polymerization of 3, 4 and 5+ were scratched from the plate and the fructan for each band extracted from the silica gel with water. The extract was pushed through a 0.45 um syringe tip filter and run through the HPLC to determine retention time. TLC samples were also quantified for the degrees of polymerization of each band by Dr. N. Jerry Chatterton, USDA Forage and Range Research Laboratory,

Logan, Utah. Retention times for DP 3, 4, 5 and 6+ were 11.3, 9.7, 8.6 and 6.9 minutes respectively. Sucrose, glucose and fructose eluted at 13.3, 16.0 and 19.3 minutes respectively.

Statistical Analyses

Analysis of variance was computed for variables from each week combined over runs, temperatures and varieties. A combined analysis of variance was also computed for run, temperature, variety and weeks of acclimation after emergence. The individual varietal means and means for weeks of acclimation were compared using the LSD calculated with the appropriate mean square error from the ANOVA table.

Multiple regression analysis was used to examine the relationships between osmotic potential and carbohydrates.

RESULTS AND DISCUSSION

Analyses of variance were computed for osmotic potential, fructan index (FI), glucose, fructose, sucrose, GFS (Glucose + Fructose + Sucrose), total nonstructural carbohydrate (TNC) and percent crown moisture (% CM). Varieties differed in period I for sucrose, fructose, GFS, TNC, and % CM with differences among weeks occurring for FI, sucrose, fructose, GFS, TNC and % CM (Table 3).

Table 3. Winter wheat crown tissue variety and week mean squares for eight variables from acclimation period I.

Source	df	Osmotic							
		Potential	FI	Sucrose	Glucose	Fructose	GFS	TNC	% CM
Run	1								
Var (V)	1	.114 ns	.575 ns	.0001 *	.000005 ns	.000006 *	.0002 *	31.8 *	27.3 *
Week (W)	1	.438 ns	.982 *	.0002 *	.000005 ns	.000015 *	.0001 *	46.3 **	28.9 *
V x W	1	.0009 ns	.006 ns	.00003 ns	.000041 ns	.000003 ns	.0002 ns	.62 ns	.37 ns
Resid	3	.136	.059	.000007	.000002	.0000005	.00002	1.16	1.07

FI = Fructan Index (fructan moles/mg H₂O);

GFS = (Glucose + Fructose + Sucrose) (molality);

TNC = Total nonstructural carbohydrate (peak area(mV sec)/mg H₂O);

% CM = Percent Crown Moisture

*, ** = significant at P < .05 and P < .01, respectively.

ns = Not Significant.

Varieties also differed in periods II and III combined for osmotic potential, FI, GFS, TNC and % CM (Table 4). A significant variety x temperature interaction was detected for osmotic potential only (Table 4). This interaction is

explained by Froid, the more winterhardy variety reacting differently at the -2 C treatment than at the +2 C treatment. For periods II and III all the variables except fructan index (FI) and % CM differed significantly among weeks (Table 4).

Only FI and glucose differed significantly between the +2 C and the -2 C treatment implemented during period II (weeks of acclimation 5, 6 and 7) (Table 4). So few detectable differences may have resulted from reduced degrees of freedom due to the experimental design.

Table 4. Winter wheat crown tissue temperature, variety and week mean squares for eight variables during plant growth periods II and III.

Source	df	Osmotic Potential	FI	Sucrose	Glucose	Fructose	GFS	TNC	% CM
Run	1								
Temp (T)	1	13.05 ns	6.34 *	.041 ns	.013 *	.006 ns	262.0 ns	.159 ns	13.1 ns
Err a	1	.244	.026	.002	.00004	.0001	7.2	.004	.566
Var (V)	1	1.40 *	9.91 *	.004 ns	.00002 ns	.00008 ns	10.4 *	.004 *	174.0 *
T x V	1	1.97 *	.955 ns	.00001 ns	.00016 ns	.00002 ns	.18 ns	.0003 ns	16.0 ns
Err b	2	.026	.312	.0002	.00004	.000005	.494	.0002	7.05
Week (W)	4	.774 *	.406 ns	.002 *	.0034 *	.007 *	37.6 **	.033 *	7.8 ns
T x W	4	.060 ns	.067 ns	.00002 ns	.0013 *	.0001 ns	1.96 ns	.002 ns	4.7 ns
V x W	4	.111 ns	.101 ns	.0001 ns	.00006 ns	.0002 ns	1.10 ns	.0009 ns	1.01 ns
TxVxW	4	.012 ns	.118 ns	.0001 ns	.00003 ns	.00007 ns	.34 ns	.0002 ns	.43 ns
Err c	16	.056	.236	.0003	.002	.0003	2.52	.002	.43

FI = Fructan Index (fructan moles/mg H₂O);

GFS = (Glucose + Fructose + Sucrose) (molality);

TNC = Total nonstructural carbohydrate (peak area(mV sec)/mg H₂O);

% CM = Percent crown moisture.

*, ** = significant at P < .05 and P < .01, respectively.

ns = Not Significant.

Variety comparisons for eight variables over seven sampling weeks at the +2 C and -2 C temperature regimes are shown in Tables 5 and 6, respectively. Varieties differed for only 17 of 56 measurements taken at the +2 C regime (Table 5), whereas 28 of 56 measurements differentiated varieties at the -2 C regime (Table 6). Although the literature indicates that temperatures near 0 C are adequate to induce maximum hardening, it appears the -2 C temperature regime provided a better environment than the +2 C temperature regime for a differential varietal response. Furthermore, the -2 C temperature regime was more representative of the average acclimating conditions experienced by winter wheat in Bozeman, Montana. The major purpose of this study was to examine the relationship between osmotic potential and carbohydrates in the crowns of two winter wheats varying in winterhardness. The +2 C and -2 C temperature treatments were included to assure a response. Since the best differential varietal response occurred at the -2 C temperature treatment, the bulk of the discussion is based on data from the -2 C temperature regime.

Osmotic Potential

Crown osmotic potential decreased significantly over the nine weeks of acclimation (Table 4; Figure 1). No significant differences between varieties occurred during

Table 5. Variety means of Froid and Brawny winter wheat crown tissue for eight variables and seven weekly samples from the +2 C period II temperature regime.

Variable	WEEK						
	3	4	5	6	7	8	9
Osmotic Potential	-1.77 A +	-2.22 A	-1.95 A	-2.03 A	-2.23 A	-2.30 A	-2.77 A
	-1.52 A @	-2.00 A	-2.32 A	-2.20 A	-1.93 B	-2.61 A	-2.57 A
LSD (.05)	.676	.676	.518	.542	.242	.491	.329
FI	1.95 A	2.60 A	3.02 A	2.58 A	2.63 A	2.56 A	2.41 A
	1.36 A	2.12 B	2.15 A	2.15 B	1.95 A	1.78 A	1.72 A
LSD (.05)	.447	.447	1.61	.304	1.35	1.22	1.49
Sucrose	.042 A	.048 A	.060 A	.070 A	.070 A	.094 A	.105 A
	.042 A	.044 A	.055 A	.055 B	.053 B	.069 A	.072 A
LSD (.05)	.005	.005	.025	.008	.016	.035	.038
Glucose	.023 A	.020 A	.023 A	.020 A	.023 A	.035 A	.041 A
	.017 B	.023 B	.030 B	.034 A	.023 A	.038 A	.044 A
LSD (.05)	.0025	.0025	.0055	.038	.0039	.017	.0099
Fructose	.014 A	.010 A	.021 A	.033 A	.036 A	.070 A	.088 A
	.011 B	.009 A	.022 A	.040 A	.034 A	.065 A	.081 A
LSD (.05)	.0012	.0012	.0052	.027	.018	.028	.024
GFS	.078 A	.077 A	.104 A	.122 A	.128 A	.199 A	.233 A
	.059 B	.076 A	.106 A	.128 A	.109 A	.171 A	.197 B
LSD (.05)	.008	.008	.025	.058	.036	.080	.034
TNC	12.8 A	17.1 A	20.2 A	18.2 A	18.2 A	17.6 A	17.4 A
	8.3 B	13.7 B	17.0 A	16.7 A	14.3 A	13.5 A	13.6 A
LSD (.05)	1.97	1.97	9.67	1.55	4.61	8.83	7.18
% CM	77.9 A	74.5 A	72.7 A	72.1 A	72.2 A	71.7 A	71.7 A
	82.0 B	77.8 B	75.2 A	73.8 A	74.7 A	75.2 A	74.8 B
LSD (.05)	1.8	1.8	5.3	3.1	4.4	3.7	2.9

FI = Fructan Index (fructan moles/mg H₂O);

GFS = (Glucose + Fructose + Sucrose) (molality);

TNC = Total nonstructural carbohydrate (peak area(mV sec)/mg H₂O);

% CM = Percent crown moisture;

+, @ = Froid and Brawny, respectively.

Two variety means in the same column followed by the same letter are not significantly different.

LSD (.05) = Least Significant Difference at P < .05.

Table 6. Variety means of Froid and Brawny winter wheat crown tissue for eight variables and seven weekly samples from the -2 C period II temperature regime.

Variable	WEEK						
	3	4	5	6	7	8	9
Osmotic Potential	-1.77 A +	-2.22 A	-2.85 A	-2.79 A	-3.31 A	-3.52 A	-3.80 A
	-1.52 A @	-2.00 A	-2.23 B	-2.10 B	-2.26 B	-2.72 B	-2.86 B
LSD(.05)	.676	.676	.518	.542	.242	.491	.329
FI	1.95 A	2.60 A	3.68 A	3.93 A	4.18 A	3.85 A	3.09 A
	1.36 B	2.12 B	2.79 A	2.62 B	2.50 B	2.05 B	2.24 A
LSD(.05)	.447	.447	1.61	.304	1.35	1.22	1.49
Sucrose	.042 A	.042 A	.133 A	.126 A	.137 A	.160 A	.161 A
	.032 A	.044 A	.111 A	.122 A	.110 B	.130 A	.153 A
LSD(.05)	.0048	.0048	.025	.0079	.016	.035	.038
Glucose	.023 A	.020 A	.041 A	.049 A	.040 A	.096 A	.119 A
	.017 B	.023 B	.040 A	.052 A	.046 B	.084 A	.110 A
LSD(.05)	.0025	.0025	.0055	.038	.0039	.017	.0099
Fructose	.014 A	.010 A	.049 A	.052 A	.045 A	.111 A	.126 A
	.011 B	.009 A	.048 A	.059 A	.057 A	.089 A	.108 A
LSD(.05)	.0012	.0012	.0052	.027	.018	.028	.024
GFS	.078 A	.077 A	.222 A	.227 A	.222 A	.367 A	.406 A
	.059 B	.076 A	.198 A	.232 A	.212 A	.303 A	.371 B
LSD(.05)	.008	.008	.025	.058	.036	.080	.034
TNC	12.9 A	17.1 A	24.7 A	25.1 A	25.3 A	29.2 A	26.8 A
	8.3 B	13.7 B	18.0 A	18.4 B	16.6 B	18.0 B	20.6 A
LSD(.05)	1.97	1.97	9.67	1.55	4.62	8.83	7.18
% CM	77.9 A	74.5 A	69.8 A	68.5 A	67.1 A	64.8 A	65.7 A
	82.0 B	77.8 B	74.3 A	73.8 B	72.8 B	72.0 B	70.2 B
LSD(.05)	1.80	1.80	5.30	3.14	4.44	3.69	2.91

FI = Fructan Index (fructan moles/mg H₂O);

GFS = (Glucose + Fructose + Sucrose) (molality);

TNC = Total nonstructural carbohydrate (peak area(mV sec)/mg H₂O);

% CM = Percent crown moisture.

+, @ = Froid and Brawny, respectively.

Two variety means in the same column followed by the same letter are not significantly different.

LSD = Least Significant Difference.

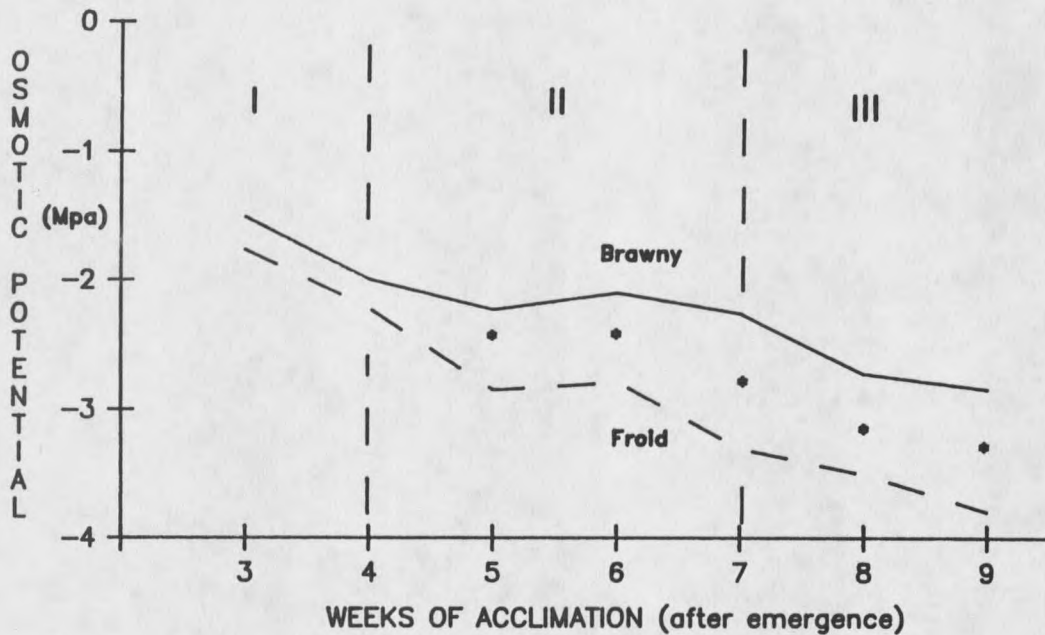


Figure 1. Weekly osmotic potentials of Froid and Brawny grown at the -2 C period II temperature regime. (I = 8 C/-3 C, II = -2 C/-2 C, III = -6 C/-6 C, day/night) * = varieties differed at $P < .05$.

period I. However, once the plants were subjected to subfreezing temperatures (-2 C in period II and -6 C in period III), the more winterhardy variety, Froid, developed a significantly lower osmotic potential than the less winterhardy Brawny (Figure 1). These results are consistent with the relationship of osmotic potential and winterhardiness of field hardened wheat found by DeNoma (1989).

CarbohydratesFructan Index (FI)

Significant differences between varieties for Fructan Index (FI) (fructan moles/mg H₂O) were measured during periods I, II and III (Table 6; Figure 2). Froid, the more winterhardy variety, had higher FI level than the less winterhardy Brawny (Figure 2).

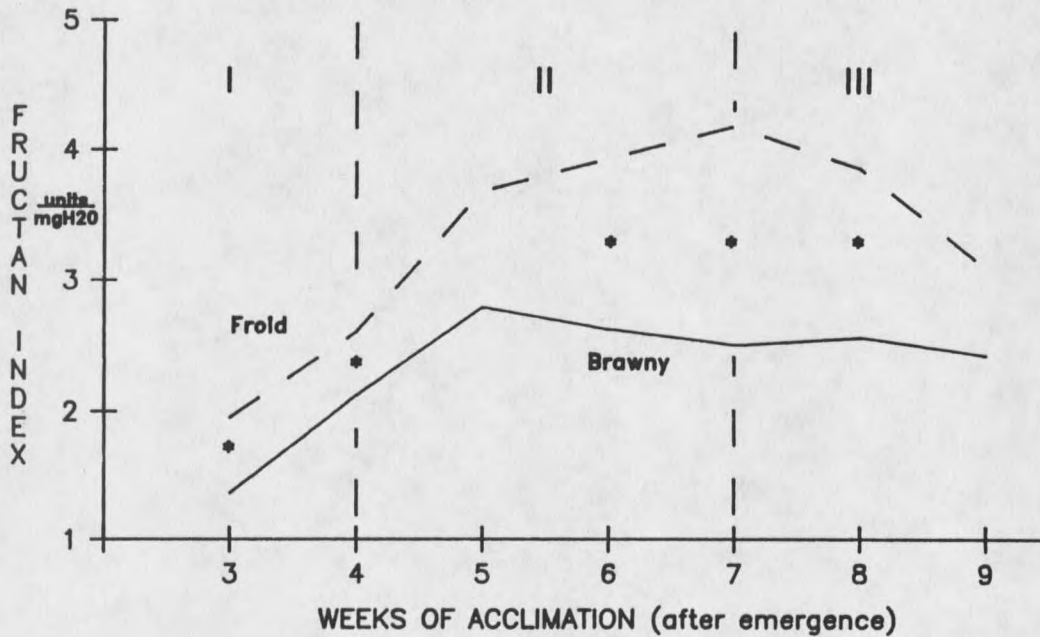


Figure 2. Weekly fructan indices (FI) for Froid and Brawny grown at the -2 C period II temperature regime. (I = 8 C/-3 C, II = -2 C/-2 C, III = -6 C/-6 C, day/night) * = varieties differed at P < .05.

These results are consistent with the report by Wille (1985) that high concentrations of fructan were associated with more winterhardy wheats.

The increased FI measured during periods II and III coincided with marked decreases in osmotic potential (Figures 1 and 2). This is logical since fructan is synthesized in response to cool temperatures (Pollock and Ruggles, 1976; Wagner et. al., 1983). However, the increase in Fructan Index (FI), especially apparent in Froid, throughout period II was interesting (Figure 2). During this time the plants were at -2 C temperature and little if any fructan synthesis should have occurred. Plant water may have influenced this relationship. Percent crown moisture (% CM) during periods I, II and III decreased significantly (Tables 3 and 4; Figure 3). The more winterhardy Froid, consistently maintained a lower % CM than the less winterhardy Brawny (Figure 3). Since FI is an estimate of the concentration of fructan per mg of water, it follows that decreased crown water content would result in increased FI. However, during period III FI decreased, but the corresponding decrease in % CM and osmotic potential seen during period II continued (Figures 1, 2 and 3). Other carbohydrates or solutes not examined in this study may also be involved in osmoregulation in the winter wheat crown.

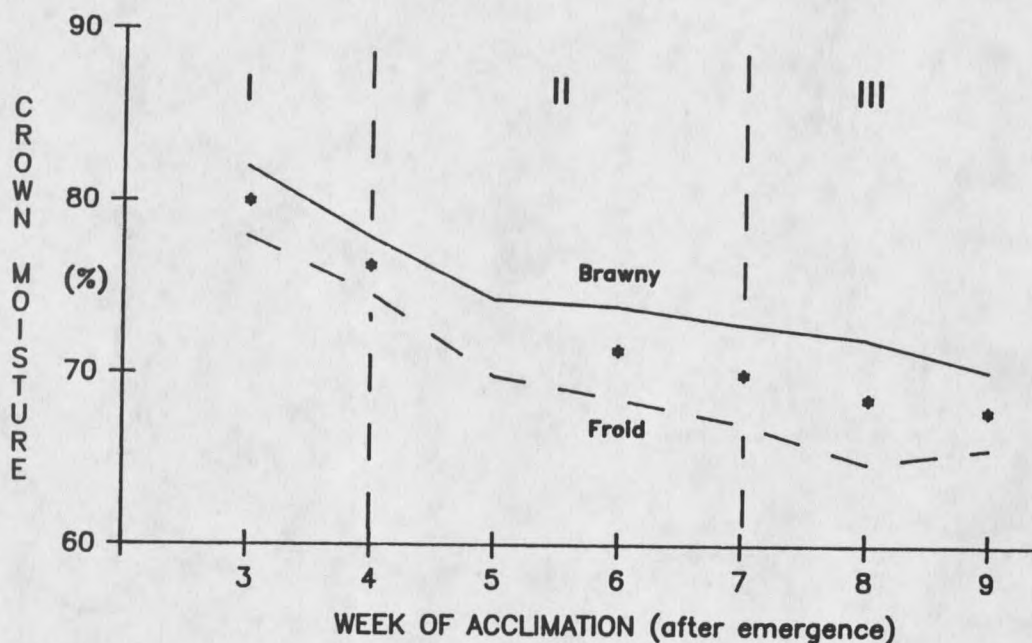


Figure 3. Weekly crown moisture percent for Froid and Brawny grown at the -2 C period II temperature regime. (I = 8 C/-3 C, II = -2 C/-2 C, III = -6 C/-6 C, day/night) * = varieties differed at $P < .05$.

Glucose, Fructose and Sucrose

Varietal differences were detected for sucrose at week 7, glucose at weeks 3, 4 and 7, and fructose at week 3 (Table 6; Figure 4 a-c). Glucose, fructose and sucrose increased significantly during the third to ninth acclimation weeks (Tables 3 and 4; Figure 4 a-c). Combining glucose, fructose and sucrose into one factor (GFS) by summing the molalities was valid since osmotic potential is a function of the number of molecules not the type of molecule per unit weight of solution. No significant

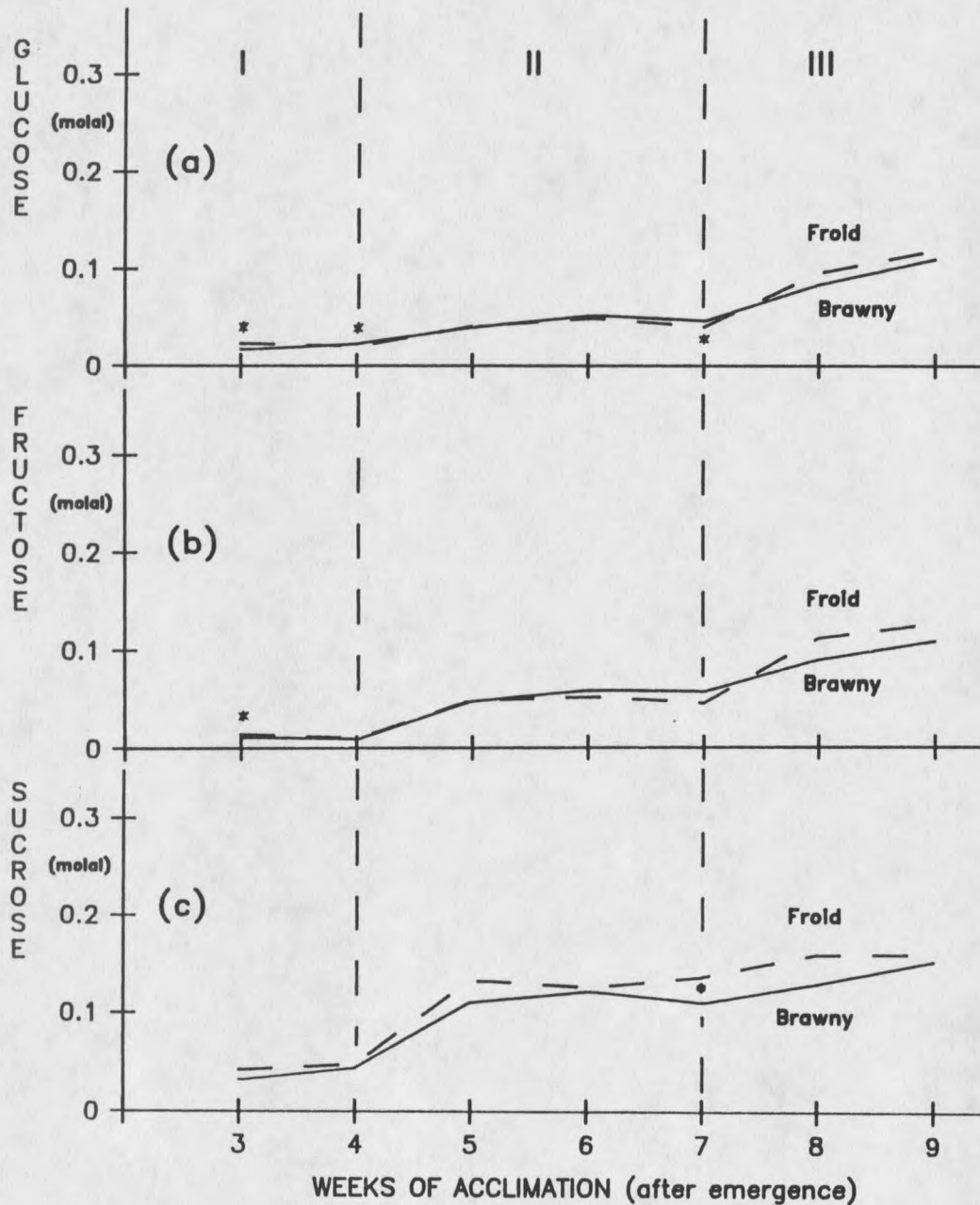


Figure 4.(a-c). Weekly glucose, fructose and sucrose concentrations for Froid and Brawny grown at the -2°C period II temperature regime. (I = $8^{\circ}\text{C}/-3^{\circ}\text{C}$, II = $-2^{\circ}\text{C}/-2^{\circ}\text{C}$, III = $-6^{\circ}\text{C}/-6^{\circ}\text{C}$, day/night)
 * = varieties differed at $P < .05$.

varietal differences were detected for GFS (Glucose + Fructose + Sucrose) except at the third and ninth weeks of acclimation (Table 6; Figure 5). Large increases in GFS occurred during the fifth (first week at -2 C) and the eighth (first week at -6 C) weeks of acclimation (Figure 5). This corresponded to decreased osmotic potential and increased Fructan Index during the fifth week of acclimation and decreased osmotic potential and decreased Fructan Index at the eighth week of acclimation (Figures 1, 2 and 5).

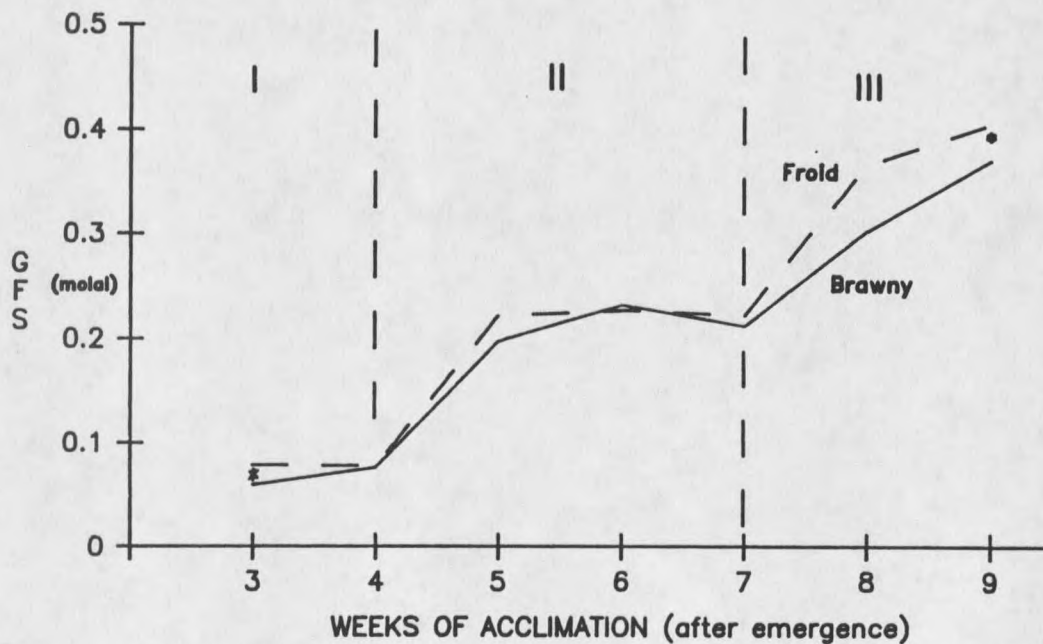


Figure 5. Weekly GFS (Glucose + Fructose + Sucrose) concentration for Froid and Brawny grown at the -2 C period II temperature regime. (I = $8\text{ C}/-3\text{ C}$, II = $-2\text{ C}/-2\text{ C}$, III = $-6\text{ C}/-6\text{ C}$, day/night) * = varieties differed at $P < .05$.

It is unlikely that photosynthesis or fructan synthesis occurred at -2 C or -6 C. The large increase in GFS during the eighth week probably resulted from the breakdown of fructan to glucose, fructose and sucrose (Edelman and Jefford, 1968).

Although no reduction in the Fructan Index (FI) was apparent during the fifth week of acclimation (Figure 2) a reduction in water content and corresponding increased fructan concentration might have obscured the actual reduction in fructan (Figures 2 and 3). When fructans were expressed on a dry weight basis, (Figure 6) this reduction in fructan became apparent during periods II and III of acclimation. Reductions in the amount of fructan during both the fifth and eighth weeks of acclimation (Figure 6) correspond to decreased osmotic potential and increased GFS (Figures 1 and 5). It appears that a highly interactive system involving the concentrations of fructan, glucose, fructose and sucrose combined with the breakdown of fructan to mono- and disaccharides and changes in percent crown moisture contribute to osmoregulation in winter wheat crowns in response to decreases in temperature.

Total Nonstructural Carbohydrate

Significant increases in total nonstructural carbohydrate (TNC) occurred during the nine weeks of

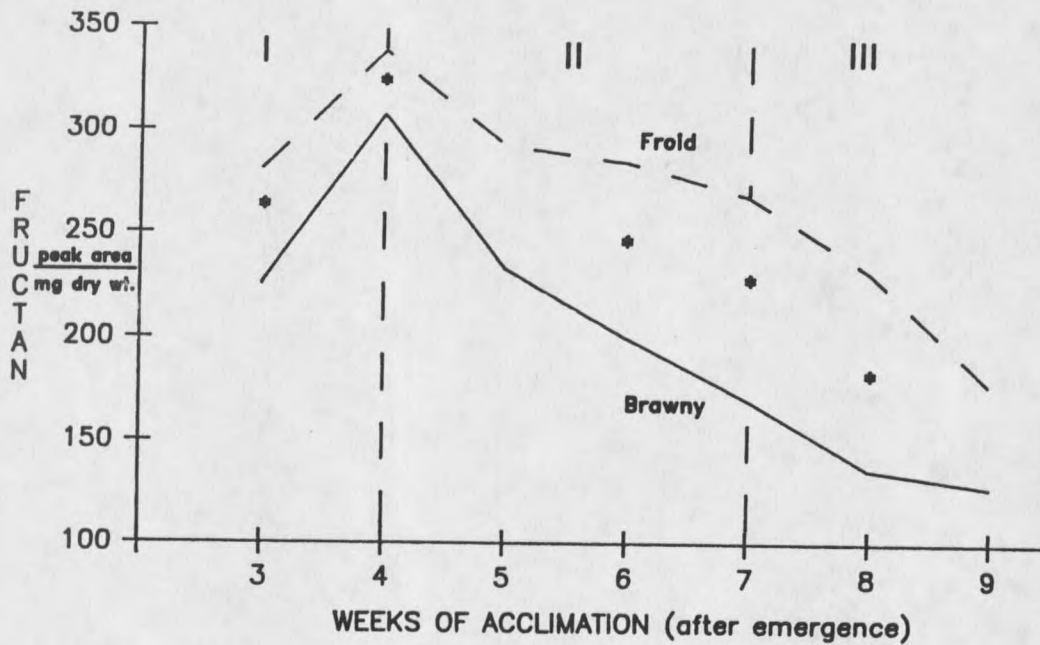


Figure 6. Weekly fructan content (peak area/mg sample dry weight) for Froid and Brawny grown at the -2°C period II temperature regime. (I = $8^{\circ}\text{C}/-3^{\circ}\text{C}$, II = $-2^{\circ}\text{C}/-2^{\circ}\text{C}$, III = $-6^{\circ}\text{C}/-6^{\circ}\text{C}$, day/night). * = varieties differed at $P < .05$.

acclimation (Table 4; Figure 7). Significant differences between varieties were detected at the third, fourth, sixth, seventh and eighth weeks of acclimation with the more winterhardy Froid always maintaining a higher concentration of TNC than the less winterhardy Brawny (Table 6; Figure 7). These results are consistent with Wille (1985) who found that high crown total nonstructural carbohydrate was correlated with increased winterhardiness in wheat. As the concentration of TNC went up during the fourth (-2°C) and

eighth (-6 C) weeks of acclimation, a corresponding decrease in osmotic potential was measured (Figures 1 and 7).

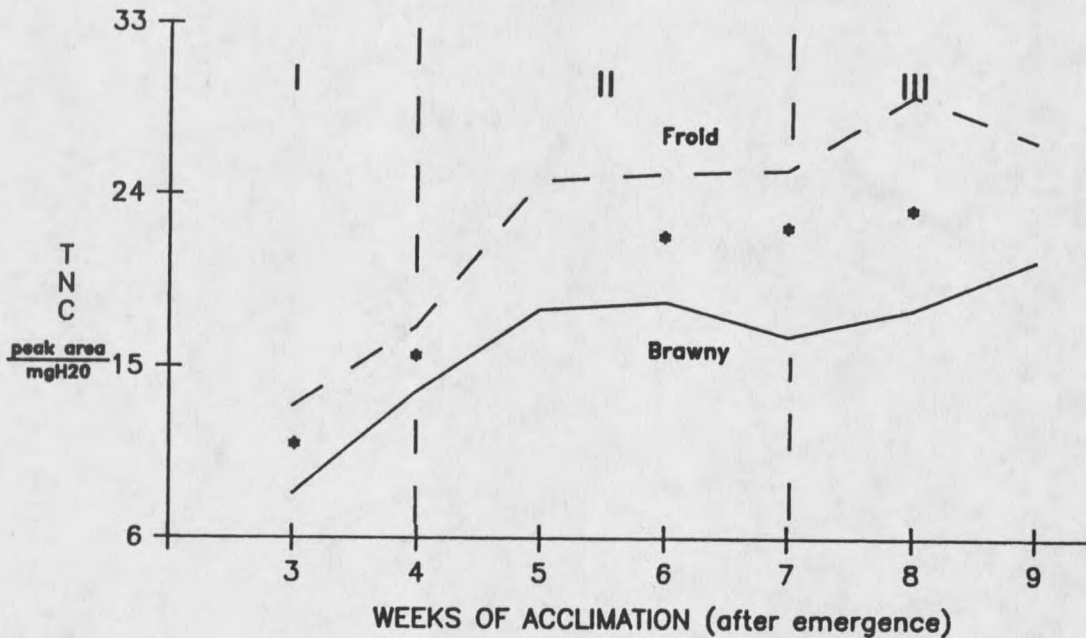


Figure 7. Weekly total nonstructural carbohydrate (TNC) concentration for Froid and Brawny grown at the -2 C period II temperature regime. (I = 8 C/-3 C, II = -2 C/-2 C, III = -6 C/-6 C, day/night). * = varieties differed at $P < .05$.

The concentration of TNC was calculated differently than the concentrations of fructan, glucose, fructose or sucrose. TNC concentration was calculated as the peak area/mg H₂O rather than starting with a molal standard or at least an estimate of molality as with fructan. This gives a good representation of TNC concentration. Correlations for concentrations of fructan, glucose, fructose and sucrose in

terms of peak area and in terms of molality were compared by simple correlation. The correlation between fructan as peak area/mg H₂O and Fructan Index was good ($r = .885$). Therefore, TNC expressed in terms of peak area/mg H₂O was a reasonable estimate of the actual concentration of TNC in the crown.

Relationships Among Traits

A negative correlation between osmotic potential and another positive measured factor shows that as osmotic potential is decreasing (becoming more negative) the level of the other factor is increasing.

Highly significant negative correlations were found between osmotic potential and all of the carbohydrate components measured (Table 7). All of the carbohydrate components were associated with osmoregulation in the winter wheat crown. Although other solutes present in the crown tissue (proteins, ions, etc.) would also contribute to osmotic potential, in this study the relationship of carbohydrates with osmotic potential and the importance of their role in winterhardiness was examined.

Osmotic potential as a function of several traits was examined using multiple regression. Fructan Index (FI), sucrose, glucose and fructose were associated with 75% of the variation in osmotic potential (Table 8). Sucrose and

Table 7. Correlation coefficients (r) among seven crown tissue variables over two winter wheat varieties, seven sampling weeks and two runs at the -2 C period II temperature regime. (n = 28)

	Osmotic Potential	FI	Sucrose	Glucose	Fructose	GFS
FI	-.66 **					
Sucrose	-.76 **	.59 **				
Glucose	-.66 **	.26 ns	.79 **			
Fructose	-.69 **	.36 ns	.87 **	.98 **		
GFS	-.74 **	.44 *	.94 **	.95 **	.98 **	
TNC	-.81 **	.90 **	.81 **	.62 **	.69 **	.75 **

FI = Fructan Index (fructan moles/mg H₂O);

GFS = (Glucose + Fructose + Sucrose) (molality);

TNC = Total nonstructural carbohydrate
(peak area(mV sec)/mg H₂O);

*, ** = significant at P < .05 and P < .01, respectively.
ns = Not Significant.

fructose, however, only contributed 5% to the regression equation (Table 8). FI and glucose were associated with 70% of the variation in osmotic potential (Table 8). Both FI and glucose regression coefficients contributed significantly to the regression equation and both were negative (Table 8). As glucose and fructan concentrations

Table 8. Multiple regression coefficients expressing osmotic potential of winter wheat crown tissue as a function of several traits for all samples from the -2 C period II temperature regime. (n = 28)

Dependent Variable	Independent Variables	Y Intercept	Regression Coefficients				R ²
			B1	B2	B3	B4	
Osmotic Potential	FI, Gluc Suc, Fruc	-.48	-.35 *	-30.4 *	-7.5 ns	24.1 ns	.75
Osmotic Potential	FI, Gluc	-.87	-.40 **	-10.8 **			.70
Osmotic Potential	FI, GFS	-.95	-.32 **	-3.4 **			.69
Osmotic Potential	TNC	-.80	-.90 **				.66
Osmotic Potential	Gluc	-1.83	-13.6 **				.45
Osmotic Potential	Fruc	-1.90	-12.0 **				.47
Osmotic Potential	Suc	-1.34	-11.4 **				.59
Osmotic Potential	FI	-1.17	-.51 **				.44

FI = Fructan Index (fructan moles/mg H₂O);

Gluc = Glucose (molality); Fruc = Fructose (molality);

Suc = Sucrose (molality);

GFS = (Glucose + Fructose + Sucrose) (molality);

TNC = Total nonstructural carbohydrate
(peak area (mV sec)/mg H₂O);

*, ** = significant at P < .05 and P < .01, respectively.

ns = Not Significant.

increased, osmotic potential decreased. It is difficult to justify excluding any of the carbohydrate components in the

regression equation since all of them contributed to osmotic potential. A regression equation using FI and GFS (Glucose + Fructose + Sucrose) accounted for 69% of the variation in osmotic potential and worked as well as using FI and glucose (Table 8). TNC (total nonstructural carbohydrate) concentration is also closely associated with osmotic potential since it accounted for 66% of the variation (Table 8). We suggest that these regression equations might allow prediction of actual or at least relative osmotic potentials of diverse winter wheat cultivars.

Since all carbohydrates contributed to osmotic potential, several combinations of carbohydrate components were closely related to osmotic potential; however, which particular carbohydrate component affected osmotic potential to the greatest degree? When sucrose was used in a regression equation 59% of the total variation was accounted for while FI only accounted for 44% of the variation (Table 8). It appears sucrose affects changes in osmotic potential more than any other single carbohydrate component. But where does the sucrose responsible for decreasing the osmotic potential in the winter wheat crowns originate?

Using a technique similar to that of Smith (1981), Dr. Jarvis Brown of Montana State University determined the amount of total nonstructural carbohydrate as mg of TNC/mg dry sample weight on several selected crown tissue samples.

Subtracting the amount of sucrose, glucose and fructose, obtained by HPLC analysis, from the total nonstructural carbohydrate gave an estimate of the actual concentration of fructan in the crowns. After 4 and 5 weeks of acclimation, crowns of both Froid and Brawny contained substantially more fructan than GFS (Table 9). As high as 84% of the total carbohydrate in the crown was fructan by week 4 of acclimation. A decrease in percent fructan and a corresponding increase in percent GFS occurred between week 4 (8 C day/-3 C night) and week 5 (-2 C day/-2 C night) of acclimation. It appears a substantial amount of fructan was broken down to GFS in response to a reduction in temperature. Glucose, fructose and sucrose increased by about 2X, 5X and 3X, respectively, but the largest increase in amount of sugar, as expected, was in sucrose (Table 6).

Table 9. Fructan and GFS concentrations expressed as percent of total nonstructural carbohydrate (TNC) for Froid and Brawny at two weeks of acclimation for run number 2 at the -2 C period II temperature regime.

Week of Acclimation	Variety	Fructan ----- % of TNC-----	GFS
4	Froid	84 %	16 %
	Brawny	81 %	19 %
5	Froid	69 %	31 %
	Brawny	69 %	31 %

GFS = (Glucose + Fructose + Sucrose).

Although sucrose, and therefore GFS, appears to affect osmotic potential of winter wheat crown tissue to the greatest degree, virtually all increases in GFS during period II (-2 C day/-2 C night) and period III (-6 C day/-6 C night) were probably due to fructan breakdown. It is unlikely photosynthesis is very active at subfreezing temperatures.

Also, FI is more than an estimate of fructan concentration. It is an estimate of the number of molecules of fructan per mg of water. The metabolic system that builds and breaks down fructan has the unique ability to convert long chain fructans to shorter chain fructans, thereby increasing the number of fructan molecules, and hence, decreasing the osmotic potential. This reduction of fructan chain length along with hydrolysis of fructan to sucrose, then in turn to glucose and fructose constitutes a major force behind osmoregulation and, therefore, winter survival of winter wheat crown tissue.

SUMMARY

The objective of this research was to determine the relationship of osmotic potential and carbohydrates in the crowns of acclimating winter wheat.

Measurements of concentrations of fructan index (FI), sucrose, glucose, fructose and total nonstructural carbohydrate (TNC) and readings for percent crown moisture (% CM) and osmotic potential were taken on crowns from a controlled environment study beginning with the third and ending with the ninth week of acclimation following emergence. Concentrations of fructan (FI) and TNC were consistently higher in the more winterhardy variety, Froid, than in the less winterhardy variety, Brawny. This agrees with the relationship between winterhardiness and carbohydrates discussed by Wille (1985).

Percent crown moisture was consistently lower in the more winterhardy Froid than in the less winterhardy Brawny. Furthermore, Froid maintained a significantly lower osmotic potential than the less winterhardy Brawny in agreement with DeNoma (1989). The varieties did not differ significantly for levels of sucrose, glucose or fructose.

It appears a highly interactive system involving concentrations of fructan, glucose, fructose and sucrose combined with the breakdown of fructan from long to short chains and to mono- and disaccharides and changes in crown moisture content contribute to osmoregulation in winter wheat crowns in response to reduced temperature.

Highly significant negative correlations were found between osmotic potential and all the carbohydrate components measured ($r = -.66$ to $-.81$). Several combinations of the carbohydrate components in multiple regression equations were closely associated with osmotic potential. Sucrose in a regression equation accounted for 59% of the total variation and was the single carbohydrate component affecting changes in osmotic potential to the greatest degree. However, after the seedlings were placed at subfreezing daytime temperatures (week 5 of acclimation), the breakdown of fructan was responsible for virtually all increases in sucrose concentration. The breakdown of fructan also corresponded to increases in glucose, fructose and FI. FI is proportional to the concentration of fructan molecules in the crown tissue. Measurements on dry weight concentrations of TNC showed that fructan constituted up to 84% of the total nonstructural carbohydrate in winter wheat crowns.

Since fructan concentration and fructan chain length (degree of polymerization) appear to be the controlling forces behind osmoregulation in winter wheat crowns, it follows that wheat with the ability to store larger amounts of fructan and, therefore, larger amounts of TNC will, in general, be more winterhardy. Froid, the more winterhardy variety, always maintained a higher concentration of fructan and total nonstructural carbohydrate as well as lower osmotic potential and percent crown moisture than the less winterhardy variety Brawny.

These data strongly suggest that osmotic potential, fructan concentration, total nonstructural carbohydrate and crown moisture content can be used in breeding for improved winterhardiness in wheat.

Any or all of the above mentioned factors could be used in a screening technique for winterhardiness in wheat. However, the expense and difficulty associated with measuring carbohydrates currently prohibits their use, but osmotic potential and percent crown moisture differentiated varieties very well.

Although the simple measurements will work for a screening technique, the major emphasis for future research should be towards understanding the mechanisms more winterhardy varieties employ for accumulating higher amounts of fructan than less winterhardy varieties. Research should

include studies on enzyme differences and other possible genetic controls the wheat plant may use in response to cold temperature to increase fructan content and winterhardiness.

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APPENDIX

Table 10. Winter wheat crown tissue temperature and variety mean squares for eight variables at week 5 of acclimation.

Source	df	Osmotic							
		Potential	FI	Sucrose	Glucose	Fructose	GFS	TNC	% CM
Run	1								
Temp (T)	1	.322 ns	.840 ns	.0084 ns	.0004 ns	.0014 ns	.022 ns	15.0 ns	6.92 ns
Err a	1	.088	.020	.0009	.00003	.0004	.003	4.7	.858
Var (V)	1	.030 ns	1.52 ns	.0004 ns	.00002 ns	.00001 ns	.0002 ns	49.5 ns	23.7 ns
T x V	1	.486 ns	.029 ns	.0001 ns	.00003 ns	.00001 ns	.0004 ns	5.85 ns	1.94 ns
Err b	2	.044	.427	.0001	.000005	.000005	.0001	15.4	5.56

FI = Fructan Index (fructan moles/mg H₂O);

GFS = (Glucose + Fructose + Sucrose) (molality);

TNC = Total nonstructural carbohydrate (peak area(mV sec)/mg H₂O);

% CM = Percent crown moisture.

ns = not significant.

Table 11. Winter wheat crown tissue temperature and variety mean squares for eight variables at week 6 of acclimation.

Source	df	Osmotic							
		Potential	FI	Sucrose	Glucose	Fructose	GFS	TNC	% CM
Run	1								
Temp (T)	1	.217 ns	1.64 ns	.008 ns	.001 ns	.0007 ns	.022 ns	37.2 *	11.8 ns
Err a	1	.075	.040	.001	.00003	.0001	.0026	.047	.180
Var (V)	1	.133 ns	1.51 *	.0002 *	.0001 ns	.0001 ns	.0007 ns	33.1 *	34.0 *
T x V	1	.377 ns	.388 *	.00006 ns	.00007 ns	.00001 ns	.00001 ns	13.3 *	3.15 ns
Err b	2	.048	.015	.00001	.0002	.0001	.0006	.395	1.26

FI = Fructan Index (fructan moles/mg H₂O);

GFS = (Glucose + Fructose + Sucrose) (molality);

TNC = Total nonstructural carbohydrates (peak area(mV sec)/mg H₂O);

% CM = Percent crown moisture.

* = significant at P < .05; ns = Not Significant.

Table 12. Winter wheat crown tissue temperature and variety mean squares for eight variables at week 7 of acclimation.

Source	df	Osmotic								
		Potential	FI	Sucrose	Glucose	Fructose	GFS	TNC	% CM	
Run	1									
Temp (T)	1	.989 ns	2.18 ns	.008 ns	.0008 ns	.0005 ns	.019 ns	43.7 *	24.8 ns	
Err a	1	.029	.0009	.0001	.00006	.00003	.0006	.222	.48	
Var (V)	1	.905 *	2.78 ns	.0009 *	.00002 ns	.00004 ns	.0004 ns	79.4 *	33.8 ns	
T x V	1	.277 *	.504 ns	.00006 ns	.00002 ns	.0001 ns	.00005 ns	11.9 ns	4.86 ns	
Err b	2	.01	.30	.00004	.000003	.00005	.0002	3.5	2.39	

FI = Fructan Index (fructan moles/mg H₂O);

GFS = (Glucose + Fructose + Sucrose) (molality);

TNC = Total nonstructural carbohydrate (peak area(mV sec)/mg H₂O);

% CM = Percent crown moisture;

* = significant at P < .05; ns = not significant.

Table 13. Winter wheat crown tissue temperature and variety mean squares for eight variables at week 8 of acclimation.

Source	df	Osmotic							
		Potential	FI	Sucrose	Glucose	Fructose	GFS	TNC	% CM
Run	1								
Temp (T)	1	.884 ns	1.21 ns	.008 ns	.006 ns	.002 ns	.044 ns	129.6 ns	49.9 **
Err a	1	.118	.073	.0003	.0004	.0001	.0024	6.2	.001
Var (V)	1	.119 ns	3.31 ns	.002 ns	.00005 ns	.0004 ns	.0042 ns	116.8 ns	57.2 *
T x V	1	.606 ns	.52 ns	.00001 ns	.0001 ns	.0001 ns	.0006 ns	25.4 ns	6.9 ns
Err b	2	.039	.24	.0002	.00005	.0001	.001	12.8	2.31

FI = Fructan Index (fructan moles/mg H₂O);

GFS = (Glucose + Fructose + Sucrose) (molality);

TNC = Total nonstructural carbohydrate (peak area(mV sec)/mg H₂O);

% CM = Percent crown moisture.

*, ** = significant at P < .05 and P < .01, respectively.

ns = not significant.

Table 14. Winter wheat crown tissue temperature and variety mean squares for eight variables at week 9 of acclimation.

Source	df	Osmotic										
		Potential	FI	Sucrose	Glucose	Fructose	GFS	TNC	% CM			
Run	1											
Temp (T)	1	.881 *	.726 ns	.009 **	.01 ns	.002 ns	.06 ns	132.9 ns	56.6 ns			
Err a	1	.0008	.364	.000001	.0005	.0007	.002	14.6	5.62			
Var (V)	1	.657 *	1.17 ns	.0008 *	.00003 ns	.0003 ns	.003 ns	50.1 ns	29.5 *			
T x V	1	.279 ns	.013 ns	.0003 ns	.00007 ns	.00006 ns	.00001 ns	2.9 ns	.904 ns			
Err b	2	.017	.365	.00002	.00002	.00009	.0002	8.5	1.36			

FI = Fructan Index (fructan moles/mg H₂O);

GFS = (Glucose + Fructose + Sucrose) (molality);

TNC = Total nonstructural carbohydrate (peak area(mV sec)/mg H₂O);

% CM = Percent crown moisture;

*, ** = significant at P < .05 and P < .01, respectively.

ns = not significant.

Table 15. Weekly fructan average degree of polymerization for Froid and Brawny at the +2 C and -2 C period II temperature regimes.

Variety	Temperature Regime	Week of Acclimation						
		3	4	5	6	7	8	9
Froid	+2 C	5.1	5.4	5.3	5.1	4.9	3.8	3.5
	-2 C	5.1	5.4	4.2	4.0	3.8	3.9	3.7
Brawny	+2 C	4.4	5.0	6.0	5.5	5.2	4.0	3.8
	-2 C	4.4	5.0	3.5	3.3	3.1	3.1	2.9

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