Carbohydrates, regrowth, and chlorophyll as physiological indicators of winterhardiness in winter wheat (Triticum aestivum L.)
by Michael Joseph Wille

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

Abstract:
Winter wheat offers approximately a 20% yield advantage over spring wheat, provided it can successfully overwinter. In northeastern and north central Montana, winter wheat is often injured during winters of low temperatures and minimal snow cover. The development of hardier winter wheat is a major objective of the Montana winter wheat breeding program. Current methods of assessing the winterhardiness potential of new genotypes are not precise, and are subject to unpredictable winter conditions. The objective of this study was to examine the relationship of several physiological characteristics with winterhardiness of wheat, and to develop a winterhardiness screening procedure. Eight or ten wheat cultivars of varying hardiness were sampled from autumn to early spring in 1981-82 and 1982-83 for crown carbohydrate concentrations, crown regrowth after controlled freezing, and leaf chlorophyll concentration. ' Carbohydrates: Crown tissues were assayed for glucose plus fructose, sucrose, and fructosans. Total available carbohydrates were estimated as the sum of the individual fractions. No starch was detected. Concentrations of glucose plus fructose (r = 0.645* to 0.780*), fructosans (r = 0.704* to 0.915**) and total available carbohydrates (r = 0.757* to 0.895**), but not sucrose, were correlated with winterhardiness scores. The technique is highly sensitive and specific for each carbohydrate.

Regrowth: In the regrowth experiment 10 crowns were placed in a freezer for successive periods of 12 hrs at 0°C, -10°C, -20°C, and switched to a growth chamber for 72 hrs at 21°C/10°C (day/night temp.). Measurements included regrowth (mm) per plant, regrowth (mm) per survivor, and number of survivors. While at times the correlation between winterhardiness and regrowth was high,the data were inconsistent. Evidence suggests this may be due to fluctuating hardiness levels as a response to environmental changes. It is concluded that with the proper temperature regime the method may prove valuable.

Chlorophyll: Fresh weight and leaf area were determined using twenty fully expanded leaf blades from each cultivar. Chlorophyll was extracted and the amount determined by the method of Argon(3). Results were expressed in terms of mg chlorophyll per dm -2 Although the results of this experiment do not reveal any significant correlations between leaf chlorophyll concentration and winterhardiness score, difficulties in obtaining accurate non-destructive measurements of leaf areas may have masked actual differences among cultivars.

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

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ABSTRACT

Winter wheat offers approximately a 20% yield advantage over spring wheat, provided it can successfully overwinter. In northeastern and north central Montana, winter wheat is often injured during winters of low temperatures and minimal snow cover. The development of hardier winter wheat is a major objective of the Montana winter wheat breeding program. Current methods of assessing the winterhardiness potential of new genotypes are not precise, and are subject to unpredictable winter conditions. The objective of this study was to examine the relationship of several physiological characteristics with winterhardiness of wheat, and to develop a winterhardiness screening procedure. Eight or ten wheat cultivars of varying hardiness were sampled from autumn to early spring in 1981-82 and 1982-83 for crown carbohydrate concentrations, crown regrowth after controlled freezing, and leaf chlorophyll concentration.

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Chlorophyll: Fresh weight and leaf area were determined using twenty fully expanded leaf blades from each cultivar. Chlorophyll was extracted and the amount determined by the method of Arnon(3). Results were expressed in terms of mg chlorophyll per dm^{-2}. Although the results of this experiment do not reveal any significant correlations between leaf chlorophyll concentration and winterhardiness score, difficulties in obtaining accurate non-destructive measurements of leaf areas may have masked actual differences among cultivars.

INTRODUCTION

In Montana, winter wheat has a yield advantage of about 20% over spring wheat. In the northeastern and north central sections of the state, this yield advantage is often not realized because of winter injury. The development of new winter wheat cultivars with improved winterhardiness is a major objective of the winter wheat breeding program.

Assessing the hardiness of new winter wheat lines is imprecise due largely to the mercurial nature of the weather. Winters of either unusual severity, which cause complete mortality, or of unusual temperance, in which no mortality occurs, do not allow the breeder to detect relative differences amoung cultivars. Therefore all lines must be carried forward until a winter of appropriate severity causes differential winter injury. It was the objective of this study to examine several physiological characteristics and their relationship to winterhardiness. This could provide a more objective basis for selection of winterhardy wheats.

REVIEW OF LITERATURE

In any discussion of freezing injury, it is necessary to define the nature of the injury. While the precise nature of freezing injury is unknown, several theories have been proposed to explain it.

The ice crystalization theory, championed principally by Olien (21, 30, 31, 32, 33), holds that freezing injury is the result of the mechanical disruption of cells or tissues through the formation of crystalline ice. This may occur intracellularly or extracellularly. In intracellular freezing, which is typical of tender plants, ice crystals grow through the protoplast, destroying its integrity, and therefore causing death. In winter wheat, intracellular freezing is unlikely under field conditions.

In hardier species, ice appears to propagate intracellularly and does not form in the cytoplasm or vacuole (30). There are two types of extracellular freezing which Olien (30) calls equilibrium and non-equilibrium freezing. Non-equilibrium freezing (a thermodynamically irreversible reaction) involves the explosive growth of extracellular ice crystals which results in large distortions of tissues and which generates great shearing forces between the growing ice crystals and anatomical structures of the plant such as in the vascular tissue. Such disruptions have been noted in crown tissue of winter cereals (29) and are a common cause of injury in the eastern United States where mid-winter thaws cause an increase in cell water

content which is then followed by a period of freezing (30). In equilibrium freezing the system is never significantly displaced from thermodynamic equilibrium (hence it is a reversible reaction). Since a state of near equilibrium is maintained, ice crystals grow slowly and there is time for redistribution of water between the cell interior and exterior. This type of freezing is accompanied by cell desiccation and plasmolysis. Injury from equilibrium freezing does not generally occur until protoplasts are severely dehydrated at low temperatures. Equilibrium freezing appears to be the most common form of injury to cereals in the western United States (30, 51, 52, 53). In effect, equilibrium freezing may be considered a form of drought, since the removal of water into the extracellular spaces imposes a severe desiccation stress on the protoplast (56). However, freezing injury cannot be explained solely as a water stress as was demonstrated by Sukumaran and Weiser (48) who found that slow freezing of potato leaves caused more injury than desiccation at corresponding desiccation energies. Olien obtained similar results with barley (31). Hence, freezing injury must be considered as an interaction between desiccation and low temperature.

The current consensus is that freezing injury is essentially a biochemical event. Most theories consider the inactivation of enzymes and/or membrane damage. These include direct low temperature inactivation (16), the sulfhydryl-disulfide hypothesis (23), the salting-out hypothesis (28), the vital water hypothesis (62), and the bound water hypothesis (5).

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Levitt (23) proposed that freezing injury is due to the formation of intermolecular disulfide bonds induced by the close proximity of protein molecules due to dehydration. This results in a conformational change in the protoplasmic proteins. However, Heber and Santarius (17) have demonstrated that while the oxidation of sulfhydryl groups may be a secondary event, such oxidation is not the primary cause of freezing injury.

Based on theoretical calculations Heber (16) proposed that tertiary protein structure becomes unstable both above and below optimum temperatures, but experimental evidence (16, 58) led Heber to conclude that "while there is ample evidence for cold denaturation in vitro... it appears presently difficult to invoke these effects as determining factors in freezing injury of cells".

Protein tertiary structure is intimately associated with the properties of the aqueous medium in which it exists. Therefore, Weiser (62) has proposed that as water molecules become more highly ordered with decreasing temperature and water-to-water hydrogen bonding increases, hydrogen bonding of water to macromolecules decreases and thus the forces which hold hydrophilic residues to the exterior and hydrophobic residues to the interior also decrease. This causes a conformational change and therefore loss of activity.

Along a similar line, Merryman (28) and Lovelock (26) feel that denaturation occurs as a result of the increased ionic strength of the cytoplasm as water is withdrawn during freezing. As salt concentration rises, water-to-protein hydrogen bonds are replaced by ions, resulting in a "salting-out" effect.

Heber and Santarius (17) have proposed that the primary cause of freezing injury is the disruption of the membrane system. Irreversible uncoupling of oxidative phosphorylation has been demonstrated in isolated mitochondria of spinach (Spinachia oleracea L.) (16, 17), beet (Beta vulgaris L.) and winter wheat (Triticum aestivum L.) (17) and of cyclic photophosphorylation in isolated spinach chloroplasts (16, 17, 38, 41) as well as those of winter wheat and beet (17). Similar results have been shown to occur in vivo in spinach (17). In addition, the release of plastocyanin and chloroplast coupling factor CF_1 during a slow freeze-thaw cycle has been reported (12). Whereas the uncoupling of photophosphorylation may not be immediately catastrophic, since the plant could live for a time on stored food reserves, the uncoupling of oxidative phosphorylation is a very serious metabolic disorder and is sufficient to explain the rapid death caused by freezing. The phosphorylating systems of chloroplasts and mitochondria are almost identical in design and therefore inferences may readily be made from one to the other.

What is the chemistry of the disorder? Although this has yet to be resolved, current theories center around structural changes in either the lipid system or the membrane-bound proteins or both. Neither lipids nor specific proteins need be directly involved in phosphorylation reactions since any change which disrupts the order of the phosphorylating system (which is entirely embedded within the membrane) is sufficient to cause uncoupling. Dehydration of bound water and high salt concentration may be considered as prime agents of this process. In fact, the distinction between desiccation and high

salt concentration may be meaningless since salts themselves act as dehydrating agents.

Carbohydrates

Parker (35) studied seasonal changes in the carbohydrates of Eastern White Pine (Pinus strobus L.) and found that the raffinose concentration increased in the fall, rising from undetectable amounts in September to 1.5% of fresh weight by December; and was closely associated with increased hardiness. This was followed by a steep decline in the spring which was nearly concomitant with a decrease in hardiness. A similar pattern was found to occur in cloudberry, (Rubus chamaemous L.) (20).

In wheat, Barnell (6) observed that total ethanol-soluble carbohydrates increased with decreasing temperature during freezing periods and declined during warmer temperatures. Sucrose, in particular, showed a sharp increase during freezing periods. Glucose and fructose showed a pattern similar to that of sucrose, but muted. Sucrose (as a percentage of dry weight) did not quickly attain maximal values with cold weather, but continued to increase while temperatures remained low, and declined rapidly as the temperature rose.

Using two ecotypes of orchard grass (Dactylis glomerata), one from Norway and the other from Portugal, Eagles (8) found a highly significant negative correlation between temperature and fructosan content. Both ecotypes showed a significant increase in fructosans with lower temperature, with the Norwegian type increasing to a much greater extent than the Portugese type.

Conversely, Steponkis and Lanphear (47) could not demonstrate any direct parallelism between cold hardiness and any of the soluble sugars of English ivy (Hedera helix L.) when sampled weekly. They concluded that while carbohydrates do play a role in the acclimation process, cold hardiness is not simply an accumulation of sugars, nor does the carbohydrate level limit either the rate or the degree of hardiness.

Siminovitch et al. (46) found that the increase in frost hardiness of black locust was often accompanied by the disappearance of starch and an increase in soluble sugars. A subsequent study (45) showed that while there appears to be no primary relationship between hardiness and soluble sugar content, there was a negative correlation between starch and hardiness. It was, therefore, concluded that the increase in winterhardiness of black locust was due, not to an increase in soluble sugars, but to the disappearance of starch, since the presence of starch was viewed as a deterent to hardening.

Sakai (37) demonstrated that when mulberry twigs were gradually desiccated at room temperature for two to three days, they exhibited a decrease in starch content, and in water content, and an increase in sucrose and in osmotic concentration. When twigs were frozen at -10°C or -20°C for 24 hours, desiccated twigs had a survival rate of 100% and 70%, respectively, compared to 25% and 0% for the control. It was also established that without starch reserves sucrose content does not rise, nor does frost resistance increase.

More specifically with wheat, it has been observed that at hardening temperatures there is an increase in mono- and disaccharides, in

oligosaccharides (4, 55, 56) and in fructosans (55, 56) with a proportionally greater increase in the amount of fructosans (55, 56). Contrary to the general trend, Green and Ratzlaff (14) found that at low, positive temperatures (in °C) there existed an inverse relationship between the quantities of soluble sugars, particularly sucrose and fructose, and winterhardiness as determined by a comparison between two hardy and two nonhardy genotypes. However, since they did not measure any of the higher molecular weight saccharides, they acknowledge their results could be an artifact of the procedure and that the hardy cultivars could be converting simple sugars into oligo- and polysaccharides.

Babenko (4) reported that when hardened wheat plants are exposed to freezing temperatures, the level of oligosaccharides falls while sucrose and fructose levels increase. It was suggested that the hydrolysis of oligo- and polysaccharides with the concurrent increase in the quantity of simple sugars is an important mechanism of freezing resistance.

Dexter (7) observed that winter wheat plants could be hardened in the dark at 0°C if there were sufficient food reserves. On the other hand, more succulent greenhouse plants hardened little in the dark at 0°C, but hardened more completely with 7 hrs. of daily illumination, and still more completely with 15 hrs of illumination daily. None, however, could be hardened under any regime of light and temperature without CO_2.

Tumanov and Trunova (56, 57), using a 12% sucrose solution, were able to produce hardened winter wheat plants in the dark at low temperature which were equal to or hardier than the control group kept at the same temperature in the light. The rates of sucrose accumulation from the sucrose solution and by photosynthesis were approximately equal.

A wide variety of simple sugars as well as some other compounds has been found effective in increasing the hardiness of several species. Levitt found both glucose and sucrose effective in hardening cabbage leaves (22) as was glycerine in hardening both cabbage and sassafrass (Saxafragia cordifolia L.) (21). Sakai (37) reported that hardiness is enhanced in gardenia (Gardinia asminoides, Ellis) by glucose, sucrose, xylose, maltose, raffinose, ethylene glycol, glycerol, mannitol and acetoamide; inorganic salts, however, were ineffective.

In winter wheat, Green (13) determined that plants grown on glucose or sucrose survived freezing better than control plants. Conversely, mannitol was ineffective. In one experiment (56), Tumanov and Trunova report that sucrose, raffinose, and maltose were effective in hardening wheat coleoptiles while glucose was appreciably weaker, and lactose was ineffective. Yet in another paper (57) they report sucrose and glucose were nearly equal in inducing hardening of seedlings while rhamose and maltose were considerably less effective, and the effects of lactose and galactose were negligible. Trunova (54) determined glucose and sucrose to be equally effective; rhamose, less so; and xylose and arabinose, ineffective.

The confusion generated by contradictory results for glucose, sucrose and maltose may be somewhat ameliorated by bearing in mind that the solution concentrations, hardening regimes, and freezing conditions were not necessarily comparable between experiments. The substances which were ineffective in inducing hardening either do not enter the cells or cannot be further metabolized into useful compounds (54, 56).

The involvement of oligo- and polysaccharides in cryoprotection has often been broached, but few details are available. Eagles (8), as noted earlier, reported a strong interaction between temperature and fructosan levels in cocksfoot. Green (13) observed the presence of a "higher molecular weight oligosaccharide" on paper chromatograms of winter wheat grown on nutrient solution with either glucose or sucrose. Voblikova (59) discovered that a new fructose-based oligosaccharide rapidly appeared on paper chromatograms of wheat which had been exposed to hardening temperatures. Babenko (4) established that when wheat plants were exposed to hardening temperatures, the total amount of carbohydrate increased sharply, oligosaccharides accounting for 25% to 50% of the total. Trunova (55) stated that while the sum of mono- and disaccharides doubles during hardening, oligosaccharides triple. In all the previously mentioned work the term "oligosaccharides" is used in a generic sense to indicate unspecific compounds of D.P. (degree of polymerization) > 3.

The importance of fructosans in cold hardiness has generally been overlooked. Little is known about fructosans either chemically or physiologically. They appear in an array of taxonomically diverse

families and are common among the Asteraceae and Poaceae. They occur frequently among species which must endure either cold or dry periods during their life cycle (2, 8, 10). While little work has been done on the fructosan physiology of Poaceous species, the literature is more complete for the Asteraceae.

Tuber discs of Jerusalem artichoke (Helianthus tuberosus L.) or chicory (Chichorium entybus L.) treated with 2,4-dichlorophenoxyacetic acid (2,4-D) increased greatly in water content and osmotic potential (60). These increases were accompanied by extensive depolymerization of fructosans. After an initial decrease, the molar concentration of total soluble sugars remained constant in spite of respiration, and considerable changes in water content and concentration of individual sugars.

Rutherford and Weston (36) found that cold storage of roots of Jerusalem artichoke, chicory or dandelion (Taraxacum officinale, Weber) induces the depolymerization of high molecular-weight (D.P. > 10), oligosaccharides into lower D.P. compounds, and the simultaneous increase in total soluble sugars, while the amount of dry matter remains constant.

Similarly, Jefford and Edelman (18) demonstrated that storage of Jerusalem artichoke tubers at 2°C induces a decrease in the average chain length while the amount of total carbohydrates remains constant. Storage at 20°C, however, induces a loss of total carbohydrates without a decrease in average chain length.

These experiments establish a relationship between fructosans and temperature and/or water regulation in the Asteraceae. Wheat may use

fructosans as an osmotically inert store of saccharides to be mobilized during freezing periods.

Edelman and Jefford (10) have proposed a general model of sucrose/fructosan interconversion in which fructosans are easily converted to sucrose (and by extension, into glucose and fructose), and sucrose into fructosans.

The system found in Jerusalem artichoke consists of three enzymes: SST, FFT and FFH. Sucrose-sucrose 1-fructosyl transferase (SST) forms the trisaccharide, 1F-fructosylsucrose, from sucrose as shown in the equation:

$$G\sim F + G\sim F \longrightarrow G\sim F\text{-}F + G$$

Since SST shows a high specificity for sucrose and shows little or no activity with the trisaccharide as donor or with glucose as acceptor, it is unable to promote polymerization above the trisaccharide level.

The second synthetic enzyme, B (2-1') fructan : B (2-1') fructan 1-fructosyltransferase (FFT), is highly specific for the transfer of a single terminal fructosyl residue to the same position in another molecule. It has no hydrolytic activity. The reaction is shown as:

$$G\sim F\text{-}F_n + G\sim F\text{-}F_m \longleftrightarrow G\sim F\text{-}F_{n-1} + G\sim F\text{-}F_{m+1}$$

where n is the number of extra-sucrosyl fructose residues of the donor and m the number of extra-sucrosyl fructose residues of the acceptor.

Sucrose is a special case in that it cannot donate its fructose residue but can act as an acceptor. Reactions with polymers of different length are all facilitated by the same enzyme.

The rate of reaction is greatly affected by chain length. The sucrose molecule regenerated by the transfer of a fructose residue from 1F-frustosylsucrose by the reaction

$$G\sim F + G^{*}\sim F^{*}\text{-}F \rightleftharpoons G\sim F\text{-}F + G^{*}\sim F^{*}$$

is unique in that it has a much higher affinity for the fructose residues than does the original sucrose molecule. Even though FFT continues to transfer residues at the same or an enhanced rate, the regenerated sucrose competes with the trisaccharide so successfully that the formation of polymers of high D.P. is totally inhibited.

Polymers of high D.P. also show a great affinity for fructose transferred from 1F-fructosylsucrose; and long-chain polymers are rapidly formed at the expense of short-chain polymers.

Two hydrolases, B (2-1') fructan 1-fructanohydrolase (FFH), with very similar properties have been described (9) and are referred to as hydrolases A and B. Their activities are described by the following equation:

$$G\sim F\text{-}F_n \xrightarrow{H_2O} G\sim F\text{-}F_{n-1} + F$$

Hydrolases A and B are distinguished by their mobilities on DEAE-cellulose columns and their relative activities on inulin and the fructosyl oligosaccharides.

Both hydrolases break only the B (2-1') linkage between a terminal fructosyl group and its adjacent fructose residue, the rate depends on the degree of polymerization and the nature of the group at the non-reactive end. They are both inactive against sucrose and are, in fact, inhibited by sucrose, hydrolase B being more susceptible to inhibition by sucrose than hydrolase A. Neither is inhibited by free fructose, possibly because fructose exists as a furanose in sucrose and in polymers, but as free molecules it exists cheifly as a pyranose. Neither hydrolase A nor B has any transferase activity.

A summary of the activities of these enzymes as the basis of physiological adaptation in Jerusalem artichoke is presented in Figure 1.

Sucrose is viewed as an important control point for a number of reasons. It is the currency for the import and export of carbohydrates. It is the substrate for SST which synthesizes the trisaccharides 1F-fructosylsucrose to initiate fructosyl chains. Increasing the concentration of sucrose may prevent depolymerization by direct inhibition of FFH. Because of the special property of sucrose regenerated from 1F-fructosyl-sucrose, it can divert FFT activity by accepting fructosyl residues transferred from the trisaccharide, reforming the substrate, thereby affecting the length and number of fructosyl chains.

The key enzymes for such a system have been isolated from Jerusalem artichoke, onion (Allium cepa L.) and asparagus (Asparagus officinalis L.) (10, 42, 43).

I. Polymerization

$$4G\sim F \longrightarrow 2G\sim F\text{-}F + 2G \quad \text{(SST)}$$

$$2G \longrightarrow G\sim F \quad \text{(sucrose synthesis)}$$

$$2G\sim F\text{-}F \longrightarrow G\sim F\text{-}FF + G\sim F \quad \text{(FFT)}$$

$$2G\sim F \longrightarrow G\sim F\text{-}F\text{-}F$$

Leading to:

$$(n + 2)G\sim F \longrightarrow 2G\sim F\text{-}Fn$$

II. Conversion of Fructosans to Oligomers During Cold Storage

$$2\ G\sim F\text{-}F_n \xrightarrow{2n\ H_2O} 2\ G\sim F + 2nF \quad \text{(hydrolases)}$$

$$2nF \longrightarrow n\ G\sim F \quad \text{(sucrose synthesis)}$$

$$G\sim F\text{-}F_n + nG\sim F \longrightarrow nG\sim F\text{-}F + G\sim F \quad \text{(FFT)}$$

$$3\ G\sim F\text{-}F_n \xrightarrow{2n\ H_2O} 3G\sim F + nG\sim F\text{-}F$$

III. Depolymerization

$$2G\sim F\text{-}F_n \xrightarrow{2n\ H_2O} 2G\sim F + 2nF \quad \text{(hydrolases)}$$

$$2nF \longrightarrow n\ G\sim F \quad \text{(sucrose synthesis)}$$

$$2G\sim F\text{-}F_n \xrightarrow{2n\ H_2O} (n + 2)G\sim F$$

Figure 1. Activities of SST, FFT, and FFH in Relation to Polymerization, Chain-Length Interconversion, and Depolymerization adapted from Edelman and Jefford (10).

The essential feature of this system is that there is no energy requirement for either polymerization or depolymerization, the linkages being derived from preformed sucrose molecules. In contrast, starch synthesis requires one ATP molecule for each glucosyl residue added to the polymer.

Such a system would allow a simple and flexible means of adjusting the osmotic activity of the plant cells by regulating the amount of free sugars as well as the number and chain-length of fructosan molecules. This system would work equally well if hardiness depends on specific cryoprotective effects of carbohydrates. If, as Santarius (40) demonstrated in spinach and beet, cryoprotection becomes increasingly more effective with increasing chain-length, then it would be a simple matter to adjust the chain length of the available fructosan chains for the necessary degree of protection.

In winter wheat, a similar system would provide a rapid and flexible mechanism for dealing with the daily and seasonal fluctuations of Montana weather.

Specific protective effects of a number of saccharides has been observed. Isolated chloroplast thylakoid suspensions have been protected from freeze-induced uncoupling of cyclic photophosphorylation by glucose (16, 24, 38, 39, 41), sucrose (16, 17, 24, 38, 41), raffinose (24, 38), fructose (39, 41), and dextrans (39, 40). Similarly, non-cyclic photophosphorylation has been protected by glucose, sucrose, and fructose (41).

Williams and Merryman (63) demonstrated that isolated chloroplast granna suspended in glucose solution were uninjured by any temperature down to that of liquid nitrogen and could even be freeze-dried with only 50% loss of proton uptake upon rehydration. Similar protection from freezing injury has been afforded to mitochondria by sucrose (17) and to erythrocytes by glycerol (25) suggesting a universal interaction between membrane systems and carbohydrates.

Regrowth

Mechanical refrigeration has been used to study the cold-hardiness of plants periodically since, at least, 1918. Worzella and Cutler (64), using field-hardened winter wheat cultivars, found good agreement between artificial freezing and field survival ($r = 0.73$). Weibel and Quisenberry (61), also using field-hardened winter wheat, reported close agreement between controlled freezing tests and field survival ($r = 0.87$). As the season progressed, the temperature necessary to produce a differential kill decreased, and since the plants appeared to respond to constantly variable weather conditions, careful observation of both weather and plant materials was necessary to determine the appropriate exposure temperature (61).

Andrews (1) found a significant correlation between survival rates of cold hardened sprouted seeds of winter wheat and field survival ($r = 0.85$). The sprouted seeds of the cultivars ranked about the same in their relationship to the standard check as these cultivars did under field conditions. The advantage of this method over field

hardening is that it permits the testing of more cultivars per unit of space and/or labor. The disadvantages lie in that no less than ten replicates of 20 seeds each are required to give a reasonable resolution; and careful control is necessary to stabilize the temperature slightly above freezing. Gullord et al. (15) rated wheat crowns after freezing for lower peripheral meristem damage (1 to 5 scale) and for percent survival. No significant interaction was found between cold hardiness and either meristem rating or percent survival among the 14 wheat cultivars tested. Interaction, in terms of meristem rating, did occur when eight advanced breeding lines were tested.

Suneson and Peltier (49) observed that when flats of winter wheat, hardened and wintered in the field, were sampled bi-weekly, hardiness ranking among four cultivars varied widely over the course of the winter, apparently due to short-term variations in environmental conditions.

Chlorophyll

An apparent correlation between winterhardiness and dark green coloration of over-wintering winter wheat plants has been reported (50). Leaf color is currently being used as a selection criterion for evaluating winterhardiness potential of new genotypes in the Montana state winter wheat breeding program.

Preliminary investigation into this relationship (Brown, J.H., unpublished) showed that while the chlorophyll concentration (expressed as milligrams of chlorophyll per unit leaf area) of several

winter wheat varieties closely approximated relative winterhardiness, the differences were not significant. The present study was undertaken to investigate this apparent relationship in greater detail, and to ascertain its applicability as an objective measure of winterhardiness potential.

MATERIALS AND METHODS

Cultivars

Eight cultivars of winter wheat were selected for the first year study and scored for relative winterhardiness, a score of 5 being most hardy, and a score of 1, least hardy (50). Two additional cultivars, Norwin and Nugaines, were added for the second year of the study. The selected cultivars and their scores are shown in Table 1:

TABLE 1

Winter Wheat Cultivars, Hardiness Group, and Hardiness Scores† Studied in 1981-82 and 1982-83.

1981-82

Hardy Cultivars	ID #	Score	Less Hardy Cultivars	ID #	Score
Norstar	CI 17735	5	Centurk	CI 15025	2
Froid	CI 13872	5	MT6928	MT 6928	2
Winalta	CI 13670	4	Oregon Feed Wheat	OR 69-1171	1
MT7115	MT 7115	4			
Redwin	CI 17844	3			

1982-83

Hardy Cultivars	ID #	Score	Less Hardy Cultivars	ID #	Score
Norstar	CI 17735	5	Centurk	CI 15025	2
Froid	CI 13872	5	MT6928	MT 6928	2
Norwin	MT 7877	5	Nugaines	CI 13968	1
Winalta	CI 13670	4	Oregon Feed Wheat	OR 69-1171	1
MT7115	MT 7115	4			
Redwin	CI 17844	3			

† Winterhardiness score is proportional to hardiness. Scores 3-5 are "hardy", scores 1-2 are "less hardy" (50).

Field Methods

The selected cultivars were field planted at Bozeman, Montana on September 23, 1981 and September 24, 1982, respectively, at a seeding rate of 10g per 3m of row, in four 3m rows, 0.3m between rows in a randomized complete block design with four replications. Sampling dates for the carbohydrate and chlorophyll studies are given in Table 2. Sampling dates for regrowth after controlled freezing studies are given in Table 3.

TABLE 2

Sampling Dates and Days From Planting For Carbohydrate and Chlorophyll Studies in 1981-82 and 1982-83.

1981-82 Sampling Date	Days From Planting	1982-83 Sampling Date	Days From Planting
November 3	41	December 20	87
November 16	54	March 01	158
March 23	181	March 30	187
April 14	203	April 19	207
April 25	214		

TABLE 3

Sampling Dates and Days From Planting For Regrowth Study in 1981-82 and 1982-83.

1981-82 Sampling Date	Days From Planting	1982-83 Sampling Date	Days From Planting
December 12	80	December 20	87
December 21	89	March 03	160
		March 23	180

On these dates, about 30 plants of each treatment were dug from the soil, placed in plastic boxes lined with moist blotter paper, and transported in an ice chest.

Laboratory Methods

Carbohydrate Determination

Plants selected for the carbohydrate study were rinsed, the roots removed, and the top growth excised at the soil surface. The crowns were placed in coin envelopes and oven dried at 100°C for 1 hr, and 70°C thereafter. Samples were ground to a fine powder in a ball mill, and stored in a desiccator.

Samples from each treatment were carefully weighed to 50 ± .1 mg and placed in conical 15 ml centrifuge tubes. Simple sugars were extracted in three successive 1 ml aliquots of 80% ethanol, incubated for 20 min at 80°C and centrifuged at 2000 x g for 20 min. The supernatant from each extraction was decanted into a 6 ml test tube. After three extractions, each tube was brought to 3.0 ml total volume with 80% ethanol.

Fructosans were extracted by resuspending the pellet from the ethanol extraction in 1.0 ml water and proceeding as above using water as the solvent. As with the simple sugars, fructosans were extracted three times.

Quantitative assays for all the measured carbohydrates (glucose, fructose, sucrose and fructosans) were based on the following reaction sequences (19):

Glucose + Fructose + 2 ATP $\xrightarrow{\text{Hexokinase}}$ Glucose-6-P + 2 ADP

Fructose-6-P $\xrightarrow{\text{P-Glucoisomerase}}$ Glucose-6-P + Fructose-6-P

2 Glucose-6-P + 2 NADP+ $\xrightarrow{\text{Glucose-6-P deH}}$ 2 6-P-gluconate + 2 NADPH

Carbohydrates are hydrolyzed to glucose or fructose which are the basic entry molecules to the reaction sequence. Glucose and fructose are then phosphorylated in the presence of ATP by hexokinase to yield glucose-6-phosphate. Glucose-6-phosphate is converted to 6-phosphogluconate by glucose-6-phosphate dehydrogenase with the coincident reduction of NADP to NADPH. NADPH can then be measured spectrophotometrically at 340 nm and the concentration of NADPH determined by the equation:

$A(\varepsilon 1)^{-1} = [C]$ where A = Absorbance

ε = Molar Extinction Coefficient of NADPH = 6220

1 = Light Path of Cuvette (cm)

[C] = Molar Concentration of NADPH

The concentration of glucose is then known since the concentration of NADPH is directly proportional to that of glucose (19, 34, 44).

In this experiment glucose and fructose were assayed collectively due to inherent contamination of glucose-6-phosphate dehydrogenase with hexokinase. For this determination 40µl of the ethanol extract was placed in a 6 ml test tube with 100µl phosphoglucoisomerase (EC 5.3.1.9) solution (25 U ml^{-1}), 1.0 ml of "hexose reagent" composed of a Tris-HCl buffer (360 mM) at pH 8.89 containing 1 mM ATP, 0.2 mM

NADP, 2.0 mM $MgCl_2$, 3.6 mM DTT (dithiothreitol), 0.5 U ml^{-1} glucose-6-phosphate dehydrogenase (EC 1.1.1.49), 0.8 U ml^{-1} hexokinase (EC 2.7.1.1), and 1.86 ml water giving a total volume of 3.0 ml. The contents were vortex mixed and incubated at room temperature for 30 min. Absorbance at 340 nm was measured on a Varian 634 spectrophotometer.

Sucrose was determined by placing 20µl of ethanol extract in a 6 ml test tube with 180µl water and 200µl of 0.4 N NaOH yielding a 0.2N NaOH solution. The tubes were mixed, capped and heated in an 80°C water bath for 20 min to destroy free glucose and fructose (27). The samples were cooled, neutralized with 200 µl HCl (0.4 N) and 100µl invertase (EC 3.2.1.26) solution (40 U ml^{-1}) added (19). They were then capped and incubated in a water bath at 55°C for 30 min yielding a mixture of free glucose and fructose. One ml of "hexose reagent" was added and the procedure continued as for the combined hexose assay.

Fructosans were assayed by placing 10 µl of the aqueous extract in a 6 ml test tube with 190µl water and 200µl HCl (0.2 N) yielding a 0.1 N HCl solution, and heating in a hot water bath (80°C) for 25 min to hydrolyze the polymer into fructose monomers. The samples were cooled and neutralized with 200µl NaOH (0.2 N). One ml of "hexose reagent" was added and the procedure continued as for the combined hexose assay. For all determinations, appropriate standard solutions of either glucose, sucrose, or fructosans were run simultaneously as were ethanol or water blanks.

Measurement of Regrowth After Controlled Freezing

For the regrowth test, 10 healthy plants from each treatment were selected. The roots were completely removed, and the top growth removed 25mm above the base. The trimmed crowns were placed in 1.1mm thick clear plastic sandwich bags, excess air removed, and the bags were heat sealed. Bags were placed in a freezer with the following temperature regimes (Table 4).

Table 4

Freezing Regimes for Each Sampling Date for the Study of Regrowth of Winter Wheat Cultivars After Freezing: 1981-82 and 1982-83

Sampling Date	Hours	Temperature (°C)
December 21, 1981	12	0
	12	-10
	12	-20
December 20, 1982	12	- 2
	12	-14
	12	-20
March 23, 1983	12	0
	12	- 5
	12	-10

At the end of the freezing period, bags were removed from the freezer and placed in a growth chamber for 72 hours, 21°C/10°C day/night temperature. Specimens were measured for the number of survivors, total regrowth (mm) per 10 specimens, and regrowth (mm) per survivor.

Chlorophyll Determination

Samples of each treatment were thoroughly rinsed and 20 healthy, fully expanded leaf blades from at least 10 different plants were collected by clipping each leaf immediately distal to the auricle. Fresh weight was determined and leaf area measured on a Licor Model 3100 area meter. Each fresh sample was ground in a Sorvall tissue homogenizer with a quantity of 80% acetone as determined by the formula

$$Y = 100X - X$$

where X = fresh weight (g) and Y = mls acetone. The suspension was transferred to an appropriate Erlenmeyer flask, sealed with Parafilm, and refrigerated at 4°C overnight to allow solids to settle. After settling, 1 ml of solution was carefully withdrawn, placed in a cuvet and mixed with 3 ml of 80% acetone (i.e., 1:3 dilution). Absorbance was measured at 645 nm and 663 nm on a Zeiss PMQII spectrophotometer, and total chlorophyll concentration calculated according to the method of Arnon (3). Chlorophyll concentration per unit of leaf area (mg chlorophyll dm^{-2}) was also calculated.

Statistical Methods

Cultivars with winterhardiness scores of three and greater, on a scale of one to five, are considered to be winterhardy enough for Montana; those with scores less than three are unacceptable (Taylor, G. A., unpublished data). Therefore, in both years, cultivars were

separated into "Hardy" and "Less Hardy" groups for comparison (Table 2).

Analyses of Variance were calculated for each of the three experimental techniques. Orthogonal comparisons were made for the data from each experiment and each year between the group of cultivars which are considered winterhardy (scores 3-5) and those considered less hardy (scores 1-2). Comparisons were also made between cultivars with a winterhardiness score of 5, and those with scores of 3-4. In 1982-83 the additional two cultivars allowed the comparison of cultivars with a score of 2 with those with a winterhardiness score of 1.

For the carbohydrate study, Pearson correlation coefficients were calculated for the correlations between winterhardiness score and concentrations of glucose plus fructose, sucrose, fructosans, and total carbohydrates. For the crown regrowth study, Pearson correlation coefficients were calculated for the correlations between winterhardiness score and number of survivors, average regrowth per cultivar, and average regrowth per survivor. For the chlorophyll study, Pearson correlation coefficients were calculated for the correlations between winterhardiness score and chlorophyll concentration per unit leaf area.

RESULTS AND DISCUSSION

Carbohydrates

Glucose Plus Fructose

Data for the orthogonal comparisons of combined hexoses (glucose + fructose) are presented in Tables 5 and 6 and Figures 2 and 3. In 1981-82, the hardy group contained more hexoses than the non-hardy group on both November sampling dates (November 3 and November 16) and on the earliest spring sampling date (March 23). The hardiest cultivars, Norstar and Froid, could not be distiguished from the others in the hardy group at any sampling date.

In 1982-83, the hardy cultivars contained appreciably more hexoses than the less hardy cultivars at the December 20 sampling, and on March 1 and March 30. Cultivars with hardiness scores of five (Norstar and Froid) had a higher hexose concentration than the other cultivars in the hardy group for the December 20 and March 1 samplings. Among the less hardy class, those with a winterhardiness score of two differed significantly from those with a score of one on all occasions, but in no consistent pattern.

Sucrose

For 1981-82 (Table 7, Figure 4), the hardy class contained more sucrose than the less hardy class on the first sampling date (November 3). On the last sampling (April 25) the less hardy class contained more than the hardy class. Within the hardy class, score group 5

TABLE 5

Orthogonal Comparisons of Glucose Plus Fructose Concentrations [μmoles (g dry wt.)$^{-1}$] Among Winter Wheat Cultivars Differing in Winterhardiness (WH.) Scores†: 1981-82

Sampling/ Dates / WH. Scores			3-5 vs 1-2	5 vs 3-4
November 3, 1981	Means	=	194 133	184 200
	t (51)	=	2.80 **	-0.619
November 16, 1981	Means	=	161 137	164 159
	t (54)	=	3.37 **	0.647
March 23, 1982	Means	=	212 155	217 208
	t (69)	=	11.2 **	1.32
April 14, 1982	Means	=	93.0 91.0	91.6 94.0
	t (21)	=	0.236	-0.232
April 25, 1982	Means	=	106 109	109 103
	t (21)	=	0.458	0.672

† Winterhardiness score is proportional to hardiness. Scores 3-5 are "hardy", scores 1-2 are "less hardy" (50).

Symbols "*" and "**" indicate significant differences at p=0.05 and p=0.01, respectively, according to Student's t (df).

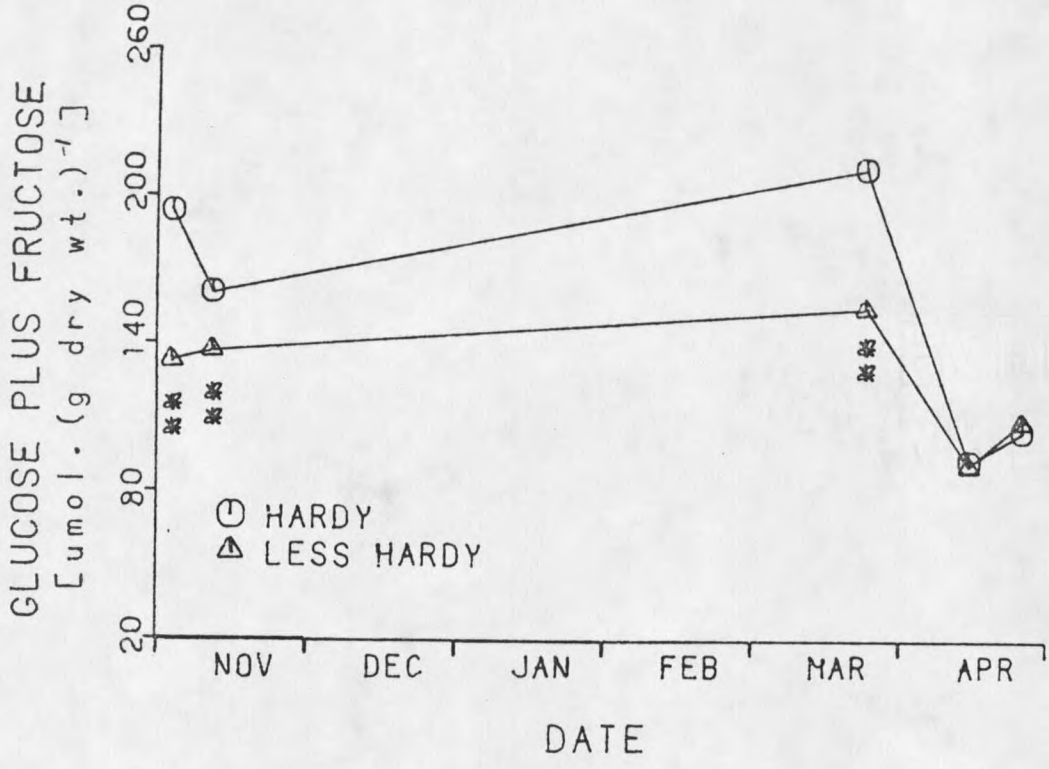

Figure 2: Average Combined Glucose and Fructose Content of the Crown Tissue of Hardy and Less Hardy Winter Wheat Cultivars Sampled During 1981-82.

TABLE 6

Orthogonal Comparisons of Glucose Plus Fructose Concentrations [μmoles (g dry wt.)$^{-1}$] Among Winter Wheat Cultivars Differing in Winterhardiness (WH.) Scores†: 1982-83

Sampling/ Dates / WH. Scores		3-5 vs 1-2	5 vs 3-4	2 vs 1
December 20,1982	Means =	254 221	274 233	231 211
	t (87) =	3.00 **	1.97 *	4.86 **
March 1,1983	Means =	84.3 67.4	86.5 82.1	69.0 65.8
	t (87) =	5.30 **	6.93 **	2.25 *
March 30,1983	Means =	35.7 27.9	35.3 36.0	26.9 28.5
	t (117) =	4.48 **	-1.25	-6.49 **
April 19,1983	Means =	38.5 33.5	39.3 37.8	30.8 36.2
	t (27) =	0.674	0.125	-2.29 *

† Winterhardiness score is proportional to hardiness. Scores 3-5 are "hardy", scores 1-2 are "less hardy" (50).

Symbols "*" and "**" indicate significant differences at p=0.05 and p=0.01, respectively, according to Student's t (df).

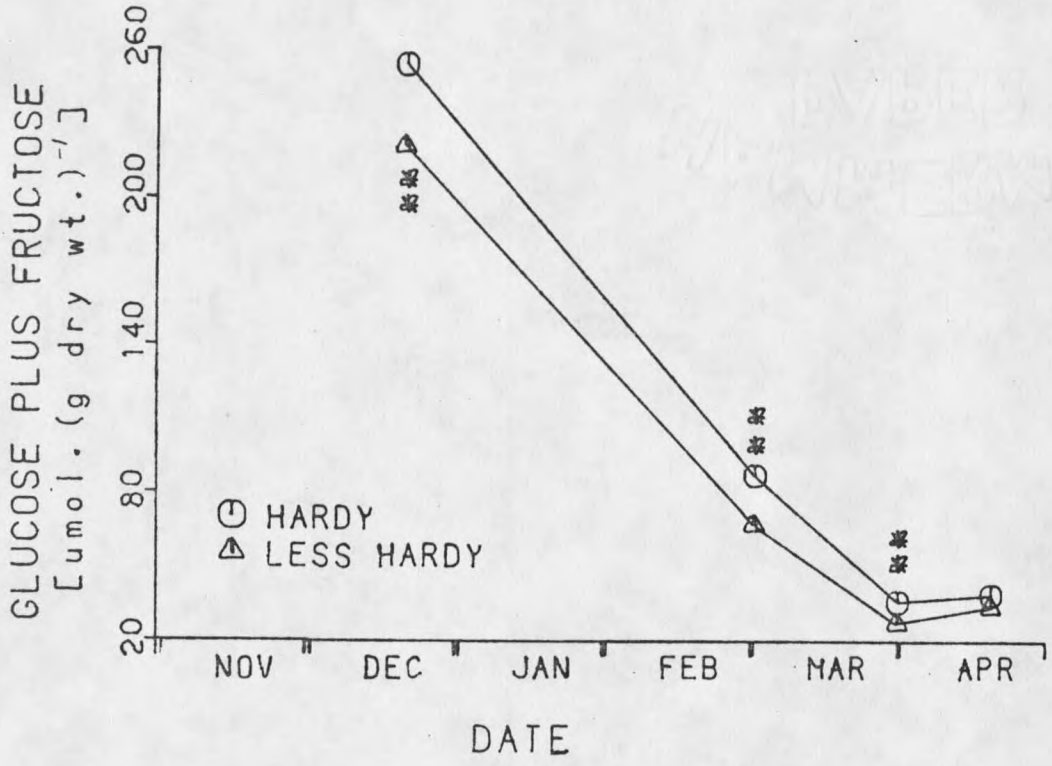

Figure 3: Average Combined Glucose and Fructose Content of the Crown Tissue of Hardy and Less Hardy Winter Wheat Cultivars Sampled During 1982-83.

TABLE 7

Orthogonal Comparisons of Sucrose Concentrations [μmoles (g dry wt.)$^{-1}$] Among Winter Wheat Cultivars Differing in Winterhardiness (WH.) Scores†: 1981-82

Sampling/ Dates / WH. Scores			3-5 vs 1-2	5 vs 3-4
November 3, 1981	Means	=	306 238	245 347
	t (51)	=	1.98 *	-2.38 *
November 16, 1981	Means	=	257 252	270 242
	t (54)	=	0.0422	0.125
March 23, 1982	Means	=	332 289	348 322
	t (69)	=	1.26	2.05 *
April 14, 1982	Means	=	110 110	99.9 117
	t (21)	=	0.0432	-1.46
April 25, 1982	Means	=	92 104	100 86.6
	t (21)	=	-2.80 *	2.67 *

† Winterhardiness score is proportional to hardiness. Scores 3-5 are "hardy", scores 1-2 are "less hardy" (50).

Symbols "*" and "**" indicate significant differences at p=0.05 and p=0.01, respectively, according to Student's t (df).

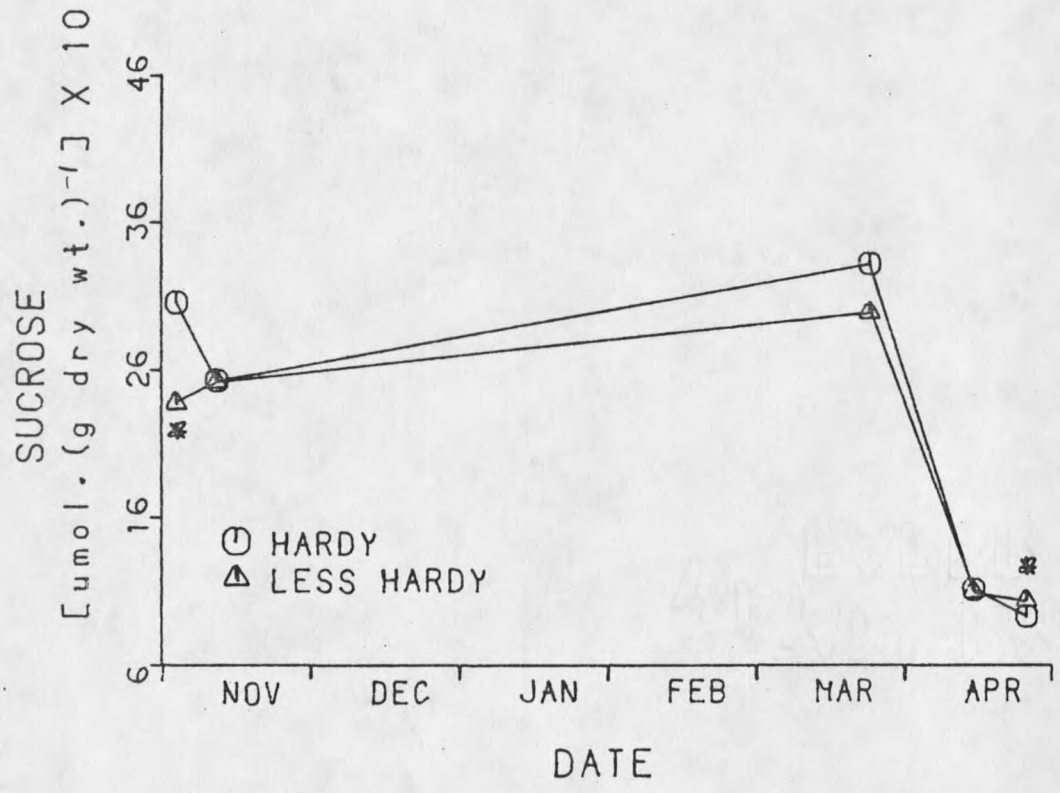

Figure 4: Average Sucrose Content of the Crown Tissue of Hardy and Less Hardy Winter Wheat Cultivars Sampled During 1981-82.

(Norstar and Froid) differed from those with winterhardiness scores of 4, 4 and 3, on three dates: on November 3 sucrose contents were lower for those of score group 5 (Norstar and Froid) than for others; and on March 23 and on April 25, sucrose concentration was greater for this group.

In 1982-83 (Table 8, Figure 5), the hardier class contained more sucrose than the less hardy class on December 20, and again in early spring on March 1. Within the hardy group, those with a winterhardiness score of five contained more sucrose than those with scores of three or four on December 20 and on April 19.

The sucrose content of cultivars with a winterhardiness score of two differed from those with a score of one on December 20 and on March 30. On both occasions, group two contained more sucrose than group one.

Fructosans

During the first year of the study, the winterhardy group was easily differentiated from the less winterhardy group on both autumn sampling dates (November 3 and November 16) and in spring on March 23, the hardier class having higher fructosan concentrations than the less hardy class. On April 14 the hardy class had a lower fructosan concentration than the less hardy class. On November 16, March 23 and April 14, hardiness score group 5 had higher concentrations of fructosans than score groups 3 and 4 (Table 9, Figure 6).

In the second year of the study, the hardier group always contained more fructosans, except on April 19. The hardy cultivars

TABLE 8

Orthogonal Comparisons of Sucrose Concentrations [μmoles (g dry wt.)$^{-1}$] Among Winter Wheat Cultivars Differing in Winterhardiness (WH.) Scores†: 1982-83

Sampling/ Dates / WH. Scores		3-5 vs 1-2	5 vs 3-4	2 vs 1
December 20, 1982	Means =	453 350	478 429	383 316
	t(87) =	5.93 **	2.86 **	3.03 **
March 1, 1983	Means =	180 158	177 183	173 142
	t (87) =	4.29 *	-1.57	1.26
March 30, 1983	Means =	216 204	216 217	206 201
	t (147) =	1.28	-0.0692	3.00 **
April 19, 1983	Means =	87.9 78.2	93.4 82.4	69.0 87.4
	t (27) =	1.34	2.47 *	0.247

† Winterhardiness score is proportional to hardiness. Scores 3-5 are "hardy", scores 1-2 are "less hardy" (50).

Symbols "*" and "**" indicate significant differences at p=0.05 and p=0.01, respectively, according to Student's t (df).

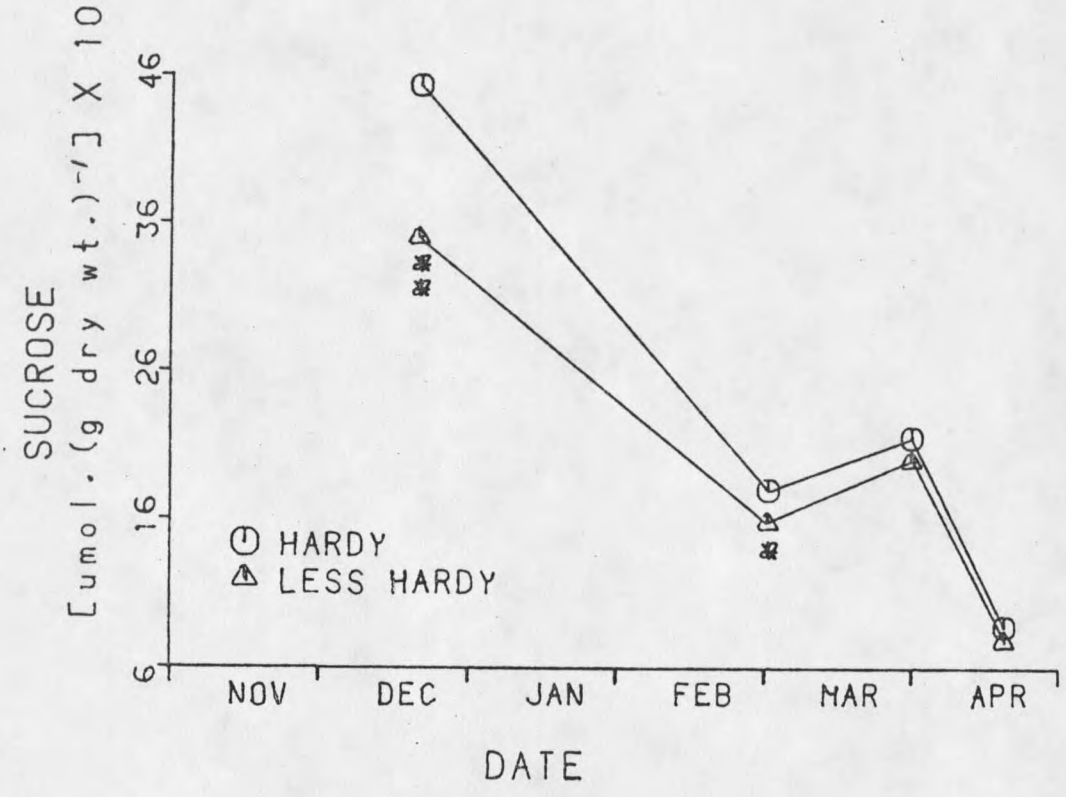

Figure 5: Average Sucrose Content of the Crown Tissue of Hardy and Less Hardy Winter Wheat Cultivars Sampled During 1982-83.

TABLE 9

Orthogonal Comparisons of Fructosan Concentrations [μmoles (g dry wt.)$^{-1}$] Among Winter Wheat Cultivars Differing in Winterhardiness (WH.) Scores†: 1981-82

Sampling/ Dates / WH. Scores			3-5 vs 1-2	5 vs 3-4
November 3, 1981	Means	=	1430 994	1250 1540
	t (51)	=	3.65 *	-1.96
November 16, 1981	Means	=	1570 1250	1700 1480
	t (54)	=	4.54 **	2.61 *
March 23, 1982	Means	=	1630 1020	1920 1440
	t (54)	=	8.36 **	5.25 **
April 14, 1982	Means	=	408 615	479 354
	t (21)	=	-4.98 **	2.36 *
April 25, 1982	Means	=	532 602	548 521
	t (21)	=	-1.22	0.372

† Winterhardiness score is proportional to hardiness. Scores 3-5 are hardy, scores 1-2 are less hardy (50).

Symbols "*" and "**" indicate significant differences at p=0.05 and p=0.01, respectively, according to Student's t (df).

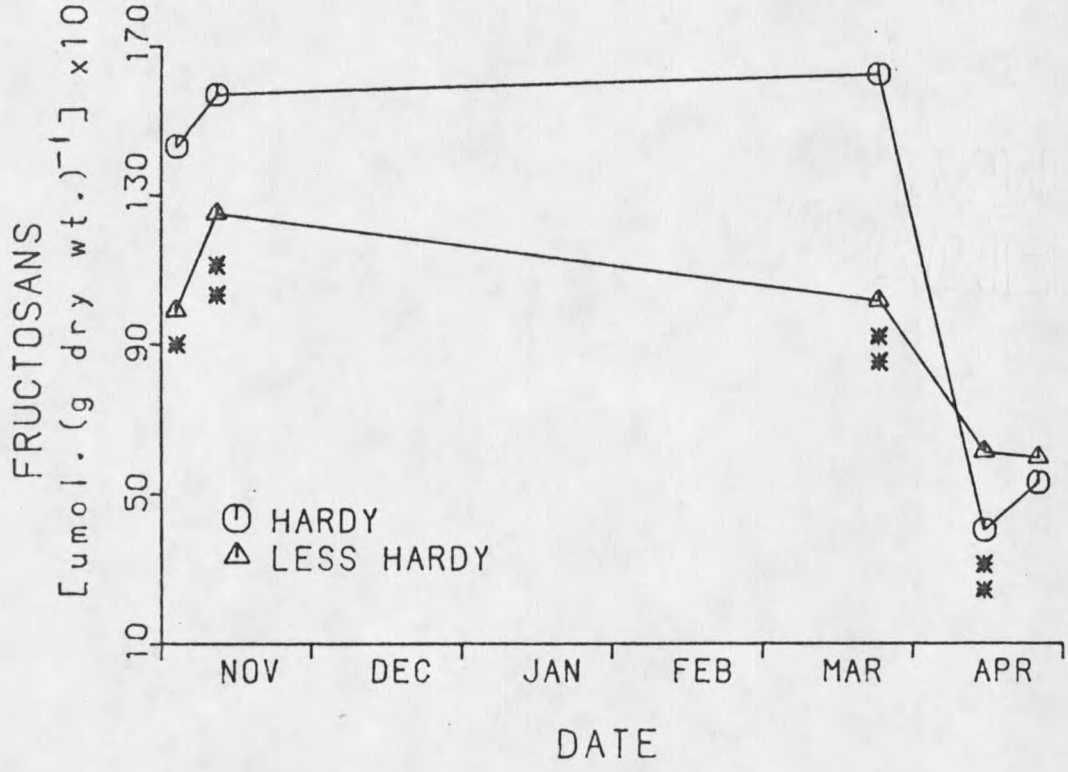

Figure 6: Average Fructosan Content (Expressed as Anhydrous Glucose Equivalents) of the Crown Tissue of Hardy and Less Hardy Winter Wheat Cultivars Sampled During 1981-82.

differed greatly from the less hardy cultivars on December 20 and on both March dates (Table 10, Figure 7). Cutivars in score group 5 had higher fructosan contents on December 20 than the other cultivars within the hardy group. Cultivars of winterhardiness score group 2 contained less fructosans than those of score group 1 on December 20, and on April 19. On March 1, however, score group 2 contained more fructosans (Table 10, Figure 7).

Total Carbohydrates

In the 1981-82 crop year the winterhardy class was differentiated from the less hardy class by higher contents of total carbohydrates on both autumn sampling dates and on the first spring sampling (March 23) (Table 11, Figure 8). The hardy group also differed on April 14; but, here the hardy cultivars had a lower total carbohydrate concentration than the less hardy group. Within the winterhardy group, the two hardiest cultivars differed from the remainder of the group on three dates. On November 3, the hardiest sub-group contained less total carbohydrates than the rest of the group; while on November 16 and March 23, they contained more.

In the 1983 crop year, the hardy class possessed more total carbohydrates than the less hardy class on December 20, and at both March samplings (March 1 and March 30) (Table 12, Figure 9). Cultivars in score group 5 contained more total available carbohydrates than the other members of the hardy class only on December 20. Winterhardiness score group 2 (Centurk and MT6928) had lower levels of total carbohydrates than winterhardiness score group 1 (Nugaines and Oregon Feed

TABLE 10

Orthogonal Comparisons of Fructosan Concentrations [μmoles (g dry wt.)$^{-1}$] Among Winter Wheat Cultivars Differing in Winterhardiness (WH.) Scores†: 1982-83

Sampling/ Dates / WH. Scores		3-5 vs 1-2	5 vs 3-4	2 vs 1
December 20, 1982	Means =	1291 960	1335 1246	893 1028
	t (147) =	5.26 **	5.14 **	-4.20 **
March 1, 1983	Means =	1390 1140	1400 1380	1280 1000
	t (87) =	3.49 **	1.90	3.48 **
March 30, 1983	Means =	851 732	851 842	693 772
	t (87) =	3.73 **	0.279	-1.90
April 19, 1983	Means =	135 141	153 117	128 156
	t (27) =	0.580	0.9935E -3	-2.111 *

† Winterhardiness score is proportional to hardiness. Scores 3-5 are "hardy", scores 1-2 are "less hardy" (50).

Symbols "*" and "**" indicate significant differences at p=0.05 and p=0.01, respectively, according to Student's t (df).

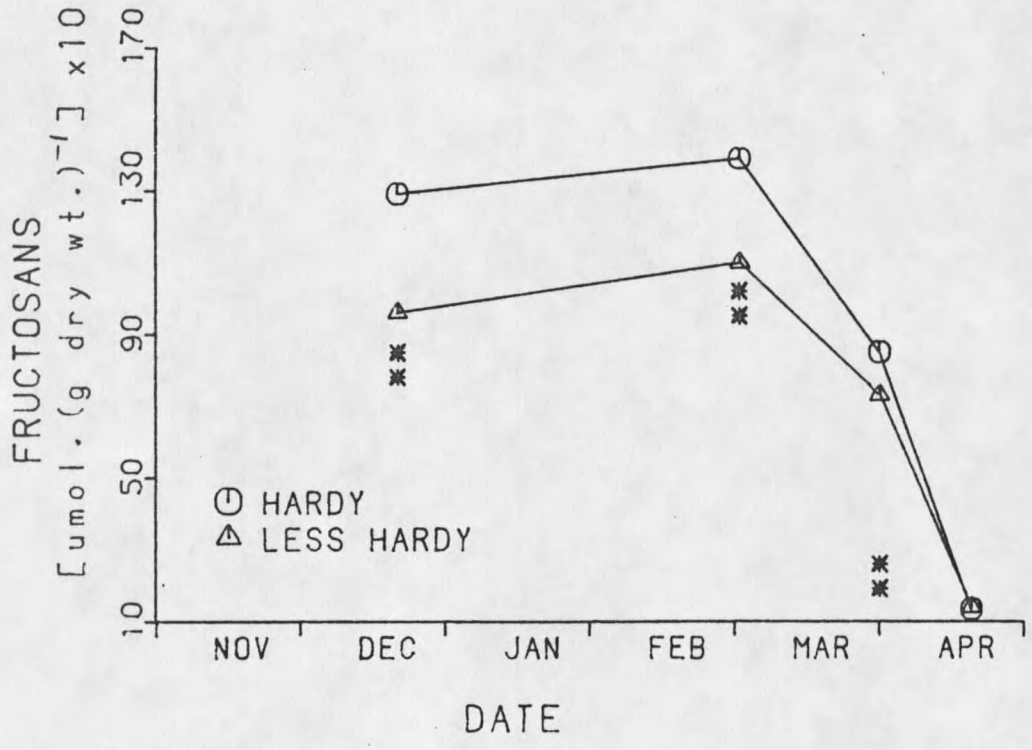

Figure 7: Average Fructosan Content (Expressed as Anhydrous Glucose Equivalents) of the Crown Tissue of Hardy and Less Hardy Winter Wheat Cultivars Sampled During 1982-83.

TABLE 11

Orthogonal Comparisons of Total Carbohydrate Concentrations [μmoles (g dry wt.)$^{-1}$] Among Winter Wheat Cultivars Differing in Winterhardiness (WH.) Scores†: 1981-82

Sampling/ Dates / WH. Scores			3-5 vs 1-2	5 vs 3-4
November 3, 1981	Means	=	1930 1360	1680 2090
	t (51)	=	3.79 **	-2.21 *
November 16, 1981	Means	=	1990 1640	2140 1880
	t (54)	=	4.13 **	2.53 *
March 23, 1982	Means	=	2170 1470	2480 1970
	t (69)	=	9.47 **	5.55 **
April 14,1982	Means	=	607 816	670 565
	t (21)	=	-4.47 **	1.80
April 25, 1982	Means	=	729 815	758 710
	t (21)	=	-1.34	0.589

† Winterhardiness score is proportional to hardiness. Scores 3-5 are "hardy", scores 1-2 are "less hardy" (50).

Symbols "*" and "**" indicate significant differences at p=0.05 and p=0.01, respectively, according to Student's t (df).

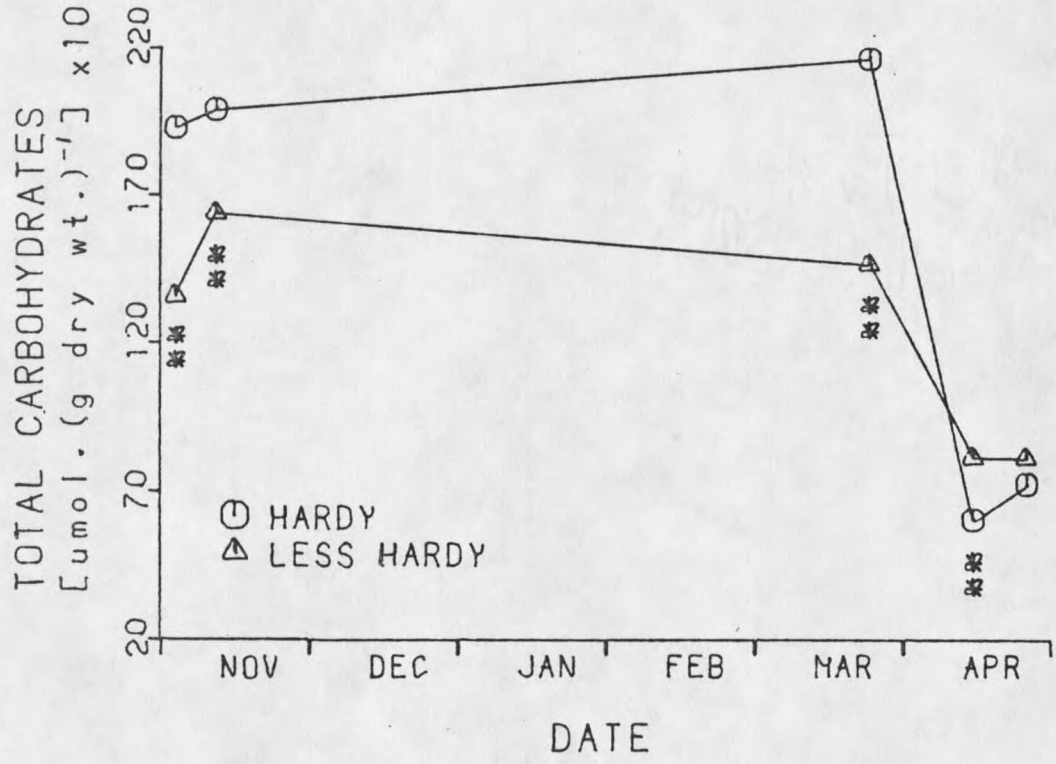

Figure 8: Average Total Available Carbohydrate Content (Calculated as the Sum of Glucose, Fructose, Sucrose, and Fructosans) of the Crown Tissue of Hardy and Less Hardy Winter Wheat Cultivars Sampled During 1981-82.

TABLE 12

Orthogonal Comparisons of Total Carbohydrate Concentrations [μmoles (g dry wt.)$^{-1}$] Among Winter Wheat Cultivars Differing in Winterhardiness (WH.) Scores†: 1982-83

Sampling/ Dates / WH. Scores		3-5 vs 1-2	5 vs 3-4	2 vs 1
December 20, 1982	Means =	1997 1530	2086 1908	1507 1554
	t (87) =	4.68 **	4.04 **	-3.90 **
March 1, 1983	Means =	1650 1360	1670 1640	1520 1210
	t (87) =	4.10 **	-1.51	3.62 **
March 30, 1983	Means =	1100 964	1103 1095	926 1002
	t (87) =	3.87 **	0.521	-2.32 *
April 19, 1983	Means =	262 254	286 237	227 280
	t (27) =	0.394	1.35	-1.84

† Winterhardiness score is proportional to hardiness. Scores 3-5 are "hardy", scores 1-2 are "less hardy" (50).

Symbols "*" and "**" indicate significant differences at p=0.05 and p=0.01, respectively, according to Student's t (df).

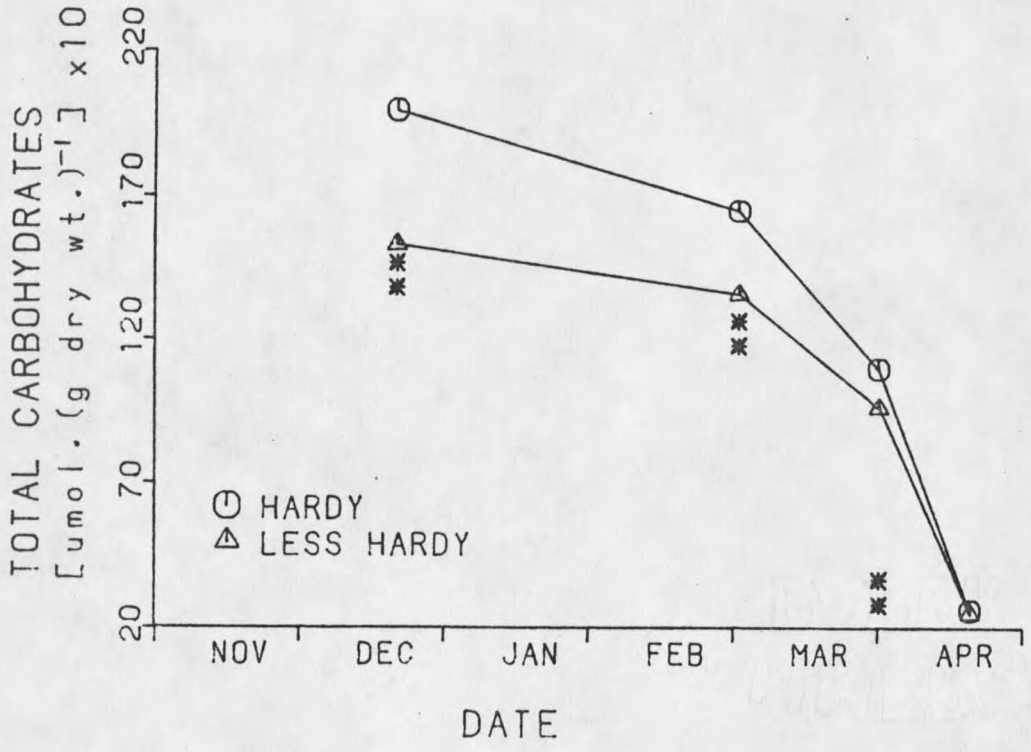

Figure 9: Average Total Available Carbohydrate Content (Calculated as the Sum of Glucose, Fructose, Sucrose, and Fructosans) of the Crown Tissue of Hardy and Less Hardy Winter Wheat Cultivars Sampled During 1982-83.

Wheat) on December 20, and March 30; but contained more total carbohydrates on March 1 (Table 12).

Considering both years, combined hexoses, fructosans and total available carbohydrates are significant indicators of winterhardiness at least in late autumn and in early spring. It is noteworthy that if the results from both years are intercalated by date, the relationship between the two classes is consistent until early April. After this time the order becomes unpredictable. Sucrose concentration does not appear to be as consistent an indicator of winterhardiness and therefore seems to be a less suitable winterhardiness index.

The segregation of cultivars with hardiness scores of 5 from the remainder of the winterhardy class was inconsistent between the two years of the study. Data for 1981-82 implied differences in fructosan and total available carbohydrate levels between the two sub-classes in the late autumn and early spring. The 1982-83 data, however, suggest that significant differences occur only in early autumn. There is a consistent relationship between the two main classes in both years, but it appears that any apparent differences between cultivars with hardiness scores of 5 and those with scores of 3 or 4 are much less predictable.

The relationship between winter wheat cultivars with a score of 2 and those with a score of 1 is more ambiguous. When the two classes could be differentiated from each other, cultivars with a score of 1 contained more of any given carbohydrate than did those with a score of 2 about half of the time.

In 1981-82, the mean total carbohydrate concentrations of the winterhardy group were always greater than those of the less hardy group until early April. In 1982-83, the means of the winterhardy group were significantly greater than the less hardy group through March. Although the concentration of each carbohydrate at any given time differs greatly between years, the relationship between the two classes is comparable for the two years (Figures 1-8).

Winterhardiness Score: Correlation of Winterhardiness Score With Various Carbohydrate Fractions

Data for the correlation of winterhardiness scores with combined hexoses, sucrose, fructosans, and total available carbohydrates are presented in Tables 13 and 14. In 1981-82, combined hexoses were correlated with winterhardiness scores on November 16 and March 23. Significant correlations were found with both fructosans and total carbohydrates on November 16 and March 23. In 1982-83, combined hexoses were correlated with hardiness scores on December 20 and March 30. Highly significant correlations of hardiness score with both fructosans and total available carbohydrates were found on December 20 and March 1. Sucrose was not correlated with winterhardiness score at any time.

Taken as a whole, the data suggest that the best times to sample field-hardened winter wheat are in late autumn/early winter or in early spring; and that either combined hexoses or fructosans are the most desirable carbohydrates to assay. The assay for total available carbohydrates is not recommended because, although they gave the highest correlation with winterhardiness scores, the marginal increase

TABLE 13

Correlation Coefficients of Winterhardiness Scores† and Four Carbohydrates Averaged Over Eight Winter Wheat Varieties: 1981-82

	November 3	November 16	March 23	April 14	April 25
Glucose + Fructose	0.572	0.708 *	0.780 *	0.009	0.059
Sucrose	0.008	0.294	0.362	0.174	0.237
Fructosans	0.461	0.915 **	0.850 **	0.613	0.304
Total	0.448	0.894 **	0.868 **	0.651	0.329

† Winterhardiness score is proportional to hardiness. Scores 3-5 are "hardy", scores 1-2 are "less hardy" (50).

Symbols "*" and "**" indicate significant differences at p=0.05 and p=0.01, respectively, according to Student's t (df).

TABLE 14

Correlation Coefficients of Winterhardiness Scores† and Four Carbohydrates Averaged Over Ten Winter Wheat Varieties: 1982-83

	December 20	March 1	March 30	April 19
Glucose + Fructose	0.645 *	0.571	0.704 *	0.426
Sucrose	0.387	0.430	-0.504	0.323
Fructosans	0.796 **	0.704 *	0.444	0.022
Total	0.895 **	0.757 *	0.513	0.243

† Winterhardiness score is proportional to hardiness. Scores 3-5 are "hardy", scores 1-2 are "less hardy" (50).

Symbols "*" and "**" indicate significant differences at p=0.05 and p=0.01, respectively, according to Student's t (df).

in correlation does not warrant the additional time and expense involved in this determination as done in this study.

These findings are in contradiction to those of Green and Ratzlaff (14) and Fowler et al. (11). In both reports, however, only those saccharides soluble in an 80% ethanol solution were examined - glucose, fructose, sucrose and raffinose.

Green and Ratzlaff (14) concluded that the occurrence of large quantities of soluble sugars is inversely related to cold hardiness. Since the concentrations of glucose, fructose, and sucrose were comparable to those in the present experiment, the presence of insufficient amounts of these carbohydrates can be ruled out. However, since fructosans were not measured, it may be that, under the environmental conditions of that experiment, the hardier cultivars were converting more of their low-molecular weight saccharides into fructosans.

Fowler et al. (11) rejected the use of ethanol-soluble saccharides as a screening method because both repeatability and heritability are unacceptably low. While these conclusions may be correct with respect to the absolute concentration of a given carbohydrate on a particular date, and therefore preclude an estimate of absolute winterhardiness, a relative estimate is not precluded. Although the absolute value of a given carbohydrate on a given date may vary widely between years, the relationshp between classes appears stable (compare Figures 2 and 3, 4 and 5, 6 and 7, and 8 and 9).

It seems clear from the evidence that carbohydrates cannot exert their protective effects by acting as freezing point depressants of

cell water. Based on the highest concentrations of total carbohydrates found in this study reduced to glucose equivalents, the maximum freezing point depression which could have been theoretically obtained by all carbohydrates in their monomeric state was 0.2°C. Temperatures of -11°C under 6 cm of snow cover, however, are not uncommon in Montana. This would have required a cell solute concentration of 5.9m to achieve the required degree of protection. This concentration is clearly outside the limits of living cells.

There is ample evidence of the destructive effects of freezing on the membranes of isolated chloroplast thylakoids (12, 16, 17, 38, 39, 40, 41, 57, 63) and isolated mitochondria (16, 17), and of the protection afforded by various carbohydrates (12, 16, 17, 38, 39, 40, 41, 57, 63). The structural and functional similarities between chloroplasts and mitochondria, and the similarities between their injury upon freezing and their protection by carbohydrates cannot be coincidence. The evidence strongly suggests a common mechanism of both injury and protection that may apply not only to chloroplasts and mitochondria but to membrane systems in general. Although the mechanisms of freezing injury are still open to speculation, it appears that carbohydrates may preserve the functional integrity of membranes. If their only beneficial role were to preserve the phosphorylating capacity of mitochondria, this, alone, would be sufficient to explain the observed protective effects. While a wheat plant can overwinter without photosynthesis, as often occurs when the top growth is winterkilled, it cannot survive without functional mitochondria. The higher concentrations of carbohydrates which we measured in the hardier

cultivars suggest an adaptation which provides a greater degree of protection against the uncoupling of vital phosphorylation reactions.

Regrowth After Controlled Freezing

Regrowth data for the 1981-82 crop year are presented in Table 15. Significant differences between the "hardy" and "less hardy" groups were found in the number of crowns which survived the freezing regime (survival count), the amount of regrowth averaged over the 10 specimens in each treatment (average regrowth per plant), and average regrowth per survivor. In each case, the hardy group had a greater value than the less hardy group. There were significantly more survivors for cultivars in score group 5 than for cultivars in score groups 3 and 4. All three measurements were significantly correlated with winterhardiness score (Table 16).

Data for the 1982-83 crop year is presented in Tables 17-18. The December 20, 1982 sampling showed significant differences between the two hardiness classes only for average regrowth per plant (Table 17). Sub-classes could not be differentiated.

An attempt was made to test the effects of artificial freezing on plants sampled in the spring. For spring sampling, however, the freezing regime which was used in autumn had been shown to be too severe for all the cultivars indicating that partial dehardening had occurred by early March. A warmer regime used for the March 23 sampling produced more favorable results (Table 18). The winterhardy cultivars differed significantly from the less hardy cultivars in

TABLE 15

Orthogonal Comparisons of Number of Survivors, Average Regrowth (mm) per Plant, and Average Regrowth (mm) per Survivor After Controlled Freezing and Winterhardiness (WH.) Score†: December 21, 1981

	Average Number of Survivors	
W.H. Scores	3-5 vs- 1-2	5 vs 3-4
Means =	7.85 2.92	9.12 7.00
t(24) =	7.12 **	2.45 *

	Average Regrowth (mm) per Plant	
W.H. Scores	3-5 vs 1-2	5 vs 3-4
Means =	1.58 0.559	1.64 1.53
t (24) =	4.37 **	0.358

	Average Regrowth (mm) per Survivor	
W.H. Scores	3-5 vs 1-2	5 vs 3-4
Means =	1.82 1.04	1.71 1.90
t (24) =	2.36 *	-0.453

† Winterhardiness score is proportional to hardiness. Scores 3-5 are "hardy", scores 1-2 are "less hardy" (50).

Symbols "*" and "**" indicate significant differences at p=0.05 and p=0.01, respectively, according to Student's t (df).

TABLE 16

Correlation Coefficients of Winterhardiness Scores† With Number of Survivors, Average Regrowth (mm) Per Plant, and Average Regrowth (mm) Per Survivor After Controlled Freezing: December 21, 1981

	Score	No. of Survivors	Regrowth per Plant	Regrowth per Survivor
No. of Survivors	0.848 **	1.00		
Regrowth (10)	0.704 **	0.910	1.000	
Regrowth (Survivor)	0.566 *	0.850	0.969	1.000

† Winterhardiness score is proportional to hardiness. Scores 3-5 are "hardy", scores 1-2 are "less hardy" (50).

Symbols "*" and "**" indicate significant differences at p=0.05 and p=0.01, respectively, according to Student's t (df).

TABLE 17

Orthogonal Comparisons of Regrowth (mm) per Plant After Controlled Freezing and Winterhardiness (W.H.) Score†: December 20, 1982

	Average Regrowth (mm) per Plant		
W.H. Scores	3-5 vs 1-2	5 vs 3-4	2 vs 1
Means =	1.90 0.689	1.93 1.85	1.24 0.138
t (27) =	2.24 *	0.598	1.496

TABLE 18

Orthogonal Comparisons of Number of Survivors, Average Regrowth (mm) per Plant, and Average Regrowth (mm) per Survivor After Controlled Freezing and Winterhardiness (WH.) Scores†: March 23, 1983

	Number of Survivors		
W.H. Scores	3-5 vs 1-2	5 vs 3-4	2 vs 1
Means =	6.75 6.50	7.08 6.42	7.75 5.25
t (27) =	3.81 **	1.29	7.90**
	Average Regrowth (mm) per Plant		
W.H. Scores	3-5 vs 1-2	5 vs 3-4	2 vs 1
Means =	2.54 2.22	2.60 2.48	3.15 1.29
t (27) =	3.68 **	1.71	0.779
	Average Regrowth (mm) per Survivor		
W.H. Scores	3-5 vs 1-2	5 vs 3-4	2 vs 1
Means =	3.41 2.93	3.46 3.37	3.67 2.21
t (27) =	3.42 **	1.32	1.17

† Winterhardiness score is proportional to hardiness. Scores 3-5 are "hardy", scores 1-2 are "less hardy" (50).

Symbols "*" and "**" indicate significant differences at p=0.05 and p=0.01, respectively, according to Student's t (df).

survival count, average regrowth per plant, and average regrowth per survivor. Hardiness group 2 had more survivors than hardiness group 1. None of these factors significantly correlated with winterhardiness scores during the second crop year. The most prominent features of the combined data are the agreement between the relationships on December 21, 1981 and March 23, 1983; and the disparity with those of December 20, 1982. Prima facie evidence suggests that artificial freezing of wheat crowns is not a viable technique for determining the winterhardiness of new genotypes; though this is not necessarily the case.

As Weibel and Quisenberry (61) pointed out, wintering plants appear to respond to changing weather patterns, and conditions of the freezing regime must be calculated with recent weather history in mind. Suneson and Peltier (49) observed sizeable changes in winterhardiness rankings which they attributed to short-term environmental changes. These findings suggest that under field conditions, the degree of correlation may vary because of the continual variation in weather. Plants respond to these changes but may not change in synchrony. Resolution of this problem requires a controlled environment capable of simulating winter field conditions and maintaining a specified state while the plants equilibrate.

Although the proper temperature regime for screening Montana wheats has yet to be determined, this method still holds promise. The high correlation between hardiness score and survival counts on December 20, 1981 and March 23, 1983 is in accord with previous literature (1, 61, 64); and the ability of the tests to discriminate

between "hardy" and "less hardy" groups under the very different conditions suggests that more than chance is involved. With the combination of a suitably-defined environment and proper freezing regimen, this method may prove to be a useful tool for winter cereal breeders.

Chlorophyll

For the 1981-82 crop year, chlorophyll concentration (mg chlorophyll dm^{-2}) differed between hardiness groups only in spring, on March 23 and April 14 (Table 19). On these two dates the hardy group contained a greater concentration of chlorophyll than the less hardy group. Within the hardy group, score group 5 could not be differentiated from score groups 3 and 4. Winterhardiness scores were not significantly correlated with chlorophyll contents on either date.

In 1982-83, chlorophyll concentrations differed on both December 20 and March 30. The data are presented in Table 20. The group of hardy cultivars (scores 3, 4 and 5) contained significantly more chlorophyll per square decimeter than the less hardy group only on March 30. Differences within the hardy class were not significant. On both dates, cultivars in score group 1 contained less chlorophyll per square decimeter than those in group 2. Correlation coefficients were insignificant on both dates.

The data suggest there may be a greater degradation of chlorophyll in the less hardy cultivars over the winter, since, as a group, they tended to have a lower chlorophyll concentration in the spring but not in the autumn.

TABLE 19

Orthogonal Comparisons of Total Chlorophyll per unit Leaf Area [mg chlorophyll (dm^2 leaf area)$^{-1}$] For† Cultivars with Differing Winterhardiness (W.H.) Scores†: 1981-82

		March 23, 1982	
W.H. Scores		3-5 vs 1-2	5 vs 3-4
Means	=	6.08 4.28	5.60 6.39
t (21)	=	2.54 *	-0.884

		April 14, 1982	
W.H. Scores		3-5 vs 1-2	5 vs 3-4
Means	=	9.9 6.88	10.3 9.58
t (21)	=	2.95 **	1.43

† Winterhardiness score is proportional to hardiness. Scores 3-5 are "hardy", scores 1-2 are "less hardy" (50).

Symbols "*" and "**" indicate significant differences at p=0.05 and p=0.01, respectively, according to Student's t (df).

TABLE 20

Orthogonal Comparisons of Total Chlorophyll per unit Leaf Area [mg chlorophyll (dm^2 leaf area)$^{-1}$] For Cultivars with Differing Winterhardiness (W.H.) Scores†: 1982-83

	December 20, 1982		
W.H. Scores	3-5 vs 1-2	5 vs 3-4	2 vs 1
Means =	10.3 8.73	10.1 10.4	9.00 8.46
t (21) =	1.78	-0.555	2.456 *

	March 30, 1983		
W.H. Scores	3-5 vs 1-2	5 vs 3-4	2 vs 1
Means =	8.73 6.83	8.46 8.99	7.22 6.44
t (21) =	6.40 **	-0.185	6.57 **

† Winterhardiness score is proportional to hardiness. Scores 3-5 are "hardy", scores 1-2 are "less hardy" (50).

Symbols "*" and "**" indicate significant differences at p=0.05 and p=0.01, respectively, according to Student's t (df).

Since the trend was inconsistent in the spring it is possible that these results indicate only responses to short-term environmental stress.

One source of error in this experiment was the determination of leaf area. The leaves tended to form helixes when detached from the plants, apparently due to desiccation, even under the best of storage conditions. This reduced the accuracy of the chlorophyll concentration assay in terms of milligrams of chlorophyll per unit leaf area.

If it is assumed that the less hardy cultivars contain less chlorophyll per square decimeter and that they maintain a lower osmotic potential as recent evidence indicates (51), then the detached leaves would be more subject to twisting from desiccation, the leaf area term would be artificially low, and chlorophyll concentration (mg chlorophyll dm^{-2}) artificially high due to the smaller denominator (leaf area). In effect, even if there were significant differences between the hardy and less hardy groups, they could be canceled by the opposing differences in "apparent" leaf area.

There may, or may not, be a relationship between chlorophyll concentration and winterhardiness, but due to inherent difficulties in accurately and easily determining leaf area for large numbers of samples, this method cannot be strongly recommended as a screening procedure.

SUMMARY

This study was undertaken to examine the relationship of several physiological characteristics with winterhardiness of wheat, and develop a winterhardiness screening procedure.

For the first year of the study (1981-82), eight winter wheat cultivars were scored for winterhardiness on a 1-5 scale (5 being the most hardy and 1 ,the least hardy). Two additional cultivars were included the second year of the study (1982-83). Each year, cultivars were seeded in late September, and sampled in late autumn and early spring for the following determinations: (1) crown tissue concentrations [μmoles (g dry wt.)$^{-1}$] of glucose plus fructose, sucrose, fructosans (expressed as anhydrous glucose equivalents), and total carbohydrates; (2) leaf chlorophyll concentration [mg chlorophyll (dm^2 leaf area)$^{-1}$]; and (3) crown regrowth following a controlled freezing regime. Crown regrowth was then estimated as the number of survivors average regrowth per plant, and average regrowth per survivor.

Winterhardiness score was significantly correlated with concentrations of glucose plus fructose (r=0.645 to 0.787), fructosans (r=0.707 to 0.915), and total carbohydrates (r=0.757 to 0.895). Sucrose was not significantly correlated with winterhardiness score.

Differences in leaf chlorophyll concentration between cultivars were observed mainly in the spring; however, these differences were

inconsistent and leaf chlorophyll concentration was not significantly correlated with winterhardiness score. Practical difficulties, including that of obtaining accurate leaf area measurements due to severe twisting of the leaves, may have masked possible relationships (standard errors of individual observations ranged between 10% and 67% of means) Refinement of this technique may increase the usefulness of this method.

Even though at times the number of survivors and average regrowth per plant, and average regrowth per survivor after the imposition of a freezing regime were significantly correlated with winterhardiness score, the results were inconsistent. This may be due to fluctuating hardiness levels as a response to short-term temperature changes. With further study to determine the proper temperature regime, this method may prove to be an effective method of evaluating the winterhardiness potential of new winter wheat cultivars.

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APPENDIX

TABLE 21

Means and Standard Deviations of Individual Observations of Concentrations [μmoles (g dry wt.)$^{-1}$] of Glucose Plus Fructose, Sucrose, Fructosans, and Total Carbohydrates of Winter Wheat Cultivars Sampled on November 3, 1981.

Cultivar	Glucose + fructose	Sucrose	Fructosans	Total carbohydrates
Norstar	179 ± 104	265 ± 53.5	1162 ± 442	1606 ± 445
Froid	188 ± 98.4	225 ± 50.3	1343 ± 225	1757 ± 191
Winalta	223 ± 129	264 ± 55.5	1349 ± 228	1836 ± 190
Mt 7115	182 ± 192	245 ± 36.2	1607 ± 427	2034 ± 397
Redwin	196 ± 80.4	532 ± 35.4	1675 ± 675	2403 ± 1106
Centurk	226 ± 158	200 ± 82.1	806 ± 478	1232 ± 588
MT 6928	113 ± 23.5	294 ± 40.0	1107 ± 680	1513 ± 649
Ore. Feed Wheat	60.7 ± 7.40	221 ± 30.9	1069 ± 294	1351 ± 306

TABLE 22

Means and Standard Deviations of Individual Observations of Concentrations [μmoles (g dry wt.)$^{-1}$] of Glucose Plus Fructose, Sucrose, Fructosans, and Total Carbohydrates of Winter Wheat Cultivars Sampled on November 16, 1981.

Cultivar	Glucose + fructose	Sucrose	Fructosans	Total carbohydrates
Norstar	170 ± 30.3	269 ± 121	1759 ± 120	2198 ± 155
Froid	158 ± 13.5	270 ± 22.9	1650 ± 256	2079 ± 233
Winalta	157 ± 41.8	245 ± 35.8	1501 ± 263	1904 ± 320
Mt 7115	156 ± 46.9	245 ± 94.5	1467 ± 629	1868 ± 758
Redwin	162 ± 37.0	236 ± 29.1	1464 ± 186	1862 ± 197
Centurk	167 ± 12.4	318 ± 168	1364 ± 76.2	1848 ± 211
MT 6928	133 ± 6.00	236 ± 21.1	1161 ± 94.0	1530 ± 82.0
Ore. Feed Wheat	113 ± 18.1	203 ± 19.7	1233 ± 118	1548 ± 121

TABLE 23

Means and Standard Deviations of Individual Observations of Concentrations [μmoles (g dry wt.)$^{-1}$] of Glucose Plus Fructose, Sucrose, Fructosans, and Total Carbohydrates of Winter Wheat Cultivars Sampled on March 23,1982.

Cultivar	Glucose + fructose	Sucrose	Fructosans	Total carbohydrates
Norstar	244 ± 23.6	355 ± 83.7	1984 ± 378	2583 ± 327
Froid	190 ± 16.4	341 ± 72.9	1852 ± 268	2383 ± 284
Winalta	202 ± 15.6	279 ± 61.7	1155 ± 494	1636 ± 521
Mt 7115	218 ± 35.6	302 ± 77.4	1950 ± 488	2470 ± 476
Redwin	205 ± 37.3	385 ± 69.2	1210 ± 390	1800 ± 352
Centurk	178 ± 25.9	270 ± 58.1	1193 ± 200	1641 ± 205
MT 6928	185 ± 34.1	298 ± 82.1	942 ± 148	1425 ± 209
Ore. Feed Wheat	101 ± 16.5	300 ± 104	921 ± 184	1323 ± 203

TABLE 24

Means and Standard Deviations of Individual Observations of Concentrations [μmoles (g dry wt.)$^{-1}$] of Glucose Plus Fructose, Sucrose, Fructosans, and Total Carbohydrates of Winter Wheat Cultivars Sampled on April 14, 1982.

Cultivar	Glucose + fructose	Sucrose	Fructosans	Total carbohydrates
Norstar	112 ± 35.5	117 ± 28.2	449 ± 141	678 ± 192
Froid	71.3 ± 19.6	82.7 ± 15.6	509 ± 26.5	663 ± 55.8
Winalta	96.8 ± 15.6	101 ± 24.0	324 ± 82.5	522 ± 47.2
Mt 7115	71.6 ± 5.55	129 ± 42.8	352 ± 79.6	553 ± 80.5
Redwin	114 ± 28.3	122 ± 11.3	385 ± 195	621 ± 220
Centurk	83.6 ± 23.2	105 ± 11.5	454 ± 188	642 ± 200
MT 6928	111 ± 29.7	118 ± 37.8	528 ± 77.4	758 ± 51.4
Ore. Feed Wheat	76.6 ± 7.54	106 ± 7.65	863 ± 61.9	1048 ± 62.0

TABLE 25

Means and Standard Deviations of Individual Observations of Concentrations [μmoles (g dry wt.)$^{-1}$] of Glucose Plus Fructose, Sucrose, Fructosans, and Total Carbohydrates of Winter Wheat Cultivars Sampled on April 25, 1982.

Cultivar	Glucose + fructose	Sucrose	Fructosans	Total carbohydrates
Norstar	125 ± 23.8	115 ± 13.0	504 ± 137	744 ± 124
Froid	93.8 ± 27.2	85.6 ± 19.7	592 ± 139	771 ± 158
Winalta	102 ± 23.6	92.5 ± 15.4	427 ± 117	622 ± 132
Mt 7115	103 ± 36.5	87.8 ± 13.7	687 ± 276	878 ± 290
Redwin	103 ± 16.1	79.5 ± 4.00	448 ± 253	631 ± 266
Centurk	95.0 ± 14.8	110 ± 10.6	577 ± 114	782 ± 111
MT 6928	99.4 ± 19.0	89.3 ± 12.3	511 ± 186	700 ± 196
Ore. Feed Wheat	133 ± 21.6	111 ± 10.0	718 ± 310	962 ± 312

TABLE 26

Means and Standard Deviations of Individual Observations of Concentrations [μmoles (g dry wt.)$^{-1}$] of Glucose Plus Fructose, Sucrose, Fructosans, and Total Carbohydrates of Winter Wheat Cultivars Sampled on December 20, 1982.

Cultivar	Glucose + fructose	Sucrose	Fructosans	Total carbohydrates
Norstar	302 ± 35.4	558 ± 72.2	1290 ± 332	2150 ± 319
Froid	254 ± 38.5	424 ± 92.4	1451 ± 243	2129 ± 244
Norwin	266 ± 41.6	451 ± 90.4	1263 ± 173	1980 ± 108
Winalta	224 ± 30.4	442 ± 68.0	1354 ± 321	2020 ± 366
Mt 7115	230 ± 52.2	418 ± 106	1415 ± 364	2063 ± 498
Redwin	245 ± 34.2	427 ± 132	970 ± 176	1642 ± 263
Centurk	245 ± 22.5	416 ± -19	958 ± 143	1619 ± 234
MT 6928	217 ± 51.4	351 ± 109	828 ± 188	1396 ± 199
Nugaines	246 ± 62.9	336 ± 120	1061 ± 291	1643 ± 387
Ore. Feed Wheat	176 ± 42.9	295 ± 107	994 ± 354	1465 ± 497

TABLE 27

Means and Standard Deviations of Individual Observations of Concentrations [μmoles (g dry wt.)$^{-1}$] of Glucose Plus Fructose, Sucrose, Fructosans, and Total Carbohydrates of Winter Wheat Cultivars Sampled on March 1, 1983.

Cultivar	Glucose + fructose	Sucrose	Fructosans	Total carbohydrates
Norstar	99.4 ± 9.05	191 ± 24.6	1227 ± 196	1517 ± 319
Froid	78.8 ± 11.5	157 ± 18.2	1396 ± 220	1633 ± 225
Norwin	81.2 ± 12.1	181 ± 23.6	1586 ± 263	1848 ± 277
Winalta	114 ± 34.8	174 ± 23.6	1273 ± 217	1561 ± 235
Mt 7115	67.8 ± 15.4	162 ± 24.3	1631 ± 92.1	1860 ± 152
Redwin	64.7 ± 10.2	214 ± 41.8	1227 ± 219	1506 ± 259
Centurk	79.4 ± 15.2	173 ± 20.0	1358 ± 157	1610 ± 152
MT 6928	58.6 ± 5.15	174 ± 12.9	1192 ± 417	1424 ± 431
Nugaines	65.4 ± 16.6	147 ± 20.8	1048 ± 214	1261 ± 191
Ore. Feed Wheat	66.1 ± 17.3	138 ± 22.3	962 ± 74.0	1166 ± 75.4

TABLE 28

Means and Standard Deviations of Individual Observations of Concentrations [μmoles (g dry wt.)$^{-1}$] of Glucose Plus Fructose, Sucrose, Fructosans, and Total Carbohydrates of Winter Wheat Cultivars Sampled on March 30, 1983.

Cultivar	Glucose + fructose	Sucrose	Fructosans	Total carbohydrates
Norstar	30.9 ± 8.01	213 ± 42.5	736 ± 218	980 ± 251
Froid	35.5 ± 8.28	214 ± 43.2	1022 ± 163	1270 ± 202
Norwin	39.7 ± 5.69	221 ± 43.9	796 ± 60.7	1060 ± 65.5
Winalta	38.0 ± 6.89	220 ± 41.6	785 ± 37.9	1040 ± 36.9
Mt 7115	34.1 ± 1.59	194 ± 26.8	867 ± 81.8	1100 ± 86.6
Redwin	35.8 ± 7.00	236 ± 46.4	875 ± 118	1150 ± 126
Centurk	30.2 ± 2.55	205 ± 41.0	771 ± 55.9	1010 ± 71.3
MT 6928	23.6 ± 3.00	206 ± 43.0	615 ± 89.3	846 ± 114
Nugaines	29.5 ± 4.62	215 ± 47.8	764 ± 117	1010 ± 119
Ore. Feed Wheat	27.5 ± 6.55	187 ± 49.3	780 ± 266	995 ± 294

TABLE 29

Means and Standard Deviations of Individual Observations of Concentrations [μmoles (g dry wt.)$^{-1}$] of Glucose Plus Fructose, Sucrose, Fructosans, and Total Carbohydrates of Winter Wheat Cultivars Sampled on April 19, 1983.

Cultivar	Glucose + fructose	Sucrose	Fructosans	Total carbohydrates
Norstar	36.4 ± 2.24	114 ± 32.5	140 ± 39.6	290 ± 57.7
Froid	41.0 ± 6.94	89.6 ± 45.6	166 ± 24.8	296 ± 60.9
Norwin	40.6 ± 8.95	77.0 ± 36.0	154 ± 22.1	272 ± 49.2
Winalta	35.3 ± 6.90	108 ± 35.4	99.0 ± 40.2	243 ± 51.6
Mt 7115	49.6 ± 3.22	84.2 ± 24.2	130 ± 41.0	264 ± 35.4
Redwin	28.3 ± 7.21	54.5 ± 22.2	122 ± 55.1	205 ± 66.1
Centurk	33.4 ± 8.90	84.2 ± 7.51	152 ± 7.60	269 ± 21.3
MT 6928	28.3 ± 9.88	53.8 ± 11.9	104 ± 15.4	185 ± 29.0
Nugaines	40.6 ± 9.58	72.2 ± 19.6	174 ± 82.9	287 ± 84.5
Ore. Feed Wheat	32.0 ± 6.27	103 ± 27.6	138 ± 18.1	273 ± 36.0

TABLE 30

Block Effects By Analysis of Variance (ANOVA) on Concentrations of Glucose Plus Fructose, Sucrose, Fructosans, and Total Carbohydrates on Each Sampling Date.

Date	Glucose + Fructose	Sucrose	Fructosans	Total Carbohydrates
November 3, 1981	**			
November 16, 1981	**			
March 3, 1982	**	**		
April 14, 1982				
April 25, 1982		*	**	**
December 20, 1982	**	**	**	**
March 1, 1983	**	*		
March 30, 1983	**			
April 19,1983	*			

Symbols "*" and "**" indicate significant differences at p=0.05 and p=0.01, respectively, from ANOVA.

TABLE 31

Means and Standard Deviations of Individual Observations of Numbers of Survivors, Regrowth (mm) per Plant, and Regrowth per Survivor After Controlled Freezing of Winter Wheat Cultivars Sampled on December 21, 1981.

Cultivar	Number of Survivors	Regrowth per Plant	Regrowth per Survivor
Norstar	8.25 ± 0.957	0.950 ± 0.652	1.10 ± 0.753
Froid	10.0 ± 0.00	2.32 ± 0.886	2.32 ± 0.886
Winalta	7.75 ± 1.50	1.74 ± 0.694	2.20 ± 0.603
Mt 7115	9.00 ± 1.41	2.29 ± 0.567	2.54 ± 0.506
Redwin	4.25 ± 3.10	0.575 ± 0.606	0.955 ± 0.968
Centurk	7.25 ± 2.75	1.35 ± 0.764	1.86 ± 0.746
MT 6928	1.25 ± 2.50	0.250 ± 0.500	0.500 ± 1.00
Ore. Feed Wheat	0.250 ± 0.500	0.0750 ± 0.150	0.750 ± 1.50

TABLE 32

Means and Standard Deviations of Individual Observations of Numbers of Survivors, Regrowth (mm) per Plant, and Regrowth per Survivor After Controlled Freezing of Winter Wheat Cultivars Sampled on December 20, 1982.

Cultivar	Number of Survivors	Regrowth per Plant	Regrowth per Survivor
Norstar	2.76 ± 1.89	0.775 ± 0.779	3.13 ± 2.53
Froid	2.50 ± 3.32	1.62 ± 2.63	1.55 ± 1.80
Norwin	4.25 ± 2.63	3.40 ± 0.816	4.58 ± 1.79
Winalta	3.50 ± 3.32	2.28 ± 2.87	4.50 ± 3.60
Mt 7115	3.25 ± 2.22	3.05 ± 2.07	4.88 ± 3.10
Redwin	0.500 ± 0.577	0.225 ± 0.386	2.00 ± 2.31
Centurk	2.75 ± 2.22	1.28 ± 0.922	2.82 ± 1.98
MT 6928	3.33 ± 3.06	1.20 ± 1.20	2.67 ± 3.06
Nugaines	1.00 ± 2.00	0.100 ± 0.200	0.500 ± 1.00
Oregon Feed Wheat	0.750 ± 0.500	0.175 ± 0.171	1.75 ± 1.71

TABLE 33

Means and Standard Deviations of Individual Observations of Numbers of Survivors, Regrowth (mm) per Plant, and Regrowth per Survivor After Controlled Freezing of Winter Wheat Cultivars Sampled on March 23, 1983.

Cultivar	Number of Survivors	Regrowth per Plant	Regrowth per Survivor
Norstar	7.50 ± 1.00	1.92 ± 0.862	2.62 ± 1.32
Froid	8.25 ± 1.71	3.18 ± 3.32	3.51 ± 3.08
Norwin	5.50 ± 3.32	2.70 ± 2.99	4.24 ± 3.03
Winalta	4.25 ± 2.50	1.35 ± 1.37	2.73 ± 1.06
Mt 7115	8.25 ± 2.36	4.10 ± 3.74	4.42 ± 3.42
Redwin	6.75 ± 0.500	2.00 ± 0.779	2.96 ± 1.12
Centurk	9.25 ± 0.957	4.82 ± 2.14	5.16 ± 2.00
MT 6928	6.25 ± 1.89	1.48 ± 1.16	2.17 ± 0.989
Nugaines	7.00 ± 2.31	1.92 ± 1.21	2.72 ± 1.16
Oregon Feed Wheat	3.50 ± 2.08	0.650 ± 0.451	1.69 ± 0.473

TABLE 34

Means and Standard Deviations of Individual Observations of Chlorophyll Concentrations [mg chlorophyll (dm^2 leaf area)$^{-1}$] of Winter Wheat Cultivars: 1981-1982

	Chlorophyll Concentration [mg Chlorophyll (dm^2 leaf area)$^{-1}$]				
	Sampling Date				
Cultivar	November 3	November 16	March 23	April 14	April 25
Norstar	5.97 ± 3.37	7.33 ± 0.810	6.36 ± 1.52	11.4 ± 2.35	8.01 ± 2.28
Froid	4.65 ± 2.24	6.12 ± 1.60	4.84 ± 3.22	9.18 ± 3.01	7.28 ± 1.93
Winalta	4.71 ± 1.15	7.08 ± 1.22	6.48 ± 1.75	10.7 ± 3.01	6.73 ± 4.48
Mt 7115	5.55 ± 2.67	5.89 ± 2.61	5.44 ± 3.34	8.17 ± 1.57	8.55 ± 1.35
Redwin	4.66 ± 3.43	6.14 ± 1.11	7.25 ± 0.543	9.88 ± 2.64	6.35 ± 4.11
Centurk	5.66 ± 1.69	5.81 ± 1.92	6.11 ± 1.50	8.21 ± 2.21	5.42 ± 1.83
MT 6928	4.72 ± 0.787	5.52 ± 2.74	2.08 ± 1.06	4.19 ± 0.618	4.10 ± 0.41
Oregon Feed Wheat	6.55 ± 1.04	5.30 ± 1.18	4.65 ± 0.167	8.25 ± 2.28	7.06 ± 1.06

TABLE 35

Means and Standard Deviations of Individual Observations of Chlorophyll Concentrations [mg chlorophyll $(dm^2$ leaf area$)^{-1}$] of Winter Wheat Cultivars Sampled in 1982-83.

	Chlorophyll Concentration [mg Chlorophyll $(dm^2$ leaf area$)^{-1}$]		
	Sampling Date		
Cultivar	December 20	March 1	March 30
Norstar	10.1 ± 3.36	5.58 ± 2.80	8.29 ± 0.899
Froid	10.6 ± 3.84	6.11 ± 2.03	8.31 ± 0.286
Norwin	9.70 ± 1.82	6.80 ± 2.39	8.78 ± 0.660
Winalta	10.6 ± 2.67	8.74 ± 5.21	8.62 ± 0.574
Mt 7115	9.22 ± 2.16	7.24 ± 3.94	9.39 ± 0.932
Redwin	11.4 ± 2.37	6.01 ± 3.86	8.97 ± 0.546
Centurk	8.78 ± 2.40	6.64 ± 3.23	7.16 ± 0.609
MT 6928	9.22 ± 1.79	7.29 ± 3.49	7.28 ± 0.0757
Nugaines	9.38 ± 1.75	4.16 ± 1.34	7.82 ± 0.405
Oregon Feed Wheat	7.55 ± 1.63	4.23 ± 1.32	5.07 ± 0.877