COLD RESISTANCE IN THREE VARIETIES OF WINTER WHEAT AS RELATED TO NITROGEN FRACTIONS, TOTAL SUGAR AND OPTICAL DENSITY OF DILUTED CELL SAP

by

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INTRODUCTION

The ability of certain species of plants to develop a resistance to injury by cold has long been a subject of interest to plant scientists. Early investigations involved a biochemical approach to the problem of cold hardiness in plants. It was soon learned, however, that physical and anatomical changes of the plant cell undergoing cold hardening had to be understood before the problem could be approached from the biochemical view. Work by such outstanding plant physiologists as Levitt and Scarth has yielded a rather thorough understanding of the physical and anatomical changes that take place within the plant cell during the development of cold hardiness.

Now that the physical and anatomical behavior of the cell undergoing cold hardening is more fully understood, the plant scientist can resume the study of cold hardening from the biochemical point of view. During the past few decades much attention has been given to the changes in carbohydrates during hardening and their role is now fairly well understood and established with most species of plants possessing the ability to undergo cold hardening.

A knowledge of the role of nitrogenous constituents during the development of cold hardiness in plants is still obscure. Some research involving a study of woody plants and perennial legumes has shown that there is an increase in water soluble organic nitrogen constituents during hardening. The purpose of this experiment has been to further investigate this phenomenon with winter wheat. An attempt has been made to measure the amount of change in total sugar and nitrogenous constituents in different parts of three varieties of winter wheat in relation to the degree of

hardiness achieved during the fall and winter months. It was thought that such an experiment would yield a more thorough understanding of the mechanism of cold hardiness as it would apply to one of our most important crop plants--winter wheat.

REVIEW OF LITERATURE

A complete review of literature on the hardiness of plants has been made by Levitt (1956). However, the author of this thesis wishes to discuss further the results obtained by several workers cited in Levitt's review, and to also discuss the work of several investigators not cited by Levitt or those that have published papers since Levitt's review.

An outstanding contribution toward the understanding of hardiness in winter wheat was made by Kneen and Blish (1941) at the Nebraska Agricultural Experiment Station. Their study involved the metabolism of carbohydrates in winter wheat during the hardening and dehardening process. Sucrose was found to increase in the living tissue of both crowns and leaves during hardening and to decline sharply upon the onset of dehardening. The varieties possessing the greatest amount of cold resistance showed the greatest increase in sucrose in both the crowns and leaves during the winter months. At the onset of dehardening, the resistant varieties were shown to decrease in sucrose content more slowly in both their crowns and leaves than the more susceptible varieties. Sucrose content decreased more readily in the crowns than in the leaves during dehardening. From their observations Kneen and Blish concluded that the increase in sucrose during the winter months was essential for the survival of the living tissue of the wheat plant, particularly the crowns, and that the

more rapid decrease in sucrose in the crowns during dehardening was due to growth in the meristematic region.

Siminovitch et al. (1953) in their studies with bark tissue of the cambium of Robinia pseudo-acacia L. the black locust, found an interconversion of starch to sucrose occurring during the fall. Sucrose content then remained high throughout the winter and spring until May when active growth was resumed with the unused sucrose then reconverted back to starch. They hypothesized that an enzyme system, presumably phosphorylase, was responsible for carbohydrate interconversions during hardening and dehardening in the black locust.

Siminovitch and Briggs (1949) analyzed living tissue of the bark of the black locust during the winter months for three different nitrogen fractions; non-soluble protein nitrogen, soluble protein nitrogen, and soluble non-protein nitrogen. Of the three nitrogen fractions studied, only the water soluble proteins showed a direct and consistent correlation with changes in resistance of cells to cold. They concluded that the increase in soluble protein could either prevent dehydration of the cell during exposure to cold by increasing the amount of hydrophilic colloids, or prevent intracellular freezing which is usually lethal.

In an experiment with cold resistance in overwintering alfalfa, red clover and sweetclover, Bula and Smith (1954) found a steady decline in starch percentage and a steady increase in total carbohydrate and reducing sugar percentages in the crowns and taproots from October through December. The change in carbohydrates was less pronounced with red clover which in turn was shown to be less hardy by electrical conductivity tests. Studies involving changes in total nitrogen were also made. Total nitrogen

percentage increased in the crowns and taproots of all legumes studied from September to April, after which it dropped sharply. They attributed the increase in total nitrogen to changes occurring in the total available carbohydrates rather than to any change in cold resistance.

Bula et al. (1956) studied the relationship of nitrogenous constituents and total available carbohydrates to cold resistance as measured by electrical conductivity of crown and taproot tissue of three varieties of alfalfa grown at Madison, Wisconsin and Palmer, Alaska. They reported highly significant correlations of non-soluble protein nitrogen, soluble protein nitrogen, soluble non-protein nitrogen and total nitrogen with cold resistance of two hardy varieties grown at Palmer. No correlation was reported for the soluble protein in the unhardy variety. Similar results were shown for the alfalfa grown at Madison, Wisconsin, although the percentages of nitrogen fractions were somewhat higher than those of the alfalfas grown at Palmer. They reported that maximum hardiness was attained slightly after the soil surface froze, and after the average weekly air temperature remained below freezing. Their conclusion states that the water soluble proteins may be related to the development of cold resistance.

In an experiment with sweetclover grown at Palmer, Alaska, Hodgson and Bula (1956) reported highly significant correlations between total water soluble nitrogen, soluble protein nitrogen, or non-protein nitrogen and specific conductivities and total available carbohydrates in sweetclover roots grown in drilled rows sampled on October 18. They found highly significant differences for all nitrogen fractions among varieties and among dates. The variety by date interaction for soluble non-protein

nitrogen and total soluble nitrogen was thought to be due to the varieties having the least cold resistance exhibiting a faster proportional gain in non-protein nitrogen. They believed that the non-hardy varieties were able to produce amino acids and peptides, but were not able to convert them into soluble protein as rapidly as the hardy varieties, consequently they were slower in developing cold resistance.

MATERIALS AND METHODS

Three varieties of winter wheat were used for this study. The varieties selected were Minturki, Pawnee and Ponca. Minturki was selected because of its ability to develop cold resistance to a high degree. Ponca, a relatively less hardy variety than Minturki, was selected to represent the least hardy variety for the study, while Pawnee, a variety possessing less cold resistance than Minturki but more than Ponca, was selected as an intermediate.

Seed was obtained from plants grown at the Agronomy Farm in 1958, and was planted outdoors in 4-inch clay pots on September 29, 1958. Ten kernels were planted in each pot, and the pots later thinned to five plants per pot where possible. The pots were watered as often as necessary to keep the soil moist during the fall and winter.

The degree of hardiness in each of the varieties during the course of the experiment was found by determining the temperature required to kill 50 percent of the plants during a 16-hour exposure in a freezing chamber. The 50 percent killing temperature index was used rather than the 100 percent killing temperature index, in order to more accurately estimate the degree of hardiness possessed by a variety at a given temperature. When

the 100 percent kill method is used, it is difficult to determine the exact temperature required to kill the plants. Freezing tests were conducted during three consecutive days during each sampling period. Four pots of each variety were brought into the greenhouse at 1:00 p.m., watered and allowed to stand till 4:00 p.m., at which time the soil temperatures became somewhat equalized from one date to another. Sometimes the soil in the pots was frozen hard at the time they were first brought into the greenhouse and they were often covered by snow and ice. Leaving them stand for three hours before placing them into the freezer allowed the soil to thaw along with any ice or snow that might have been present, thereby preventing undue differences in results from one freezing test to the next. The pots were placed into a thermostatically controlled freezer for 16 hours beginning at 4:00 p.m. All freezing was accomplished without lights. Upon removal from the freezer, the plants were allowed to recuperate on a bench inside the greenhouse. Survival counts were made one week later. Plants were sampled for chemical analysis on the second freezing day during each sampling period. Ten pots of plants of each variety were used for chemical analysis at each sampling; four pots for nitrogen determinations, four pots for total sugar determinations, and two pots to determine the optical density of the diluted cell sap of the whole plant. All analyses were made in duplicate.

At each sampling date, the plants which were to be analyzed chemically were brought into the laboratory and allowed to stand until the soil was thawed and they could be removed with roots, crowns and leaves intact. The plants were then thoroughly washed with tap water to remove all soil from the crown and roots. Nitrogen and total sugar determinations were

made on leaves and crowns. Content of each component in the whole plant was taken as the sum of the leaf and crown determinations. Separation of the non-soluble protein nitrogen, water soluble protein nitrogen, and water soluble non-protein nitrogen fractions was done in a manner similar to that outlined by Siminovitch, et al. (1949). Total nitrogen was estimated by summation of individual nitrogen fractions.

Material used for total sugar determinations was autoclaved for 5 minutes at 5 lb pressure, dried at 70° C. for 24 hours and weighed. The dried material was finely ground with a mortar and pestle. Sugars were extracted in a Soxhlet extractor for 6-8 hours using 80 percent ethyl alcohol according to the A.O.A.C. method (1955). Following clarification, 5 ml concentrated hydrochloric acid was added to a 50 ml aliquot of the solution, allowed to stand overnight, neutralized, and made to 100 ml. Total sugar of the leaves and crowns was then quantitatively determined by using a Coleman Jr. Model 6A Spectrophotometer and measuring the optical density at 420 mu of 2 ml of each solution after it had been mixed with 3 ml potassium ferricyanide solution and 3 ml of water, heated for 5 minutes in boiling water, and made to a volume of 15 ml. The optical density readings were then recorded for each sample and referred to a standard glucose curve. Results were then computed on the basis of mg of sugar per gm of oven-dry tissue.

Optical density of the diluted extract of leaves, crowns and whole plants was determined in each variety. Whole plants were macerated with a mortar and pestle with 50 ml of distilled water, filtered and made to a volume of 100 ml with distilled water. Optical density readings were taken immediately with a Coleman Jr. Model 6A Spectrophotometer at 570 mu.

Optical density readings of crown and leaf tissue were made on 50 ml aliquots of leaf and crown extracts that had been diluted to 200 ml volume with distilled water during the process of nitrogen fractionation. The optical density readings were then expressed as optical density per gm of fresh tissue.

The correlation coefficients and the analyses of variance for nitrogen determinations, total sugar and optical density were computed as outlined by Snedecor (1956).

EXPERIMENTAL RESULTS

Cold Resistance of Varieties Studied

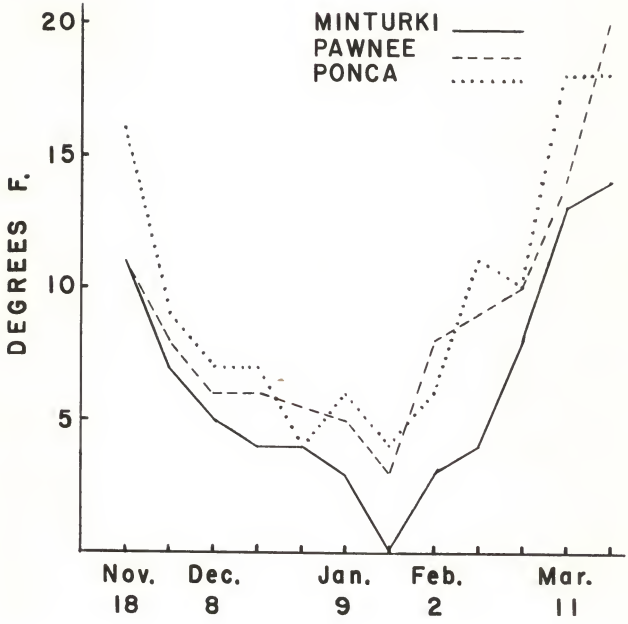
The temperature required to give a 50 percent kill at a particular date for each variety is shown in Plate 1. Minturki, Pawnee and Ponca fell into their proper order of hardiness in accordance with observed field behavior.

Cold resistance in Minturki was much more stable and therefore yielded a smoother curve than did Pawnee and Ponca. There was a gradual increase in cold resistance among the varieties to December 30, when all three varieties were equally cold resistant. Slight dehardening occurred in Pawnee and Ponca by January 9, but Minturki continued to increase in hardiness to January 20 when it reached its maximum. Pawnee reached its maximum by January 20 also, but Ponca had reached its maximum on December 30 and after some dehardening and hardening the same maximum was reached again on January 20. A rather marked decline appeared after January 20 among all varieties, but with considerable fluctuation among varieties. Minturki followed a rather gradual dehardening pattern. Frequent warming

EXPLANATION OF PLATE I

Hardiness curves of Minturki, Pawnee and Ponca winter wheat as expressed by the temperature required to obtain a 50 percent kill.

PLATE I


MINTURKI
PAWNEE
PONCA
DEGREES F.
20
15
10
5
Nov. 18
Dec. 8
Jan. 9
Feb. 2
Mar. 11

spells followed by colder temperatures during the latter part of January and through February were influential in producing this irregular pattern in the two less resistant varieties. Cold resistance in Minturki apparently was not affected by changing temperatures to such an extent as were the less resistant varieties.

Nitrogen Studies

The average total nitrogen content for each plant part of each variety at each date is reported in Table 1. Total nitrogen in Minturki and Pawnee fluctuated more widely in the plant during the period of hardening and dehardening than in Ponca.

Table 1. Average mg of total nitrogen per gm fresh tissue of each plant part of each variety at each sampling date.

Samp.	Minturki			Pawnee			Ponca		
Date	Leaves	Crowns	Plants	Leaves	Crowns	Plants	Leaves	Crowns	Plants
11-18	7.1	7.1	2.6	7.6	6.2	7.3	7.4	6.6	7.1
11-28	8.5	7.7	8.3	8.3	8.2	8.3	7.6	6.5	7.3
12-8	9.0	8.3	8.9	8.5	7.6	8.2	1.5	6.1	7.5
12-19	9.7	11.8	10.1	9.0	10.7	9.2	7.9	7.2	7.7
12-30	9.7	9.1	9.5	9.1	6.6	8.4	8.7	5.9	7.7
1-9	10.7	10.3	10.5	9.5	8.5	9.2	9.9	8.5	9.5
1-20	11.7	9.1	10.7	12.3	8.4	10.9	8.6	8.3	8.5
2-2	12.0	7.5	10.6	14.0	10.5	13.0	8.8	6.9	8.0
2-12	9.8	9.0	9.5	10.0	7.7	9.3	8.8	6.7	8.1
2-23	10.0	12.3	10.6	10.5	12.5	11.2	10.1	7.0	9.6
3-11	9.9	10.0	9.7	7.8	9.1	8.8	8.9	9.5	8.7
3-20	7.4	5.8	7.0	7.3	6.1	7.0	6.8	5.8	6.5

With the onset of hardening, total nitrogen in the parts of all varieties began to increase and continued to increase beyond January 20 when all varieties had attained maximum cold resistance. Total nitrogen

content remained fairly constant from January 20 to March 11 in the parts of all varieties. Substantially lower values for crowns of all three varieties on March 20 may be attributed to the resumption of growth. Minturki and Pawnee showed a greater total nitrogen content in all parts during the winter than did Ponca. Apparently more total nitrogen was taken up by the varieties possessing a higher degree of hardiness than by the variety possessing a lower degree of hardiness, but the trend of total nitrogen in any part of any variety does not coincide with hardening and dehardening trends.

The average total non-soluble protein nitrogen for the plant parts of each variety at each date is given in Table 2. Considerably less fluctuation between dates is noted among the parts of all three varieties.

Table 2. Average mg of non-soluble protein nitrogen per gm fresh tissue of each plant part of each variety at each sampling date.

Samp.	Minturki			Pawnee			Ponca		
Date	Leaves	Crowns	Plants	Leaves	Crowns	Plants	Leaves	Crowns	Plants
11-18	3.6	2.5	1.2	3.8	2.4	3.5	3.8	2.8	3.6
11-28	4.3	3.2	4.1	4.1	3.2	3.9	3.8	2.7	3.5
12-8	4.2	3.5	4.0	4.2	3.2	4.0	0.7	2.2	3.6
12-19	3.4	5.7	4.1	2.7	4.9	3.3	2.6	2.8	2.7
12-30	3.1	2.4	3.0	3.1	1.8	2.7	3.5	1.6	2.9
1-9	3.1	3.8	3.3	2.7	2.8	2.7	3.4	2.8	3.2
1-20	4.3	3.6	4.0	4.3	3.0	3.8	4.3	3.1	3.9
2-2	4.4	2.9	3.9	4.3	4.5	4.4	3.6	2.4	3.1
2-12	3.3	3.6	3.4	3.3	2.5	3.1	3.0	2.9	3.0
2-23	3.3	5.2	3.9	2.9	6.0	4.0	3.3	1.9	3.5
3-11	3.3	4.1	3.3	2.6	2.6	3.0	4.0	3.3	3.2
3-20	2.6	2.8	2.7	2.6	3.2	2.8	2.1	2.6	2.3

Non-soluble protein nitrogen does not increase noticeably during hardening, nor does it decrease to any great extent upon dehardening.

Table 3 shows the average soluble protein nitrogen contained by the different parts of each variety at each sampling date. There was a gradual increase in soluble protein in the crowns, leaves and whole plants of all varieties from November 18 to January 20, when a maximum in cold resistance

Table 3. Average mg of soluble protein nitrogen per gm fresh tissue of each plant part of each variety at each sampling date.

Samp. Date	Minturki			Pawnee			Ponca		
	Leaves	Crowns	Plants	Leaves	Crowns	Plants	Leaves	Crowns	Plants
11-18	2.2	1.1	1.2	2.2	0.8	1.9	2.0	0.8	1.7
11-28	2.7	1.9	2.5	2.5	1.6	2.3	2.1	0.9	1.8
12-8	3.1	2.1	2.9	2.6	1.9	2.4	0.5	1.3	2.2
12-19	3.3	2.1	3.0	3.4	1.9	3.0	2.2	1.4	2.0
12-30	3.8	2.9	3.6	3.3	2.1	3.0	3.0	1.8	2.6
1-9	4.2	2.9	3.9	3.5	2.2	3.2	3.0	2.3	2.8
1-20	4.5	3.0	3.9	3.5	2.7	3.2	2.7	2.3	2.6
2-2	4.1	2.6	3.6	3.3	2.1	2.9	2.3	2.2	2.3
2-12	3.4	2.1	3.0	2.8	2.1	2.6	2.2	1.3	1.9
2-23	3.1	2.7	3.0	2.8	1.8	2.5	3.0	1.5	1.9
3-11	3.0	2.2	2.7	2.5	1.7	2.2	2.5	1.5	2.1
3-20	3.4	0.9	2.7	3.0	0.7	2.4	2.9	0.7	2.2

was reached for all three varieties. After January 20, a rather gradual decline in soluble protein nitrogen content occurred in all parts of all varieties, but it was not as marked as was the increase during the time of hardening. Considerably lower values were found for crown tissue on March 20, at which time much of the soluble protein may have already been used for the resumption of growth.

More soluble protein nitrogen was found in the parts of Minturki at maximum hardiness than in Pawnee or Ponca. Pawnee plant parts yielded

more soluble protein nitrogen at maximum hardiness than did Ponca. The hardier variety seemed to have the ability to synthesize more soluble protein nitrogen during hardening than did the less hardy varieties. In the less hardy varieties, soluble protein content seemed to fluctuate considerably more in the crowns than in the leaves or whole plants during hardening and dehardening.

Average soluble non-protein nitrogen content for each plant part of each variety at each date is given in Table 4. There was a rather gradual increase in the parts of Pawnee and Ponca from November 18, but the increase extended to February 2 which was beyond the date at which maximum resistance was reached. Soluble non-protein nitrogen did not fluctuate so noticeably in the plant part of Minturki as it did in Pawnee and Ponca. Leaf tissue generally seemed to show more variation than did crown tissue, whereas with soluble protein nitrogen crown tissue seemed to show more variation between dates.

Table 4. Average mg of soluble non-protein nitrogen per gm fresh tissue of each plant part of each variety at each sampling date.

Samp.	Minturki			Pawnee			Ponca		
Date	Leaves	Crowns	Plants	Leaves	Crowns	Plants	Leaves	Crowns	Plants
11-18	2.3	3.5	0.7	1.5	3.1	1.8	1.6	3.0	1.9
11-28	1.5	2.5	2.5	1.6	3.4	2.1	1.7	2.9	2.0
12-8	1.8	2.8	4.0	1.6	2.4	1.8	0.3	2.6	1.7
12-19	3.0	3.9	3.1	2.9	3.9	2.9	3.1	3.0	3.0
12-30	2.7	3.8	3.0	2.6	2.7	2.6	2.3	2.5	2.3
1-9	3.4	3.5	3.4	3.2	3.4	3.3	3.5	3.5	3.5
1-20	3.0	2.5	2.8	4.4	2.8	3.8	1.6	2.9	2.0
2-2	3.5	2.1	3.0	6.4	4.0	5.7	3.0	2.2	2.7
2-12	3.1	3.3	3.2	3.8	3.1	3.7	3.6	2.5	3.3
2-23	3.6	4.4	3.8	4.8	4.7	4.7	4.5	3.7	4.2
3-11	3.6	3.7	3.7	2.8	4.9	3.5	2.5	4.8	3.4
3-20	1.4	2.1	1.6	1.7	2.2	1.8	1.8	2.6	2.0

According to the analysis of variance on nitrogen studies, there was a highly significant difference at the .01 level among dates, varieties and nitrogen fractions. Means are shown in Table 5. There was a highly significant difference between the mean of total nitrogen and all other

Table 5. Average mg nitrogen in each fraction, in each variety, and at each sampling date.

Nitrogen fractions		Varieties		Dates	
Total nitrogen	8.6	Minturki	4.6	11-18	3.3
				11-28	3.9
Non-soluble nitrogen	3.3	Pawnee	4.5	12-8	3.6
				12-19	4.6
Soluble protein nitrogen	2.4	Ponca	3.8	12-30	4.2
Soluble non-protein nitrogen	2.9			1-9	4.8
				1-20	4.9
				2-2	5.1
				2-12	4.4
				2-23	5.2
				3-11	4.6
				3-20	3.3
L.S.D.	0.45		0.39		0.78

nitrogen fractions. Both non-soluble protein nitrogen and soluble non-protein nitrogen were significantly greater than soluble protein nitrogen. Significant differences were found between Minturki and Ponca and between Pawnee and Ponca.

Correlations. The nitrogen fractions of each variety were individually and collectively correlated with the degree of hardiness for that variety. The correlation coefficients are given in Table 6. No significant correlations were obtained for non-soluble protein nitrogen and soluble non-protein

Table 6. Correlation coefficients of each nitrogen fraction of each part of each variety with cold resistance.

Part and nitrogen fraction	Minturki	Pawnee	Ponca	All vars. pooled
Leaves				
Non-sol. prot. N.	+0.488	-0.074	-0.266	-0.251
Sol. prot. N.	-0.741**	-0.543	-0.210	-0.495**
Sol. non-prot. N.	-0.382	-0.275	-0.067	-0.209
Total N.	-0.727**	-0.463	-0.027	-0.389**
Crowns				
Non-sol. prot. N	+0.250	+0.030	-0.063	-0.041
Sol. prot. N.	-0.742**	-0.794**	-0.705**	-0.760**
Sol. non-prot. N.	+0.025	+0.056	+0.418	+0.141
Total N.	-0.304	-0.190	+0.067	-0.253
Plants				
Non-sol. prot. N.	+0.104	+0.045	-0.130	-0.133
Sol. prot. N.	-0.676*	-0.704*	-0.560	-0.617**
Sol. non-prot. N.	-0.297	-0.214	+0.029	-0.142
Total N.	-0.530	-0.291	-0.191	-0.553**

**Significant at the .01 level.
*Significant at the .05 level.

nitrogen. It is apparent that there is no linear relationship between these two fractions and cold hardiness with the varieties of winter wheat used in this study. Total nitrogen was significantly correlated with cold hardiness in Minturki leaves and with pooled data of all varieties. Non-significant correlations were obtained for all other fractions in all other plant parts both between and for all varieties. Increases in total nitrogen were associated with hardiness only in Minturki leaves. Apparently total nitrogen plays no part in cold hardiness of these varieties of winter wheat.

Significant negative correlation coefficients were obtained for soluble protein nitrogen in all the parts of Minturki. Significant negative correlation coefficients were obtained for Pawnee crowns and plants, but

in Ponca, only crowns gave significant negative correlation coefficients. Since a high degree of association existed with soluble protein and cold resistance in all parts of Minturki, and since Minturki is a variety that possesses cold resistance to a high degree, it seems conceivable that soluble protein content might be used as a factor in determining the amount of cold resistance in the fall and winter months.

Salmon (1933) found that with winter cereals, survival after exposure to severe cold was dependent solely upon the condition of the crown. Where the crown was in good condition after exposure to severe cold the plants usually recuperated fully, even though the leaf tissue was severely damaged. Pauli (1952) in his study of recurrent freezes on Pawnee wheat, found a high degree of association between percent of top growth killed and plant mortality. Injury occurring to the crown during exposure to low temperatures affected greatly the chances for the wheat plant to recuperate. Although some plants showed little leaf damage after exposure to severe cold, they often died after a few days because of severe crown injury.

It is interesting to note that there was a rather high degree of association between soluble protein nitrogen and cold resistance in the crowns of all three varieties. From these results it appears that soluble protein is essential for the survival of plant tissue exposed to severe cold, and that it is associated with the development of cold resistance in winter wheat. Ponca, the least resistant variety studied suffered severe leaf damage from severe cold occurring in late December and early January. Considerable leaf damage occurred to Pawnee, although not as severe as to Ponca. The leaves of Minturki showed the least damage by severe cold during this period. Apparently the more cold resistant variety had the ability

to synthesize more soluble protein during hardening and was able to distribute it more uniformly throughout the plant.

It is fortunate, however, that the soluble protein synthesized within the less hardy varieties is stored in the crowns rather than in the leaves. If the crown remains alive during the winter, the plant will shoot forth new leaves with the resumption of warm temperatures and other favorable growing conditions to complete its normal life cycle.

Newton (1924) found that the quantity of hydrophilic colloids in the press-juice of leaf tissue tended to be directly proportional to hardiness, and that in hardy varieties it fluctuated less with changes in weather conditions. Since proteins are colloidal in nature, it seems conceivable that they play an important role in the colloidal behavior of the cell. Colloids have the ability to attract water molecules to their electrically charged surfaces, thereby acquiring a "water shell". As more water is attracted to the colloids, less free water is available within the cell to freeze and cause injury by distortion of the protoplasm when the cell is exposed to severe freezing temperatures. Hardened cells with their greater quantity of soluble protein may have considerably more colloidal surface area to attract water molecules. This may be due to the protein molecules of soluble protein being considerably smaller than the molecules of non-soluble protein.

Since soluble protein content is related with cold hardiness in winter wheat, important questions come to mind. What is the mechanism responsible for this increase in soluble protein? Is the soluble protein synthesized from amino acids and peptides? Is the soluble protein a product from the hydrolysis of higher molecular weight insoluble proteins? Do proteolytic

enzymes play a role in the increase in soluble protein? All of these questions remain unanswered and warrant the need for further research involving the role of nitrogenous substances during the development of cold resistance in plants.

Total Sugar Studies

A study was made of the changes in total sugar during cold hardening with the same varieties of winter wheat used for nitrogen studies. Total sugar in the leaves, crowns and whole plants was first calculated as mgs of total sugar per gram of oven dried tissue, and the values then computed on a percentage basis.

The average percent total sugar for individual parts of each variety at each date is given in Table 7. There was a marked increase in total sugar in the parts of all varieties during hardening, with the highest percentage occurring on January 20 in Minturki and Ponca and on February 2 in

Table 7. Average percent total sugar of fresh tissue of each plant part of each variety at each sampling date.

Samp.	Minturki			Pawnee			Ponca		
Date	Leaves	Crowns	Plants	Leaves	Crowns	Plants	Leaves	Crowns	Plants
11-18	7.5	23.7	11.2	7.6	31.9	12.3	7.8	22.9	11.5
11-28	10.9	34.8	16.3	8.5	20.9	11.5	10.2	25.7	13.9
12-8	9.6	24.8	13.0	11.5	25.9	14.2	11.6	30.5	15.6
12-19	13.7	25.8	16.3	14.5	33.1	18.8	13.2	36.8	18.0
12-30	13.5	24.4	16.2	13.7	29.2	17.2	13.8	32.0	17.0
1-9	11.9	43.0	18.4	10.3	24.8	13.2	11.8	36.5	15.9
1-20	18.8	47.2	27.8	14.5	32.5	18.8	16.4	34.0	22.1
2-2	13.5	31.2	18.2	16.9	28.2	19.8	14.4	30.3	20.0
2-12	15.9	34.2	22.1	17.7	19.5	17.8	13.3	23.7	15.4
2-23	14.6	34.4	18.9	28.3	22.7	19.8	13.7	23.0	15.9
3-11	13.5	30.4	17.8	12.5	24.2	15.8	13.8	25.0	17.3
3-20	6.8	25.6	10.6	9.9	25.8	13.6	10.0	25.6	14.7

L.S.D. between any 2 figures = 7.3

Pawnee. By January 20 all varieties had achieved maximum resistance to cold and the trends in percent total sugar for the individual parts of the individual varieties seemed to coincide fairly well with hardening trends.

The analysis of variance of the total sugar data gave a significant "F" at the .05 level for the varieties by dates and parts interaction indicating that there were differences between the total sugar percentages of different parts of different varieties at different dates. The L.S.D. for this interaction was 7.3 percent. Minturki, the hardiest variety, contained considerably more total sugar in its crown tissue than did the less hardy varieties. Differences in percent total sugar of the leaves or plants between varieties at maximum cold resistance were not significant.

There was a gradual decrease in total sugar in all parts of all varieties except Pawnee leaves after January 20. The decrease in total sugar seemed to somewhat coincide with the decline in cold resistance. The erratic behavior of Pawnee leaves is difficult to explain.

Correlations. Correlation coefficients between the percent total sugar of the parts of each individual variety and for all varieties with the degree of hardiness were determined and are given in Table 8. All parts of Pawnee, leaves and plants of Ponca, and Minturki crowns yielded non-significant correlations. On the other hand, Minturki leaves and plants and Ponca crowns gave significant negative correlations.

It is difficult to explain the diversity in correlations between parts within a variety and between varieties. From the results of other workers, the sugar content of crowns of all varieties might have been expected to be closely associated with winter hardiness. It is possible that Ponca crowns were dependent upon high total sugar content as well as high soluble

Table 8. Total sugar correlation coefficients of individual parts of each variety with the hardiness of the variety involved.

Variety	Parts		
	Leaves	Crowns	Plants
Minturki	-0.661*	-0.537	-0.645*
Pawnee	-0.271	-0.063	-0.330
Ponca	-0.538	-0.719**	-0.560
All vars. pooled	-0.449*	-0.480*	-0.519**

**Significant at the .01 level.
*Significant at the .05 level.

protein content for survival upon exposure to severe cold, while Minturki and Pawnee crowns were dependent only on a high soluble protein content when exposed to similar conditions. This is a puzzling question and cannot be answered by the data obtained in this study. However, Kneen and Blish (1941) felt that the survival of the wheat plant was entirely dependent upon the ability of the crown to resist frost injury. Since total sugar in Minturki and Pawnee crowns did not give significant correlations with cold resistance, it would seem reasonable to believe that other factors associated with the crowns of the more cold resistant varieties, perhaps soluble protein, might have been more responsible for their survival during the winter months.

Although there was a considerable difference in the correlations between the parts of individual varieties, significant negative correlations were obtained when the data of individual parts of all varieties were correlated with pooled freezing data. As with the correlation for nitrogen fractions, pooling the data gave more degrees of freedom which made it easier to detect a significant correlation. Again this is misleading, for it does not display the

behavior of total sugars with cold resistance of the individual varieties.

Differences in correlations (both of nitrogen fractions and carbohydrates) could indicate differences in the inheritance of winter hardiness in winter wheat. Worzella (1935), with studies of cold resistance in winter wheat and preliminary studies on the technique of artificial freezing, stated that cold resistance is inherited in the same manner as other quantitative characters. The theory of quantitative characters could possibly apply to the synthesis of more soluble protein during hardening. It could very well be that the more hardy variety possesses more genes responsible for the synthesis of soluble protein content during hardening than the less hardy varieties. The same could be true with sugars. Such genes could influence the production of enzymes responsible for the hydrolysis of proteins and starch during hardening to soluble proteins and soluble carbohydrates.

If it were true that the mechanism of hardiness in wheat is dependent upon these characters they might be used by the plant breeder as a basis for the selection of varieties and strains in breeding for winter hardiness. Considerably more research must be conducted to more fully understand the complexity of biochemical genetics involved with the hardiness of winter wheat.

Optical Density Studies with Diluted Cell Sap

Newton (1924) found that the moisture content of hardened tissue on winter wheat tended to be inversely proportional to hardiness, and the quantity of hydrophilic colloids contained in the press-juice to be directly proportional to hardiness.

Martin (1927) stated that when growth was retarded by low temperature,

the cell sap concentration increased due to the formation of sugars from starch and, after freezing, to the splitting of proteins to amino acids as reported by Newton. He further stated that with an increase in solids the freezing point of the juice was lowered, resulting in a possible decrease in ice crystal formation within the cell to protect the protoplasm from precipitating. Martin also mentioned the fact that at low temperatures, hardy varieties formed more sugar resulting in an increase in the concentration of the cell sap.

In this study an attempt was made to compare changes in the density of the diluted cell sap from the leaves, crowns and whole plants of wheat with trends in cold hardening and dehardening. It was thought possible that a trend of increased density might be noted with hardening and decreased density with dehardening. No direct quantitative measurements of specific compounds were actually made. Instead, the determination included the colloids and other solute particles, such as sugars and salts. Readings of all parts were expressed on the basis of optical density per gram fresh tissue.

The mean optical density values for each part of each variety at each date are given in Table 9. A considerable amount of variation between parts, varieties, and dates resulted in a highly significant varieties by dates and parts interaction when the data were analyzed statistically. The L.S.D. for comparison of any two means was 0.071. No trend was shown with hardening nor dehardening with any of the varieties studied.

Correlations. The optical density readings for each part of each variety were individually correlated with the degree of hardiness expressed as the temperature required to kill 50 percent of the plants of that variety.

Table 9. Average optical density per gm fresh tissue of each part of each variety at each sampling date.

Samp.	Minturki			Pawnee			Ponca		
Date	Leaves	Crowns	Plants	Leaves	Crowns	Plants	Leaves	Crowns	Plants
11-18	.133	.170	.113	.151	.147	.162	.114	.125	.157
11-28	.208	.229	.299	.192	.218	.253	.171	.199	.217
12-8	.149	.159	.238	.144	.155	.205	.133	.160	.223
12-19	.378	.275	.331	.356	.229	.405	.311	.183	.283
12-30	.297	.164	.254	.249	.180	.206	.206	.129	.213
1-9	.302	.173	.244	.213	.156	.239	.211	.156	.124
1-20	.183	.095	.223	.203	.103	.166	.134	.066	.215
2-2	.178	.190	.235	.221	.226	.202	.183	.113	.203
2-12	.237	.219	.163	.268	.245	.259	.191	.177	.245
2-23	.249	.234	.225	.288	.231	.220	.254	.193	.194
3-11	.208	.165	.206	.208	.169	.235	.194	.136	.216
3-20	.140	.032	.179	.144	.020	.137	.137	.024	.123

L.S.D. between any 2 figures = .071.

The correlation coefficients obtained are shown in Table 10. Non-significant negative correlations were obtained for the parts of all varieties; however, when the data from all varieties were collectively correlated with the degree of hardiness for all varieties, significant negative correlation coefficients were obtained. Again this likely is due to the greater number of degrees of freedom resulting from pooling data from all varieties.

From this study nothing definite was learned with regard to solute change or colloidal changes. With leaves and whole plants, optical density readings of the diluted cell sap could have been affected by the chlorophyll concentration changing from one sampling date to the next either with or between varieties depending upon the extent of injury received by exposure to severe cold. This would cause wide fluctuations in optical density readings of the cell sap of leaves and plants. In addition to changes in chlorophyll concentration of leaves and whole plants, many other factors

could affect the density of the diluted cell sap of any individual plant part. Colloidal content could vary independently from colloidal pigment content during the hardening and dehardening of any plant part to cause extreme fluctuations in optical density readings. From the results obtained in these studies, it becomes apparent that optical density of the diluted cell sap of any plant part of any variety was not related to hardening.

Table 10. Optical density correlation coefficients of individual parts of each variety with the hardiness of the variety involved.

Variety	Parts		
	Leaves	Crowns	Plants
Minturki	-0.406	-0.284	-0.442
Pawnee	-0.346	-0.432	-0.323
Ponca	-0.270	-0.243	-0.347
All vars. pooled	-0.369*	-0.356*	-0.381*

*Significant at the .05 level.

SUMMARY

Determinations were made of nitrogenous constituents, total sugars, and optical density of diluted cell sap in leaves, crowns and whole plants of Minturki, Pawnee and Ponca wheat from November 18 to March 20.

The degree of hardiness possessed by each variety at a given sampling date was determined by the temperature required to kill 50 percent of the plants when exposed to cold for 16 hours.

Nitrogenous constituents studied consisted of non-soluble protein nitrogen, soluble protein nitrogen, soluble non-protein nitrogen and total nitrogen. The change in each nitrogen fraction of each part of each variety

during hardening and dehardening was correlated with the temperature required to kill 50 percent of the plants of that variety.

No significant correlation was found between non-soluble protein nitrogen or soluble non-protein nitrogen and cold hardiness, indicating no relationship between these two fractions and winter hardiness.

Total nitrogen was significantly correlated with hardiness of Minturki leaves. Total nitrogen in other individual parts of Minturki and all individual parts of Pawnee and Ponca was not significantly correlated with cold resistance.

Of the three varieties studied, only Minturki gave a significant correlation with soluble protein and cold hardiness in all three plant parts. Pawnee gave significant correlations with crowns and whole plants, while Ponca gave significant correlations with crowns only. The hardy variety showed a greater change in soluble protein in all parts during hardening and dehardening than did the less hardy varieties. When pooled data of nitrogen fractions of individual parts of all varieties were correlated with cold resistance, no significant correlation was obtained with non-soluble protein or soluble non-protein nitrogen of any of the plant parts. Crowns did not give a significant correlation between total nitrogen and cold resistance. Total nitrogen gave significant correlations with leaves and whole plants and soluble protein gave significant correlations with all parts. Apparently soluble protein content in living tissue of the wheat plant, particularly in crown tissue, is related to cold resistance.

The synthesis of soluble protein in the more hardy variety of winter wheat during hardening was more pronounced than in the relatively unhardy variety. Inheritance of cold resistance, then, might possibly be correlated with quantitative characters responsible for the synthesis of soluble

protein. A knowledge of the quantitative changes in soluble protein of a variety or selection of winter wheat during hardening might serve as a basis for selection in a breeding program to supplement results obtained by freezing tests.

The role of soluble protein during hardening is not well understood. It is believed that the proteins are largely responsible for the colloidal nature of protoplasm and as the concentration of protein molecules increases, either as a result of hydrolysis or synthesis from amino acids and peptides, more surface area is provided for the attraction of free water. Binding of free water by the protein colloids can be largely responsible for the prevention of intracellular freezing which is lethal to the cells when the tissue of the wheat plant is exposed to severe cold during the winter months.

The mechanism responsible for the increase in soluble protein during hardening may be enzymatic, controlled by inheritance. A further study is needed to more fully understand the role of nitrogenous constituents during hardening in winter wheat and other crop plants.

Changes in the total sugar in the part of each variety were correlated with the degree of hardiness possessed by the variety concerned. Minturki leaves and plants and Ponca crowns were the only parts giving significant correlations between total sugar and cold resistance. The fact that Ponca crowns gave a highly significant correlation here may demonstrate the dependence of cold resistance in the less hardy variety upon both soluble protein and soluble carbohydrates.

Highly significant correlations were obtained for all parts when all varieties were collectively correlated with cold resistance. This may be due largely to a greater number of degrees of freedom resulting from the pooling of the data.

Correlation coefficients of the optical density of diluted cell sap of individual parts of each variety with cold resistance were not significant. However, when the optical density data of all varieties were pooled and correlated with the pooled freezing data, significant correlations resulted. Greater numbers of degrees of freedom resulting from pooling data from individual varieties probably was a factor.

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COLD RESISTANCE IN THREE VARIETIES OF WINTER WHEAT AS RELATED TO NITROGEN FRACTIONS, TOTAL SUGAR AND OPTICAL DENSITY OF DILUTED CELL SAP

by

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Minturki, Pawnee and Ponca winter wheats were subjected to freezing tests for three consecutive days, at intervals of ten days from November 18 to March 20 to determine the degree of cold hardiness (temperatures required to obtain a 50 percent kill) of each variety. Crowns, leaves and whole plants minus roots of each variety were analyzed for nitrogenous constituents and total sugar. During the time the freezing tests were conducted determinations were also made of the optical density of diluted cell sap of each part of each variety. Results of all determinations were compared with the degree of winter hardiness.

Results of freezing tests were in accordance with those obtained from field tests showing Minturki as being the most cold resistant variety while Ponca was the least cold resistant variety, and Pawnee intermediate.

Nitrogenous constituents analyzed consisted of non-soluble protein nitrogen, water soluble protein nitrogen, water soluble non-protein nitrogen and total nitrogen. No significant correlations were obtained between cold resistance and non-soluble protein nitrogen or soluble non-protein nitrogen of leaves, crowns and whole plants of each variety. These two fractions apparently have no relation to winter hardiness in winter wheat. Total nitrogen gave a significant correlation with winter hardiness only with Minturki leaves.

Soluble protein nitrogen of all parts of Minturki gave significant correlations with winter hardiness. Crowns and whole plants of Pawnee and crowns of Ponca also yielded significant correlations between soluble protein and winter hardiness. These results indicate that changes in soluble protein nitrogen content, particularly in wheat crowns, are associated with changes in cold resistance. The more hardy variety also produced more

soluble protein within the plant with distribution in all parts, while the less hardy varieties produced less soluble protein within the plant and concentrated the quantity mainly in the crowns.

Pooled soluble protein data of each part of all varieties were significantly correlated with pooled freezing data.

The role of soluble protein in preventing freezing injury to the cells of cold hardened tissue is not well understood. It is believed that soluble protein is produced by enzymatic hydrolysis of non-soluble protein as a result of enzymes controlled by inheritance associated with winter hardiness. Since proteins are colloids, they have a tremendous ability to absorb water molecules to their molecular surface. Hydrolysis of non-soluble protein to a greater number of smaller protein molecules may result in a greater total surface area for adsorption of water molecules to their molecular surface. Less free water might then remain within the cell to cause intracellular freezing and consequent death to the cell. A more intensive study of the role of soluble protein during cold hardening is needed.

Significant correlations between total sugar and cold resistance were obtained only with Minturki leaves and whole plants and Ponca crowns. Pooling the data from all varieties and correlating it with pooled freezing data gave significant correlations with all parts.

Since Ponca crowns yielded significant correlations with both total sugar and soluble protein, it may be that the tissue of the less hardy variety is dependent upon both high soluble protein content and high total sugar content for survival against severe cold, whereas tissue of the more hardy variety may be dependent mainly upon high soluble protein content for

survival against severe cold.

Since the quantity of soluble protein possessed by a hardened wheat plant is related to its cold resistance, it is possible that the ability to synthesize soluble protein during hardening is quantitatively inherited. Knowledge of the soluble protein content of varieties or selections during hardening and dehardening, might be used as a criterion for selection in a breeding program along with results obtained from freezing tests.

No significant correlations were obtained between optical density of cell sap and winter hardiness with the parts of any variety. Apparently the optical density of diluted cell sap is not related to cold hardiness. The inconsistency of the optical density of the diluted cell sap may be dependent upon the concentration of colloids, sugars and salts changing differently at different times during hardening and dehardening. Greater numbers of degrees of freedom resulting from pooling the data were influential in producing significant correlation coefficients when all varieties were considered as a unit.