THE RELATIVE LOSS OF HARDINESS IN WINTER CEREALS WHEN SUBJECTED TO WARM TEMPERATURES DURING WINTER AND EARLY SPRING

by

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B. S., Kansas State College of Agriculture
and Applied Science, 1932

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1934

TABLE OF CONTENTS

INTRODUCTION

Most of the research on cold resistance has dealt with hardened plants and the increase in hardiness while little consideration has been given to the decrease or loss of hardiness in plants.

Observation and the experience of farmers have shown that winterkilling of wheat in many cases is not the result of continued cold during midwinter when the plants are well hardened, but occurs with even moderately low temperatures following several days of temperatures that are favorable for growth during which the wheat loses hardiness.

An examination of the weather records shows that a minimum temperature of 10° F. or lower, following periods of five days or longer during which the mean temperature was above 40° F. every day, occurred in February or March at Manhattan and Hutchinson in 1920, 1922, 1932, 1933 and 1934 and at Garden City, Hays, and Colby in 1920, 1922, 1927, 1931, 1932, 1933 and 1934. Such changes in temperature do not always injure wheat especially if covered by snow. However, minimum temperatures even higher than 10° F. have been observed to cause serious injury to wheat that has been stimulated to active growth.

It is of considerable importance to know the relative resistance of varieties of crops at various stages of spring growth. It is also of value to know the rate at which hardiness is lost by different crops when influenced by conditions that stimulate growth. Cases have been reported where winter wheat was injured more severely than oats by low temperatures in the early spring in southeastern Kansas.

This problem deals with the relative cold resistance of winter varieties of rye, wheat, oats, and barley at different stages of hardiness, the rate of loss of hardiness in the different crops, and some of the factors which influence their cold resistance.

REVIEW OF LITERATURE

Many authors, including Akerman (3), Chandler (6), Harvey (14), Newton (27), Maximov (25), Salmon (37), Weigand (46), and others, have presented thorough reviews of papers on winter-hardiness, thus the literature reviewed here will relate principally to methods of studying the resistance of plant tissue to low temperatures and to the decrease of winter-hardiness or loss of resistance to cold in cereals.

Literature on Winter-hardiness in General

De Buffon and Du Hamel presented the first theory on ice formation and plant death. They assumed that expansion due to ice formation burst the cells causing them to come together forming large masses of ice. In 1830, Goeppert pointed out that ice was formed in the intercellular spaces instead of in the cells. This was verified by Sachs in 1860 when he pointed out that the expansion of the ice would not be sufficient to rupture the cells. Since 1860 many investigators have observed that ice rarely forms in the cells.

According to Weigand (46) hardy tissue upon thawing draws water back into its cells while less hardy tissue is not capable of regaining the water and thus death of the cells results. He concludes that death is due to the actual withdrawal of water and not to cold. Abbe (1) and Akerman (3) have reported that rapid thawing is not so detrimental when evaporation takes place slowly.

Harvey (14) found the moisture content of wheat decreasing during the fall months due to rapid evaporation of water from thawing plants. This process tends to leave the cell sap more concentrated as the season progresses.

Martin (22) called attention to the fact that when wheats are growing actively in the fall or spring the hardy varieties have a lower moisture content and a juice with a higher percentage of total solids than the non-hardy varieties.

Akerman (3) concluded on the basis of his data that low temperature in itself may cause injury, that is, living cells have a certain minimum temperature to which they can be subjected and live. Dessication of cells by freezing temperatures is a well established fact. With increasing cold the water must be withdrawn or ice will form in the cells. Not only the loss of water but the coagulating of plasmic colloids is important in winterkilling.

Maximov (25) assumes that the basis of frost resistance lies in the capacity of the protoplasm to withstand the dehydrating influence of a direct or indirect deprivation of water. This theory has been upheld by many authors since 1880 when first presented by Muller-Thurgan. Maximov (25) considers that the death of cells is due to the mechanical injury to the protoplasm caused by the compression of the cells by ice crystals which accumulate in the intercellular spaces.

The work of Janssen (19) shows that rapid changes take place in the protein molecule due to freezing. The more hardy dates of seeding seem to have a greater capacity to change the nitrogenous material into more simple and soluble forms which are less rapidly precipitated by cold.

Newton (29) and Harvey (14) conclude that as water is withdrawn death is caused by the chemical influence of the increased concentration of salts and acids on the colloids of the protoplasm.

Newton and Brown (31) investigating seasonal changes in composition of wheat plants show that the more hardy varieties, in general, contain less moisture during the dormant season. They conclude that one of the most important changes in quantitative relations of the various plant constituents during the hardening process is the reduction in moisture content. The resulting concentration of hydrophilic colloids and sugars in the cell fluid increases the resistance to freezing.

According to Jumelle as referred to by Abbe (1), plants cease respiring at a temperature of -40° C., but in the presence of light they can assimilate carbon dioxide. Thus the so-called sleep or vegetative repose is due to the dessication of the plant and not to cold.

Akerman (2) reports that a close correlation exists between sugar content and hardiness, in such a way that plants which are more resistant to frost contain more sugar than less resistant plants.

Martin (22) found hardy varieties of rye and wheat to have lower rates of respiration than non-hardy varieties when subjected to low temperatures. The hardy varieties thus retain their sugars longer or to a greater extent.

Dexter (8) concluded on the basis of electrical conductivity of water into which injured tissue had exosmosed its mineral matter, that hardening of plants is favored by conditions which promote photosynthesis and lessen respiration and vegetative growth.

Baroulina (5) found a direct correlation between cold resistance and osmotic pressure. He cited rye as being more cold resistant than wheat in spite of its lower osmotic pressure.

Salmon (37) subjected winter rye, winter wheat, winter barley and winter oats to artificial refrigeration and found them to rank in hardiness in the order named. Dakold rye was the most hardy cereal and the winter barley was as hardy as the least hardy wheats. Rosen rye appeared to rank close to Kanred wheat in hardiness.

Hill and Salmon (18) report that "plants grown in a dry soil were injured more by artificial freezing than plants grown in a wet soil, due to the high specific heat of water which in a wet soil prevents a rapid change of temperature while plants in a dry soil are exposed to a lower temperature than those in a wet soil."

Klages (20) found that moist soils gave a greater plant survival, especially during prolonged low temperatures. Low soil moisture gave protection during the first part of the exposure due to the retardation of the life processes but when killing did start it progressed more rapidly than on the moist soils.

Harvey (15) working with cabbages found that subjecting them to alternate temperatures (0° C. and 12° C. or 20° C.) for 12 hour periods caused them to acquire more hardiness than when subjected to an average of these temperatures. He suggests that hardiness in plants is a cold shock response.

Weigand (46) points out why resistance to cold is an accumulative process. Colloidal substances dry out more slowly after some water is lost due to the higher concentration. Forces of imbibition will increase in plant tissue as water is lost. The concentration of materials other than colloids increase with the loss of water. All of these factors tend to exert an increasingly strong force against dehydration.

Literature Related to Determination of Winter-hardiness

All the theories of winter-hardiness are built primarily around the idea of the water-relations in the plant. The structural, osmotic, or colloidal conditions as relate to protection against withdrawal of water or formation of ice, form the basis of explanations for individual or varietal differences in hardiness.

Several authors have attempted to define "bound water", but at present there is no universally accepted definition. Sayre (39) in defining bound water states that "all water that is not free water, that is, that does not show some of the common properties of liquid water, may be considered as bound water." From a more technical standpoint Newton and Gortner (33) assume that "bound water will not dissolve sucrose", that is, it is water held in such a way as to be unavailable for the solution of sugar. According to Sayre (39) "bound water does not exist in definite proportions relative to the solid material of the system, but as a ratio between bound water and free water."

Newton (29) studied the colloidal properties of winter wheat plants and worked out correlations between the imbibition pressure of fresh leaves, the quantity of hydrophilic colloids contained in the press juice, the volume of press juice from hardened leaves, and the winter-hardiness of the wheat. Newton defines "press juice" as "the fluid expressed from the tissues, with or without previous grinding." Newton (28) after showing a close correlation between hardiness and press juice concludes, "the hardier the variety the lower its moisture content and the greater the force with which it is retained."

Dexter (7) describes a modification of the Newton pressure method (27) in which the quantity of sap expressed is determined by electrical conductivity. Newton (27) has shown that the saps of various wheat varieties do not differ materially in electrical conductivity.

Tysdal (45) found that viscosity determinations gave a more reliable indication of hardiness than press juice and that moisture was fairly reliable but not as indicative as the viscosity reading.

Salmon and Fleming (38) found no relation between the cryoscopic value of the extracted sap of field grown winter cereals and their ability to resist winterkilling. However, greenhouse grown plants did show a definite relation between the freezing point of cell sap, turgidity of tissue, and their resistance to low temperatures.

Newton (27) found that all varieties of wheat with which he worked increased in amino nitrogen and water soluble nitrogen during hardening, which is in harmony with the evidence of Harvey (14) that splitting of the proteins is associated with the hardening process. In a more recent report Newton and Brown (32) concluded that dehydration is the basic cause of frost precipitation of proteins and that ice formation, acidity, salt concentration, and possibly pressure, are all contributing factors.

Dexter, Tottingham, and Graber (10) upon the assumption that dead tissue loses its capacity to regulate the diffusion of its soluble contents suggested that a correlation would exist between the degree of injury and the quantity of exosmosed plant fluids as measured by electrical conductivity. Heald (16) found plant juices to be good conductors due in a large part to dissolved mineral substances.

Dexter, Tottingham, and Graber (11) working with rye, oats, barley, and wheat have shown by means of the electrical conductivity method, that differences between the crops and different varieties within each crop, could be obtained which were closely correlated to the known hardiness.

Many attempts have been made by various authors to correlate such factors as sugars, osmotic pressure, freezing point depression, hydrophilic colloids, moisture, press juice, coagulable nitrogenous compounds, bound water, etc., with winter-hardiness in order to obtain a quick, reliable, and practical method of determining cold resistance of plants. However, up to the present time many authors, including Akerman (3), Salmon (37), Suneson (42), Martin (23), Steinmetz (41), Peltier and Tysdal (34), Lebedincev, Borodin and Brovcine (21), and Hill (17) consider artificial refrigeration as the most practical and reliable method of determining winter-hardiness in plants.

Literature on the Decrease of Hardiness

The question of loss of winter-hardiness or decrease of hardiness has been suggested by several authors.

Salmon (37) called attention to the rapid loss of hardiness in wheats when field grown material was taken into a greenhouse maintained at 50° to 55° F. A perceptible loss could be observed in 12 to 24 hours and in certain cases hardiness was retained for a period of 96 or even 120 hours.

Tumanov (44) and Suneson (42) working with hardened wheat plants found a definite loss of hardiness in a single day with plants maintained at greenhouse temperatures. Tumanov (44) reports that the rate of loss of hardiness is much greater than the rate of acquisition of hardiness. A warm period of only a few days will greatly decrease the ability of wheat to survive low temperatures. Suneson (42) found a marked change in the relative cold resistance of certain wheat varieties by comparing lots artificially frozen in November with those frozen in December.

Anderson and Kiesselbach (4) report that the increase of cold intensity in the field causes a like increase in hardiness of wheat plants and that a decrease in cold resistance may follow a few warm days in winter.

Harvey (14) concluded on the basis of artificial freezing of cabbages, that the hardiness acquired in one night may be lost the next day if the temperatures are warm. He found an accumulative effect in hardening only when the average temperature was low. Under controlled hardening he found a striking acquisition of hardiness in one to five days, and thus concluded that resistance to cold increased as rapidly as it was lost. It is likely that cabbages can not be compared to wheats in this respect, since a fully hardened condition in wheats is seldom attained before midwinter of normal years. When Newton and Anderson (30) found the rate of respiration under low temperature to be inversely proportional to winter-hardiness in wheat, they noted that the physiological activity of the plant responded very rapidly to an increase in temperature. Their data support the theory that varieties of wheat do not have a stable dormancy and thus are easily awakened into a frost susceptible condition by occasional warm periods during the winter.

Suneson and Peltier (43) noted marked changes occurring in the relative rank in hardiness of Blackhull, Kawvale, Nebraska No. 60, and Minhardi during the winter, and attributed these changes to hardening adjustments which probably were caused by temperature changes.

In a recent study Dexter (9) reported that hardiness was developed best when the environment tended to conserve and accumulate the organic food supply. Defoliation was found to decrease hardiness markedly, due to the after production of new leaves which used the organic food reserves. Dexter, by the use of electrical conductivity tests, found that the growth of new leaves or the killing of partially depleted tissues resulted in weakened and non-hardy plants which rapidly decreased in hardiness when moved into the greenhouse.

Govorov (13) after finding a correlation between winter hardiness of wheat (by field observations) and the content of glucose, suggested that iw was not sufficient to consider only the quantity of soluble sugars in a hardened plant but that one should note the changes in the quantity of sugar with changes in temperature. He placed hardened wheat plants under warm temperatures and found that spring varieties lowered their sugar content sharply (over 50% in five days), whereas winter varieties decreased their sugars very little, if any. Upon hardening, winter varieties increased their sugar content much more sharply than the spring varieties. Contrary to the general opinion at present, Govorov concluded that winter wheat plants enter a state of anabiosis and do not respond to an increase in temperature.

Rosa (35) working with vegetable plants proved that the hardening process in plants is accompanied by a marked increase in water retaining power and that hardy plants actually retain larger amounts of unfrozen water than the less hardy. He concluded that, "hardy plants possess the ability to initiate changes whereby the stability and water-retaining power of the protoplasm and consequently of hardiness are increased, while more tender species possess this ability to a very slight degree if at all."

MATERIALS AND METHODS

This study which deals with the loss of hardiness in winter cereals when subjected to periods of warm temperatures during winter and early spring, was conducted at the Kansas Agricultural Experiment Station in 1932-33 and 1933-34. Four methods were employed in attempting to determine the relative cold resistance of Dakold and Rosen rye, Kanred wheat, Sporen Gray Winter oats, and Tennessee Winter barley, after subjection to different periods of warm temperatures.

A quantitative measure of press juice as proposed by Newton (23) and Dexter (7), electrical conductance of exosmosed plant fluids from frozen tissue as suggested by Dexter, Tottingham, and Graber (10 and 11), total moisture, refraction of expressed juice, and artificial refrigeration which has been used by many investigators, were used in an effort to determine some of the reasons for cold resistance as well as to show the relationship of the four crops when subjected to such environmental changes as occur in fall, winter, and spring.

In 1932-33, 420 four-inch pots and 10 boxes (24"x12") were planted to each of the four crops. In 1933-34, 560 pots and 10 boxes (18"x18") of each crop were planted, along with 400 additional pots of both Kanred wheat and Tennessee Winter barley. Rosen rye was used in 1932-33, and Dakold rye, a more winter-hardy variety, was used in 1933-34. Rosen rye was found to be little or no more resistant than Kanred wheat and thus it was decided to obtain a strain more hardy than the wheat. The C. I. number, planting date and number of pots are given in Table I.

Table I. C. I. Number of Each Crop, Date of Planting, and Number of Pots Planted in the Years 1932-33 and 1933-34.

		1932-33		1933-34	
Crop	C.I. No.	No. pots	Date planted	No. pots	Date planted
Rosen rye	195	420	9-29	----	----
Dakold rye	242	---	----	560	9-28
Kanred wheat	5146	420	9-29	1000	9-27
Tennessee Winter barley	3543	420	9-29	1000	9-27
Sporen Gray Winter oats	2506	420	9-28	560	9-29

The material planted in 1932-33 was started in the greenhouse and after emergence was moved to a fenced enclosure east of the greenhouse where it was allowed to "harden" under natural conditions. In 1933-34 the material was moved from the greenhouse to the enclosure immediately after planting.

Eight to ten seeds were planted in each pot and later the plants were thinned to five plants per pot. Approximately 125 seeds were sown in each box both years.

The winters of 1932-33 and 1933-34 were unusually dry therefore, watering of the plants was necessary. The plants were watered only when the temperatures of the soil and air were approximately that of the water used, which was often enough to provide favorable moisture conditions at all times. When the plants were moved to the greenhouse the soil moisture was maintained near the optimum.

The pots and boxes were set in the enclosure in four groups according to crops. During short periods of severe weather the barley and oats were covered with muslin held above the plants by a frame of laths. It was thought that the covering might save the weaker crops from complete killing during extremely low temperatures, the occurrence of which were relatively few during the two years. The short periods during which the plants were covered probably had no influence on the experimental results.

Recording thermometers were used to obtain temperatures of the greenhouse and the fenced enclosure where the plants were allowed to harden.

The border effect which was noted in 1932-33 was prevented during the winter of 1933-34 by banking soil level with the tops of the pots around each group.

During 1932-33, the crops made about normal fall growth. In December the temperatures reached -14° F. which caused some injury, especially in the pots around the edges of each group. This low temperature increased the injury in the oats which already had appeared to a slight extent. Barley showed a slight leaf injury following this extremely low temperature while the wheat and rye were slightly tipped.

In 1933-34 all the crops made a heavier vegetative growth and entered the winter in a good condition for this study. The oats survived the winter in fine shape which very likely was due to the greater vegetative growth and a somewhat milder winter.

In order to obtain some field data on this problem replicated series of the crops were grown in the Kansas State College cereal-breeding nursery and winterkilling notes were taken.

The plants grown in the boxes were used in determining the quantity of expressed juice, the total moisture, the refractive index, and the electrical conductance of frozen tissue while the plants in the pots were subjected to artificial refrigeration.

Two boxes of each crop were used to provide a series of the samples. The first samples were taken immediately after the boxes were moved to the greenhouse. The tests were repeated at intervals of one to three days over a period of seven to 15 days as will be indicated later.

In 1932-33, leaves were used for expressed juice determinations and the crowns were used for electrical conductivity, while in 1933-34, leaves were used for both tests. In making the electrical conductivity test in 1932-33 the plant was pulled up, and washed free of soil. The surface water was absorbed with a cloth, the dead leaf sheaths removed, and the fibrous roots trimmed off. The crowns or stems used were approximately three-fourths of an inch long. Samples weighing two and one-half grams were placed on a cheesecloth rack to be frozen. The material was frozen for one hour at a definite temperature which will be stated for each experiment. After freezing, the tissue was allowed to thaw in test tubes before adding 20 cc. of conductivity water. The tubes were tightly stoppered and exosmosis allowed to proceed at 20° C. for two hours after which the solution was poured off the tissue. The electrical conductance of the solutions was read as soon as possible.

Resistance in ohms was measured by the Kohlrausch method which is a modification of the wheatstone-bridge method. An alternating current was sent through the solution which was in a cell containing two platinum electrodes. The resistance was balanced against a rheostat on a wheatstone-bridge, the point of equilibrium being determined by means of ear phones. A standard solution of potassium chloride was used for determining the cell constant which was used in calculating the specific conductivity.

The same procedure was followed in 1933-34 except that the samples were composed of uninjured leaf tissue instead of plant crowns, since samples of leaves were found to have less variability than crowns. The leaves were taken off close to the ligule, and cut into two parts to aid in rapid handling of the tissue. The samples were increased to three grams, which required the use of 30 cc. of conductivity water to make the proper concentration for the cell.

As previously stated samples for total moisture, electrical conductivity, indices of refraction, and expressed juice were taken at the same time of day for all the tests reported. The tests were made as quickly as possible and the material exposed only when necessary. Quart fruit jars provided the best means for holding the samples under constant humidity and temperature conditions while making the tests.

The method for determining the quantity of expressed juice was the same for both years during which this study was conducted. Duplicate three gram samples, wrapped in weighed filter paper (usually eight sheets, nine cm. in diameter) were subjected to a constant pressure of 1400 pounds per square inch (by use of a Laurie hydraulic press), for five minutes in 1932-33. The pressure was increased to 1666 pounds per square inch in 1933-34. The leaves were discarded and the filter papers which had absorbed the expressed plant juice were placed in weighing bottles and weighed. All weights were recorded in grams to the third decimal place, which was considered sufficiently accurate. Having the weight of the bottle and the weight of the filter papers in each sample the quantity of juice expressed could easily be calculated.

An Abbe-Zeiss refractometer was used to determine the refractive index of the plant juices. Duplicated, four gram samples of leaves were placed in a small press and five drops of juice expressed. The refractive index was determined at 15° C. for all the tests reported.

The sample for total moisture, collected at the same time as those taken for expressed juice and electrical conductance, was dried at 95° C. until a constant weight was attained.

The artificial refrigeration was accomplished by means of the carbon-dioxide direct expansion refrigeration machine described by Sellschop and Salmon (40). The pots were placed in boxes holding 16 pots each to facilitate handling them before and after freezing. The freezing chamber (10' x 4') held 80 pots or five of the boxes.

All freezing periods were twelve hours, beginning and ending at eight o'clock. Salmon (37) and Suneson (42) have shown that it requires nearly twelve hours for the temperature of the soil in a four inch pot to approach the temperature of the freezing chamber. Salmon (37) pointed out the advisability of thorough watering before freezing to prevent undue variations in injury due to the soil moisture content. This was carried out except in a few cases, when pots in which the soil was frozen solid were moved from outdoors directly into the refrigerator. These cases were of little consequence however, since the moisture content of the soil in the pots outdoors was kept relatively high by frequent watering.

Several hours are required to lower the air temperature of the freezing chamber to the minimum when a quantity of warm soil is put into it. In all instances the temperature reported for each freezing lot is the minimum temperature of the chamber. The variability of temperature control in the chamber was ±1° F.

Temperatures at which the different freezing lots were frozen varied considerably due to the wide differences in the degree of hardiness of the plants. Judging the temperatures that certain plants could stand was one of the most difficult problems throughout the study. Hardy plants might be frozen at a temperature as low as 4° F. while plants which had been kept in the greenhouse for 15 days were severely injured by temperatures of 20° to 22° F.

Changes in resistance to cold that are induced by favorable growth temperatures were measured by comparing plants that had grown outdoors and hardened under natural conditions with similar plants that had been in the greenhouse for varying lengths of time and had lost some of their hardiness. In some cases plants of the four crops were moved into the greenhouse at intervals of 24 or 48 hours and all tested at the same time, while in other cases a large unit of the four crops was moved in at one time and portions of it tested at intervals.

The former plan was used principally in 1932-33, while both plans were followed in 1933-34. The smallest unit in each freezing test consisted either of four or eight pots of each crop that had been treated alike.

When pots of the four crops were moved into the greenhouse at intervals and all frozen at the same time each freezing lot would include 20 pots of each crop. For example, four pots would be brought directly from outdoors to the refrigerator, four others would have been in the greenhouse one day, another group of four pots would have been in two days, another group three days, and still another four days, making five groups or 20 pots of each crop to be frozen at the same time.

The other plan was to move a large unit into the greenhouse at one time and test portions of it at intervals of one or three days. In such cases, 40 pots of each crop were moved into the greenhouse and divided into five portions of eight pots each. This provided material for five tests in which the four crops were compared directly. The first test which included eight pots of each of the four crops was made as soon as the plants were moved into the greenhouse and the others were made at intervals of one or three days as will be indicated in the data.

The method used in obtaining the percentage injury is the same as described by Salmon (37). It may be open to criticism since it is only an estimate based on the judgment of two people. However, the personal error of judgment is very small as was shown by Salmon (37), when independent estimates of injury were made on 230 pots of Kanred and 150 pots of Blackhull wheat. It was found that a probable error of the difference of not to exceed five per cent on single pots may be expected, due to random variation in making estimates. In this study the injury on several groups of material was re-estimated and the error found to be negligible.

The percentage injury will be used for the major portion of the data since the percentage survival, which is an actual count of surviving plants, is of more or less questionable value due to the occurrence of a phenomenon mentioned by Salmon (37) which also occurred in this experiment. Salmon (37) noted that plants may die as the result of a secondary effect which may be attributed to a physiological after-effect of injury to the roots. He pointed out that obvious and marked differences in injury sometimes occur without the death of plants, a fact also noted in this study.

A high correlation usually exists between the percentage survival and the percentage injury when plants survive normally.

The probable error was calculated for each freezing test by calculating the standard deviation from the original values. This method seemed desirable since it is well adapted to machine calculation. The probable error of the mean of comparable pots in a freezing test was determined by the equation:

$$\frac{.6745}{(N-1)} \cdot \sqrt{\frac{x^2}{N} - Mx^2}$$

in which x is the original value (percentage injury or percentage survival of a pot or other unit); N, the number of pots or units; and M, the mean of the values. The probable error of the mean is reported for each set of freezing data.

EXPERIMENTAL RESULTS

Results of 1932-33

The methods used in attacking this problem in 1932-33 differed materially from those used in 1933-34, therefore, it seems desirable that the experimental data be presented separately for the two years.

It was necessary to obtain accurate temperature records since the results throughout the experiment were closely associated with the temperatures to which the plants were subjected. The daily maximum, minimum, and mean outdoor temperatures are given in Table II and the weekly averages are presented in Table III.

The average maximum, minimum, and mean weekly temperatures for the greenhouse in which the plants were allowed to lose hardiness, are given in Table IV.

A brief summerization of the temperatures through the fall of 1932 indicates a gradual decrease of the mean temperature from October 1 to November 27 when it became fairly

Table II. Daily Maximum, Minimum and Mean* Temperatures for Manhattan, Kansas, Sept. 21, 1932 to Feb. 24, 1933

Date	September			October			November		
	Max.	Min.	Mean*	Max.	Min.	Mean*	Max.	Min.	Mean*
1				74	56	67	60	19	35
2				82	60	75	68	24	46
3				86	52	67	73	39	53
4				63	38	50	69	50	57
5				58	29	41	61	20	39
6				70	30	47	64	35	47
7				84	50	61	66	50	55
8				86	56	70	50	32	39
9				69	44	51	44	27	35
10				48	32	39	46	23	35
11				65	27	42	43+	26+	35
12				80	32	50	54+	20+	37
13				84	40	66	67+	22+	45
14				86	56	68	56+	28+	42
15				88	56	69	30+	17+	24
16				80	36	57	27+	5+	16
17				81	40	59	40	20	25
18				82	50	64	51	27	33
19				87	37	60	53	12	34
20				61	31	46	61	28	38
21	88	55	72	73	29	47	63	22	39
22	91	62	72	78	55	63	59	26	39
23	88	62	72	68	47	54	60	24	41
24	86	46	64	73	44	45	50	13	28
25	84	48	65	52	38	46	56	26	41
26	88	50	64	60	23	36	56	12	33
27	84	54	67	62	27	42	49	15	28
28	80	52	65	71	44	54	52	24	37
29	88	50	67	75	27	46	65+	38+	52
30	76	53	63	52	26	36	73+	23+	48
31				47	28	38			

Table II continued

Date	December Max.	December Min.	December Mean*	January Max.	January Min.	January Mean*	February Max.	February Min.	February Mean*
1	72	45	55	52	29	26	63	21	38
2	62	32	48	53	30	39	45	12	30
3	62	30	47	50	18	32	49	11	29
4	61	27	42	56	20	36	43	4	25
5	61	36	48	50	24	35	44	4	23
6	68	27	42	58	30	43	16	-6	1
7	40	5	20	59	24	41	10	-12	-3
8	24	10	14	56	30	43	18	-5	4
9	20	5	10	58	21	38	24	2	11
10	10	5	8	60	38	50	34	-2	15
11	17	8	13	60	15	35	52	20	30
12	18	-8	4	46	15	26	52	22	34
13	30	-7	14	50	16	32	26	12	18
14	32	6	21	50	14	31	40	7	22
15	39	4	18	58	38	48	50	32	40
16	20	-10	2	62	28	49	58	32	41
17	24	6	12	30	16	23	53	12	33
18	33	2	15	48	24	29	56	30	45
19	34	4	15	59	28	45	54	20	40
20	42	23	31	56	29	40	64	26	46
21	49	22	33	59	39	51	68	38	57
22	44+	24+	34	68	34	53	68	32	50
23	47	32	37	59	20	37	75	36	53
24	44	34	37	58	26	43	76	32	51
25	47	31	38	60+	23+	42			
26	46	21	32	48+	22+	35			
27	49	26	36	45+	21+	33			
28	44	18	30	42+	20+	31			
29	50	26	38	54+	33+	44			
30	39	27	33	57	15	36			
31	38+	15+	27	60	44	51			

* The mean daily temperatures were calculated by taking the time factor into consideration which prevents a sudden change in temperature of short duration from affecting the mean to a great extent.

\+ These data were taken from the official reports of climatological data at Manhattan, which usually varied not more than 2° from that recorded where the experimental plants were located.

Table III. Average of Daily Maximum, Minimum and Mean* Temperatures and Absolute Maximum and Minimum Temperatures for Weekly Periods, Sept. 30, 1932 to Feb. 24, 1933, Manhattan, Kansas

Week ending		Av.Max.	Av.Min.	Av.Mean*	Max. of week	Min. of week
September	30	83	50	65	88	46
October	7	74	45	58	86	29
	14	74	41	55	86	27
	21	79	40	57	88	29
	28	66	40	49	78	23
November	4	63	30	44	75	19
	11	53	30	41	66	20
	18	46	20	32	67	5
	25	57	22	37	63	12
December	2	61	27	43	73	12
	9	48	20	32	68	5
	16	24	0	11	39	-10
	23	39	16	25	49	2
	30	46	26	35	50	18
January	6	51	24	34	58	15
	13	56	23	38	60	15
	20	52	25	38	62	14
	27	57	26	42	68	20
February	3	53	22	37	63	11
	10	27	-2	11	44	-12
	17	47	20	31	58	7
	24	66	31	49	76	20

* The mean daily temperatures were calculated by taking the time factor into consideration which prevents a sudden change in temperature of short duration from affecting the mean to a great extent.

Table IV. Average of Daily Maximum, Minimum and Mean Greenhouse Temperatures for Weekly Periods, Sept. 26, 1932 to Feb. 27, 1933

Week ending		Average maximum	Average minimum	Average mean*
September	26	90	49	70
October	3	82	56	69
	10	89	67	78
	17	92	70	81
	24	95	70	83
	31	88	65	77
November	7	85	68	76
	14	93	66	80
	21	91	69	81
	28	89	70	79
December	5	86	71	78
	12	86	64	75
	19	93	67	80
	26	81	66	74
January	2	84	66	75
	9	84	63	74
	16	88	66	77
	23	85	65	76
	30	85	64	75
February	6	86	60	73
	13	88	69	79
	20	90	68	80
	27	87	68	78
Average		88	66	77

* The mean daily temperatures were calculated by taking the time factor into consideration which prevents a sudden change in temperature of short duration from affecting the mean to a great extent.

warm for about ten days. After December 6, the mean temperature gradually dropped till it reached -10° F. on December 16, the lowest temperature to which the plants were exposed. Temperatures recorded for the last of December and all of January were relatively high, the average weekly means ranging from 35 to 42° F. The minimum temperature for this period was 14° F. and occurred January 14.

Most of the plants were taken into the greenhouse and were frozen during the month of January which incidentally had a very uniform temperature. The daily mean temperature of the greenhouse in which the plants lost hardiness ranged from 70 to 81° F. while the weekly mean temperatures had a range of 73 to 77° F.

It is generally agreed that the daily mean temperatures play a more important part in the increase or decrease of hardiness of plants than the daily maximum or minimum temperatures. Harvey (14) working with cabbages found an accumulation of hardiness only when the average temperature was low.

<u>Freezing</u> <u>Data</u>. The artificial freezing experiments conducted during the winter 1932-33 included 1680 pots or 8400 plants, which were frozen at different times during the winter.

One phase of the experiment consisted of freezing ten lots, each of which included five groups of plants of the four crops. One of the groups in each case was brought directly from outdoors and therefore was hardened while the other four were kept in the greenhouse one, two, three, and four days, respectively, before freezing. Data as to the date, time of day, and the temperature at which each lot was frozen are presented in Table V.

Table V. Date, Time of Day, and Temperature at Which Each Lot was Frozen in 1932-33

Freezing lot No.	Date frozen	Time of day taken from refrigerator	Freezing temperatures Degrees F.
1	Jan. 3	8 P.M.	10°-12° F.
2	Jan. 10	8 P.M.	10°-12° F.
3	Jan. 15	8 P.M.	9°-11° F.
4	Jan. 21	8 A.M.	7°- 9° F.
5	Jan. 23	8 A.M.	10°-12° F.
6	Jan. 25	8 A.M.	12°-14° F.
7	Jan. 28	8 P.M.	12°-14° F.
8	Jan. 30	8 A.M.	12°-14° F.
9	Jan. 31	8 P.M.	13°-15° F.
10	Feb. 2	8 P.M.	13°-15° F.

Changes in cold resistance that are induced by favorable growth temperatures were measured by freezing in one lot the five groups of plants that were moved into the greenhouse at intervals of one day. The plants for a freezing lot were brought into the greenhouse at the same time each day for five days and were frozen immediately upon bringing in the last group. Thus, it was possible to compare the injury to the different crops when frozen after different lengths of time in the greenhouse. Also it was possible to determine the relative rate at which the crops lost their hardiness while in the greenhouse.

It is well known that rye and wheat are more resistant to cold in the hardened condition than are either barley or oats. Little is known however, as to the relative resistance to cold of these crops when they are not hardened, nor as to the rate at which they lose hardiness when conditions become favorable for growth.

The relative injury to plants of Rosen rye, Kanred wheat, Tennessee Winter barley, and Sporen Gray Winter oats artificially frozen after different lengths of time in the greenhouse are presented in Table VI. The values represent the average percentage injury of ten freezing lots or repetitions of the experiment.

The average outdoor daily mean temperature for January which is the approximate period during which the plants were frozen was 39° F. The average daily minimum was 25° and the average daily maximum 54° F.

The greenhouse in which some of the plants were kept for varying lengths of time before freezing was maintained at a weekly mean temperature range of 73 to 77° F.

The injuries reported in Table VI may be compared both for the different crops and the different periods in the greenhouse before freezing.

Table VI. Relative Injury to Plants of Four Crops Artificially Frozen after Varying Lengths of Time in the Greenhouse. (Average of 10 Freezing Lots in 1932-33)

Crop	Days in greenhouse before freezing				
	0	1	2	3	4
Kanred wheat	10±.39	29±.68	42±.55	64±.50	81±.67
Rosen rye	7±.29	27±.65	48±.83	63±.94	82±.59
Tennessee Winter barley	18±.22	44±.58	68±.75	83±.45	94±.24
Sporen Gray Winter oats	62±.46	67±.57	74±.47	80±.45	91±.35
Average	23±.17	40±.31	56±.33	71±.30	85±.23

The relative injury to the varieties when naturally hardened outdoors (represented by zero days) was what would normally be expected with the exception of oats which showed a rather high percentage injury due mainly to a slowly accumulated injury that had previously occurred outdoors. Although rye is usually considered to be more resistant to cold than wheat, these and other data show that Kanred wheat is as hardy as Rosen rye.

The decrease of hardiness in the crops, with increasing lengths of time in the greenhouse, was very pronounced. The average injury to the four crops resulting from artificial freezing was 23±.17 per cent for hardened plants (zero days in the greenhouse) and 85±.23 per cent for plants that were in the greenhouse four days.

Oats decreased in hardiness rather slowly during the four days in the greenhouse as compared to the other crops, even though the injury at zero days was relatively high. After a period of three days in the greenhouse barley was less resistant to cold than was oats.

Rye and wheat reacted much the same and proved to be more resistant at all periods than either barley or oats. However, if the plants had been kept in the greenhouse for longer periods before freezing it is doubtful whether the rye and wheat would have maintained their superiority in hardiness since later tests have shown that after four days they tended to approach oats and barley in resistance to cold.

The average injury of all plants in the experiment was 55±.12 per cent. The probable error of the experiment, based on the variability of four pots in each of ten freez-lots, a total of 40 pots, was ±0.53 per cent for the mean of the ten lots. Therefore, the probable error of the difference between any two crops or any periods of time in the greenhouse was ±0.75 and the least significant difference estimated at odds of 22 to 1 was 2.25 per cent. Thus, the increase in injury for each successive day in the greenhouse is significant in every case. The crops are significantly different in cold resistance at each period, except wheat and rye after one, three and four days in the greenhouse. Rye was slightly more resistant than wheat at the zero day test and less resistant at the two day test. Barley was more resistant than oats for the first two days in the greenhouse and less resistant after that time.

Physico-chemical Tests. Tests were made to determine the total moisture, the expressed juice, and the quantity of exosmosed plant fluids as measured by electrical conductivity to supplement the data obtained by artificial refrigeration.

Newton (28) after showing a close correlation between hardiness and press juice concluded, "the hardier the variety the lower its moisture content and the greater the force with which it is retained." The data here obtained agree closely with Newton's work. It was shown that total moisture and hardiness were closely correlated and also that the expressed juice varied directly but not proportionally with total moisture.

The per cent total moisture, per cent expressed juice and a ratio of the expressed juice to the total moisture minus the expressed juice, are presented in Table VII.

Total moisture was determined on a five gram sample of leaves dried in an oven maintained at 95° C. for 24 hours. The expressed juice determination was an average of three three-gram samples of leaves which were wrapped in filter paper and pressed at a constant pressure of 1400 pounds per square inch for five minutes.

Each crop when placed in the greenhouse increased in total moisture for seven days which was the maximum time included in the test. The total moisture of barley and rye was slightly higher than that of wheat for the first three days. Wheat contained nearly the same quantity of moisture as rye when kept at favorable growing temperatures for three to five days. Barley contained as much or more moisture than either rye or wheat at every period tested.

It was clearly evident that the per cent of expressed juice increased with an increase in total moisture. Rye, wheat, and barley when subjected to pressure lost practically the same amount of juice at the zero day period. At the one and two day periods rye lost considerably more juice

Table VII. Relative Percentage of Total Moisture and Expressed Juice, and a Ratio of Expressed Juice to Total Moisture Minus Expressed Juice, for Three Crops that were in the Greenhouse for Different Lengths of Time in 1932-33

No. days in green-house	Date of test	Per cent total moisture			Per cent expressed juice (1400 lbs. pressure per sq.in)			Ratio:Expressed juice to total moisture minus expressed juice		
		Rye	Wheat	Barley	Rye	Wheat	Barley	Rye	Wheat	Barley
0	2-25	76.2	74.5	77.7	23.1	23.2	23.2	.44	.45	.43
1	2-26	80.0	76.7	80.1	26.7	25.3	24.1	.50	.49	.43
2	2-27	81.9	80.2	82.6	27.1	25.6	25.0	.49	.47	.43
3	2-28	82.6	82.7	84.3	28.1	28.2	29.5	.52	.55	.54
5	3-2	84.3	84.0	84.8	30.9	32.2	31.8	.58	.62	.60
7	3-4	84.8	85.1	86.0	31.8	35.8	36.4	.60	.73	.73

than either wheat or barley. However, at the three, five, and seven day periods, barley and wheat lost more juice than rye, which may mean that the physiological activity of these two crops started more slowly than that of rye but increased rapidly after two days in the warm temperatures.

In order to gain a clear picture of the relationship between total moisture and expressed juice, a ratio of the expressed juice to the total moisture minus the expressed juice was calculated. This ratio which is presented in Table VII indicates that as the plants lost hardiness, the expressed juice increased in a greater proportion than total moisture.

The ratio for rye narrowed more rapidly than the ratio for wheat during the first 48 hours after the plants were placed in the greenhouse and the ratio for barley showed no change during that time. During the last five days of the period the ratio for rye increased more slowly than wheat and wheat more slowly than barley. The changes in the ratios are associated with changes in resistance to cold as shown by artificial refrigeration. In both cases the total moisture and expressed juice increased. However, the ratio indicated that during the first two days total moisture increased in a greater proportion than expressed juice.

In general, the data indicated that total moisture and expressed juice provide a good index to cold resistance when large differences are to be measured but are not so reliable in measuring small differences in hardiness.

The work of Dexter, Tottingham, and Graber (11) suggested the possibility of determining winter-hardiness by means of electrical conductivity of solutions containing exosmosed plant fluids. The method is based on the assumption that dead tissue loses its capacity to regulate the diffusion of its soluble contents. When leaves of plants are injured by artificial freezing the quantity of tissue killed depends primarily on its resistance to cold. Thus, the quantity of exosmosed plant fluids from a definite amount of tissue will depend on the cold resistance of the tissue.

The different crops show little variation in the percentage of electrolytic materials contained in their sap according to Newton (27). Thus, the specific conductivity of a solution in which the plant fluids exosmosed should vary inversely with winter-hardiness.

Specific conductivity of plant fluids exosmosed from frozen tissue was used to supplement the freezing data. Four, two and one-half gram samples of plant crowns of each crop were prepared as described in materials and methods.

The samples were frozen one hour at 14 to 16° F. and were then allowed to exosmose into 20 cc. of conductivity water for two hours after which conductivity readings were made at 25° C.

The relative specific conductivity in reciprocal ohms ($x10^6$) for rye, wheat, and barley kept in the greenhouse for different periods of time before sampling are given in Table VIII.

Table VIII. Relative Specific Conductivity in Reciprocal Ohms ($x10^6$) at 25° C. for Rye, Wheat, and Barley kept in the Greenhouse for 0 to 7 days

Crop	Days in greenhouse before freezing					
	0	1	2	3	5	7
Rosen rye	574	644	667	702	1007	907
Kanred wheat	531	696	745	886	1009	1279
Tennessee Winter barley	910	759	868	1114	1246	1299

In a recent paper, Dexter, Tottingham, and Graber (11) working with rye, wheat, barley, and oats, reported that differences between crops and varieties of a crop could be obtained which were closely correlated to the known hardiness by measuring the electrical conductivity of the solution in which frozen tissue had exosmosed.

The data in Table VIII are in close agreement with the hardiness of the crops as determined by artificial refrigeration, with the exception of barley at the zero day period and rye at the five day period. No reason is apparent to explain the relatively high conductance in these two cases.

As the period of growth in the greenhouse increased the specific conductivity increased indicating a loss of hardiness in the crops. The relative cold resistance of the three crops as indicated by the specific conductivity test in general is in agreement with the results obtained by artificial refrigeration.

Results of 1933-34

The results of this experiment depended primarily on the temperatures to which the plants were subjected preceding the different tests. Thus, a close study was made of the temperatures in the greenhouse and the outdoors where the plants were hardened.

The daily maximum, minimum, and mean outdoor temperatures are given in Table IX, and the weekly averages are presented in Table X.

Table IX. Daily Maximum, Minimum and Mean* Temperatures for Manhattan, Kansas, Oct. 1, 1933 to Mar. 19, 1934

Date	October			November			December		
	Max.	Min.	Mean*	Max.	Min.	Mean*	Max.	Min.	Mean*
1	76	38	57	86	47	69	58	44	49
2	80	32	56	66	28	46	68	48	57
3	81	37	59	58	26	40	70	44	57
4	85	40	63	50	40	44	70	42	56
5	84	40	62	42	35	39	50	34	40
6	76	44	60	55	27	41	55	22	39
7	67	33	50	50	27	37	68	38	48
8	65	30	48	67	26	48	50	28	35
9	75	32	54	48	26	37	64	27	41
10	80	38	56	72	26	50	63	20	41
11	80	37	57	75	36	56	34	16	25
12	68	44	56	77	38	58	33	20	27
13	75	36	56	65	26	50	49	23	36
14	78	60	66	65	42	52	68	32	51
15	70	50	62	60	30	40	60	28	41
16	62	35	48	58	26	44	68	28	42
17	69	35	50	62	30	49	40	12	25
18	69	30	50	70	26	48	49	9	28
19	71	40	56	79	39	56	50	22	36
20	76	43	60	82	44	61	62	20	35
21	70	36	55	62	42	51	66	23	42
22	64	32	47	62	30	48	71	28	48
23	64	34	46	62	30	42	78	38	51
24	64	30	47	62	30	48	48	18	25
25	60	26	46	74	44	55	30	9	23
26	70	35	52	65	33	48	22	10	16
27	68	30	48	76	38	57	39	14	23
28	83	42	63	72	42	56	43	13	27
29	85	55	70	75	38	58	47	28	37
30	87	60	73	64	35	50	58	40	47
31	85	62	71	--	--	--	56	22	40

Table IX continued

Date	January			February			March		
	Max.	Min.	Mean*	Max.	Min.	Mean*	Max.	Min.	Mean*
1	36	18	26	55	16	38	55	23	39
2	36	28	32	67	16	47	62	35	43
3	34	25	29	72	33	51	60	32	43
4	35	30	33	53	23	36	65	28	46
5	42	30	35	52	21	35	48	24	37
6	50	30	39	55	27	38	50	23	36
7	33	23	25	65	21	40	22	20	21
8	31	20	25	37	18	28	55	12	32
9	38	28	33	39	16	28	40	12	30
10	36	24	30	49	18	31	48	7	30
11	50	27	37	56	28	40	60	30	44
12	40	30	35	60	24	39	83	44	60
13	45	20	30	70	23	44	70	20	45
14	57	20	34	77	34	52	57	20	39
15	60	25	40	59	25	42	74	34	50
16	50	15	33	67	25	44	84	33	63
17	60	25	40	69	28	47	30	19	24
18	62	26	40	43	13	25	48	11	28
19	50	19	30	34	6	20	80	26	53
20	58	20	39	51	23	35	--		
21	48	42	45	40	10	27			
22	61	27	39	34	10	21			
23	68	24	47	41	6	26			
24	70	20	46	22	8	14			
25	38	12	23	24	4	10			
26	57	14	35	20	-16	0			
27	70	31	47	30	-16	7			
28	56	19	36	43	24	32			
29	20	3	12						
30	43	3	24						
31	58	30	42						

* Mean daily temperatures were calculated by taking the time factor into consideration, which prevents a sudden change in temperature of short duration from affecting the mean to a great extent.

Table X. Average of Daily Maximum, Minimum, and Mean* Temperatures and the Absolute Maximum and Minimum Temperatures for Weekly Periods, October 7, 1933 to March 17, 1934, Manhattan, Kansas

Week ending		Av.Max.	Av.Min.	Av.Mean*	Max. of week	Min. of week
October	7	78	38	58	85	32
	14	74	40	56	80	30
	21	70	38	54	76	30
	28	68	33	50	83	26
November	4	74	45	59	87	26
	11	58	29	44	75	26
	18	65	31	49	77	26
	25	69	37	52	82	30
December	2	68	40	54	76	33
	9	61	34	45	70	22
	16	54	24	38	68	16
	23	59	22	38	78	9
	30	41	19	28	58	9
January	6	41	26	33	56	18
	13	39	25	31	50	20
	20	57	21	37	62	15
	27	59	24	40	70	12
February	3	53	17	36	72	3
	10	50	21	34	65	16
	17	65	27	44	77	23
	24	38	11	24	51	6
March	3	42	13	25	62	-16
	10	47	18	33	65	7
	17	65	29	46	84	19

* Mean daily temperatures were calculated by taking the time factor into consideration, which prevents a sudden change in temperature of short duration from affecting the mean to a great extent.

The average maximum, minimum and mean weekly temperatures for the greenhouse in which the plants were kept while they lost hardiness are given in Table XI.

Table XI. Average of Daily Maximum, Minimum, and Mean Greenhouse Temperatures for Weekly Periods, December 7, 1933 to March 22, 1934, Manhattan, Kansas

Week ending		Average maximum	Average minimum	Average mean*
December	7	79	54	64
	14	71	52	61
	21	71	54	61
	28	71	54	63
January	4	73	58	66
	11	66	54	59
	18	72	53	60
	25	79	61	68
February	1	79	58	64
	8	76	55	65
	15	81	62	69
	22	80	59	67
March	1	77	57	65
	8	78	60	69
	15	81	61	70
	22	84	56	68

A general summarization of outdoor temperatures through the fall of 1933-34 indicated a fairly uniform mean daily temperature from October 1 to December 7. After December 7 the mean daily temperatures showed considerable fluctuation for the remainder of the winter. From December 7 to December 30 the mean daily temperatures ranged from 16 to 51° F. with a decrease during the latter part of the month.

Uniform temperatures prevailed between January 1 and February 18 during which the range in the mean daily temperatures was 30 to 51° F., with the exception of January 25, 29, and 30, when the daily mean temperatures dropped to 23, 12, and 24 degrees F. respectively. The mean daily temperatures declined from February 18 to February 28. This ten day period during which the minimum temperature reached -16° F. was the coldest period of the winter and was accompanied by a heavy snow.

The mean daily temperature of the greenhouse in which most of the plants lost hardiness ranged from 55 to 75° F. while the weekly mean temperature range was 61 to 70° F. The temperatures to which the plants were subjected before freezing are presented in connection with the corresponding tables of data.

Hardening Process in Cereals. Tests were made at intervals through the winter months in order to obtain information on some of the changes and the rapidity of these changes taking place in the plants as they become hardened under natural conditions. A study of hardening under controlled conditions would have been preferable but equipment for such work was not available.

It is a well known fact that plants in a hardy condition are low in total moisture, due probably to the lack of soil moisture or the non-availability of the moisture during the winter and to the alternate freezing and thawing which lowers the moisture content of plants. Newton and Brown (31) investigated the seasonal changes in the composition of wheat plants and found that the most important change in the quantitative relations of the various plant constituents during the hardening process was the reduction in moisture content.

Data on relative percentage of total moisture, expressed juice, and the ratio of the expressed juice to moisture minus expressed juice, as determined during the hardening process in 1933-34 are presented in Table XII. The date of testing and the temperature to which the plants were exposed for five days previous to the test are recorded in the table.

The data indicate a gradual decrease in total moisture for all the crops through the fall of 1933. The last test, made on February 21, 1934, showed an increase in total mois-

Table XII. The Relative Percentage of Total Moisture and Expressed Juice and the Ratio of the Expressed Juice to Total Moisture Minus Expressed Juice, as Determined During Hardening of the Crops in 1933-34

Date of test	Av. mean temp.*	Per cent total moisture				Per cent expressed juice (1666 lb.pressure per sq.in)				Ratio: Expressed juice to total moisture minus expressed juice			
		Rye	Wheat	Barley	Oats	Rye	Wheat	Barley	Oats	Rye	Wheat	Barley	Oats
11-16	51	81.0	80.1	82.6	81.8	40.2	34.5	45.4	41.8	.98	.76	1.11	1.05
11-27	48	80.0	80.4	84.8	82.5	30.4	30.5	36.4	33.3	.61	.61	.75	.68
12-14	34	77.4	76.6	81.9	78.2	27.9	27.2	31.6	30.2	.56	.55	.63	.63
12-17	39	77.4	75.3	81.6	79.3	33.2	27.9	33.4	30.6	.75	.59	.69	.63
1-11	30	76.2	74.7	80.4	73.3	27.8	24.9	31.6	26.9	.57	.50	.65	.58
1-24	40	74.7	74.6	81.7	77.2	25.8	23.9	32.4	28.2	.53	.47	.66	.58
2-21	34	78.2	77.2	78.3	75.0	28.8	25.1	27.0	24.8	.58	.48	.53	.50

* Average mean temperature to which plants were subjected five days preceding the test.

ture for wheat and rye, while the moisture of barley and oats continued to decrease.

The total moisture of rye and wheat was lower than that of either barley or oats during December and January. The total moisture in rye and wheat decreased during the winter to the minimum shown by the test made January 24. The moisture content of barley remained relatively high and that of oats fluctuated during the winter.

In general, the percentage of expressed juice for the different crops varied with the changes in total moisture. Wheat gave the lowest percentage expressed juice of any crop at all the dates tested, except November 27, when rye was .1 per cent lower and February 21, when oats had .3 per cent less expressible juice.

The percentage expressed juice for both rye and oats was lower at every date than that of barley except on February 21 when rye gave up more juice than barley.

Tests made in November and December showed that oats lost more juice when subjected to pressure than did rye, while in January and February the rye lost more than the oats.

The relationship of the four crops is more clearly shown by the ratio calculated from the total moisture and the expressed juice. It was interesting to find that the ratio followed closely the trend of the average mean temperature for the five days preceding the test. The excessive loss of moisture through alternate freezing and thawing combined with the inability of the plant to supply its needs for moisture, due to the effect of cold on the physiological activity of the plant and on the movement of soil moisture brought about an accumulative moisture deficit which resulted in the hardening of the plant.

The ratios widened with a decrease in the average mean temperatures up to December 17. The average mean temperature for the five days preceding December 17 was 39° F., an increase of 5° F. This increase in temperature brought about a decrease in ratio, that is, more juice was expressed in proportion to the total moisture than was expressed by the test made December 14. The following test made January 11 showed a decrease in the average mean temperature of 9° F. which brought about an increase in the ratio. The only test in which this relation did not prevail was the one made January 24. This may be explained by the fact that the total moisture of wheat and rye had reached a relatively low percentage and the plants were near their peak in hardiness. Under these conditions more heat may be required to stimulate physiological activity which would bring about an increase in total moisture and expressed juice.

Barley and oats did not reach their minimum in total moisture on January 24 thus a decrease in both total moisture and expressed juice occurred up to the last test which was made February 21.

In general, total moisture and expressed juice provided a good index for determining the increase in hardiness during the winter of 1933-34.

Comparable tests on the specific conductance of wheat, rye, barley, and oats were also made during the winter. The samples for the different tests were frozen at 10 to 12° F. for two hours. Table XIII gives the specific conductance of the crops, the date of testing, and the average mean temperature for the five days preceding the test.

The most interesting feature of the data was that the specific conductivity decreased with a decrease in the average mean temperature for the five days preceding the test, thus indicating a gain in hardiness for each of the crops throughout the period of test from November 16 to January 11.

The crop relationship for each test as was shown by specific conductivity agreed closely with the known hardi-

Table XIII. Relative Specific Conductivity on Frozen Tissue of Rye, Wheat, Barley, and Oats as they became Hardened in 1933-34 (exosmosed two hours at 20° C.)

Date of test	Av. mean temp. for previous 5 days	Specific conductivity in reciprocal ohms ($x10^6$) at 20° C.			
		rye	wheat	barley	oats
11-16	51	561	920	1127	1400
11-27	48	545	912	1071	1020
12-14	34	444	811	909	669
1-11	30	131	250	557	354
Checks*		90	82	102	134

* Not frozen, average of three samples.

ness of the crops with the exception of oats. The test made on December 14 indicated a lower specific conductivity for oats than for wheat which as shown by artificial refrigeration was not in line with the actual cold resistance of the two crops.

However, the data in general are in accordance with the artificial freezing results for hardened plants.

Rate of Loss of Hardiness in Cereals. Several authors have called attention to the decrease of hardiness in winter wheats with an increase in temperature.

It is evident that several factors may influence the rate of loss of hardiness, the most important of which are temperature, moisture and light. Temperature becomes the most important factor when the moisture and light are adequate for plant growth. This is probably due to the accelerating effect of warm temperatures on the physiological activity in plants which brings about certain changes that make the plant less resistant to cold.

The average percentage injury to plants of crops maintained at 80° F. as compared to similar plants maintained at 61° F. for different periods of time are presented in Table XIV, which includes the percentage injury and its probable error, the average probable error, and the ratio of D/E.

The ratio of D/E (difference divided by the probable error of the difference) indicates the significance of the differences. When D/E is three it indicates that the odds are 22 to 1 and the differences are considered significant. When the ratio is five the odds are 1350 to 1 and when eight the odds approach infinity.

The difference in the temperatures of the greenhouse was the only variable factor and thus it was the only one

Table XIV. Percentage of Frost Injury to Crops that were Maintained at 80° F. as Compared to 61° F. over a Period of Seven Days. (Crops from both Temperatures were frozen in the same lot)

Days in greenhouse	Percentage injury to plants from greenhouse at 80° F.	at 61° F.	Av. P.E.	Ratio of D/E
0	6±1.7	6±1.7	1.7	0
1	33±2.7	13±1.7	2.2	8.4
2	52±1.4	36±2.4	1.9	5.9
3	57±2.6	59±2.2	2.4	0.6
4	77±1.9	61±2.0	2.0	5.8
5	73±1.5	67±1.7	1.6	2.7
6	87±0.9	78±1.4	1.2	5.6
7	96±0.8	71±2.3	1.6	11.4
Av. P.E.	1.7	1.9		
L.S.D.	6.8	8.2		

which influenced the resulting difference in injury.

Variations in injury were found when comparing the crops from the different greenhouses. These variations were due to the small number of pots (16 pots or 80 plants) represented by each value.

The average rate of loss of hardiness in the crops maintained at 61° F. was decidedly less than the rate of decrease in the plants held at 80° F. The hardiness of the plants taken into the two greenhouses at the beginning of the experiment is represented by the injury for zero days.

The crops losing hardiness at 80° F. showed a greater decrease in cold resistance than the crops maintained at 61° F., except at the three and five day periods in the greenhouse when the differences were not significant as shown by the ratio of D/E.

Considerable difficulty was encountered in the effort to obtain a relative rate of loss of hardiness for the four crops. The range in cold resistance that may be measured at one temperature or by one freezing is rather narrow. For instance, the difference in cold resistance between hardened rye and hardened barley probably was as wide as could be measured by one freezing. If plants of these two crops having different degrees of hardiness were to be frozen at one time a much wider range in cold resistance would be represented. For example, it is possible that hardened plants of rye and barley would be injured five and 95 per cent respectively, whereas if plants of barley which had lost some hardiness were frozen at the same time all the plants would be killed.

Therefore, it was decided to determine for each crop separately the rate of loss of hardiness during a seven day period in the greenhouse. In freezing the different crops it was intended so far as possible to subject the plants to a temperature which would give the widest possible range in injury (0 to 100 per cent) over the seven day period.

The comparative rate of loss of hardiness in the crops depended on the range in injury produced to plants that had been kept zero to seven days in warm temperatures.

The rate of loss of hardiness in rye, wheat, barley, and oats during a seven day period in the greenhouse is given in Table XV. Probable errors and the least significant difference were calculated which will aid in evaluating the data for each crop.

It should be noted that some injury occurred in the hardened plants (0 days) and that all the crops at the seven day period were near the upper limit of injury. Wheat ranged in injury from three per cent at the zero day period to 96 per cent at the seven day period which indicated a rapid rate of loss of hardiness. This is in accord with observations throughout the experiment. The point most clearly demonstrated was the rapid decrease in the cold resistance of wheat for the first four days in the greenhouse going from 3 to 80 per cent in injury.

Rye indicated a slower rate of loss of hardiness than wheat, especially for the first three days in the greenhouse. However, rye lost its cold resistance more rapidly than barley or oats. The slower rate at which barley and oats lost

Table XV. Rate of Decrease in Cold Resistance of Rye, Wheat Barley and Oats During a Seven Day Period in the Greenhouse at 68° F. (Average of Three Freezing Lots)

Days in greenhouse	Crops and temperatures at which frozen				
	Rye 4-6° F.	Wheat 8-10° F.	Barley 15-17° F.	Oats 15-17° F.	Average
			Per cent injury		
0	11±1.3	3±0.3	7±1.5	12±4.5	8±1.0
1	23±2.0	33±2.8	9±0.9	16±3.6	19±1.2
2	34±2.2	61±1.2	34±2.9	29±2.2	37±1.1
3	55±3.9	74±1.7	55±3.0	47±1.9	58±1.3
4	72±1.9	80±2.2	55±3.9	51±2.3	64±1.3
5	73±1.9	79±1.1	62±3.4	64±2.2	72±1.1
6	80±2.1	95±1.4	67±3.2	76±1.3	78±1.0
7	77±2.8	96±1.2	74±4.0	84±1.7	85±1.2
Av.P.E.	2.3	1.5	2.8	2.5	1.1
L.S.D.	9.5	6.4	12.0	10.4	4.8

hardiness was also in agreement with numerous observations.

The loss of hardiness was sufficiently rapid that the differences in injury from day to day were statistically significant for wheat at five of the seven days, oats at four, rye at three, and barley at two.

After determining the average rate of loss of hardiness in the four crops for a period of seven days in the greenhouse, it was considered desirable to know the rate of loss over a longer period. In order to obtain injury data on plants kept in the greenhouse for a longer period it was necessary to freeze them at a higher temperature in order to prevent total killing of the plants.

Plants of the different crops were subjected to greenhouse temperatures for 5 to 12 days, a period which overlapped the previously frozen 0 to 7 day material at the five, six and seven day periods as shown in Table XVI.

The plants for the 5 to 12 day period were subjected to the same conditions as those for the 0 to 7 day period except for differences in the freezing temperatures. The difference between the percentage injury of the 0 to 7 day group and the 5 to 12 day group for the three over-lapping days (five, six and seven) was due to the difference in the freezing temperature. The average difference in injury for those days was 37 per cent which was added to all the injury percentages representing the 5 to 12 day group in order to make them comparable to the injury received by the 0 to 7 day group. In other words the relative injury was determined independently within each group. The second group was then set up 37 points in the scale in order to make the upper portion of the curve for the first group and the lower portion of the curve for the second group coincide.

The same procedure was followed with a similar group of material representing a period of 9 to 16 days in the greenhouse. In this case four days of the 9 to 16 day group over-lapped the 5 to 12 day group. The average difference in the injury for the four over-lapping days was 24 per cent. Therefore, the third group was placed 24 points higher on the scale than the second group in order to bring the over-lapping portions of the curves into closest proximity. The relative degrees of injury determined in this way placed the data on a comparable basis and showed an average rate of loss of hardiness for a period of 16 days. The average percentage injury to plants of the four crops in the groups 0 to 7, 5 to 12, and 9 to 16 days and the probable errors are given in Table XVI. The first group included 336 pots, the second 256, and the third 192. The temperatures at which the plants in the first group were frozen are shown in Table XV. The freezing temperatures of the second group were 14-16 to 16-18° F. and of the third 18-20 to 20-22° F. The four crops are represented in the first group, barley and wheat in the second and wheat in the third.

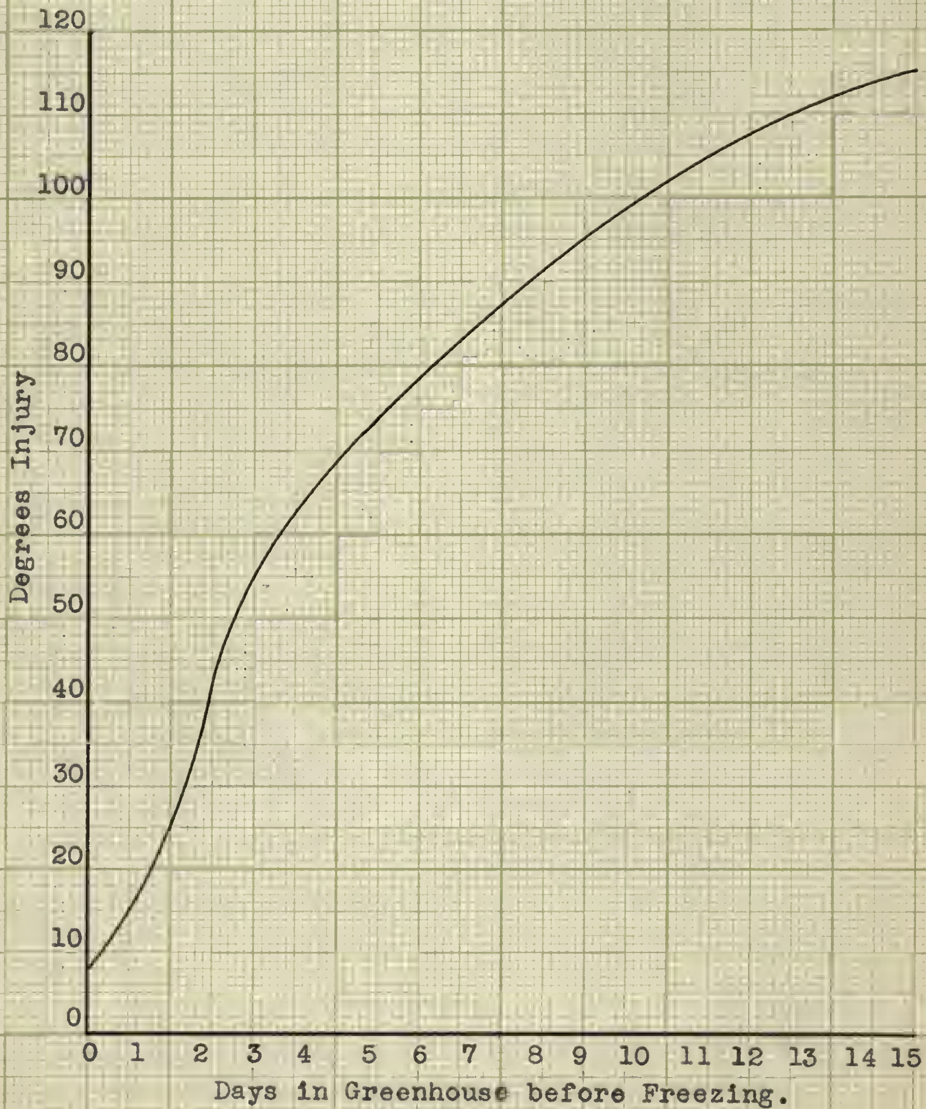
The cold resistance decreased rapidly during the first five days in the greenhouse and less rapidly during the remaining 11 days. This is well illustrated in figure 1, which shows the average rate of loss of hardiness in four crops for a 16 day period.

Table XVI. Average Rate of Loss of Hardiness over a 16 day Period in the Greenhouse at 67° F. in 1933-34

Days in green-house	Average per cent injury			Relative degrees injury
0	8±1.0			8
1	19±1.2			19
2	37±1.1			37
3	58±1.3			58
4	64±1.3			64
5	72±1.1	39±2.5		74
6	78±1.0	40±2.5		77
7	85±1.2	45±2.4		83
8		48±1.9		85
9		62±2.0	41±2.4	100
10		60±1.9	45±2.5	102
11		73±1.7	40±2.2	106
12		75±1.8	47±2.3	110
13			52±2.5	113
14			51±2.5	112
15			53±2.0	114
16			55±2.1	116
L.S.D.	4.8	8.9	9.8	

A study of the total moisture and the expressed juice over a 16 day period in the greenhouse showed an interesting relationship when compared to the injury data presented in Table XVI which was obtained from material directly compar-

Fig. 1. Rate of Loss of Hardiness.



able to that used for total moisture and expressed juice determinations.

One test was made on wheat for the 0 to 7 day period in the greenhouse. Wheat and barley were tested for the 5 to 12 day period and three tests were made on wheat for the 9 to 16 day period. An average of these tests gave an indication of the moisture changes which took place over the 16 day period when the temperatures were favorable for growth.

Table XVII includes the total moisture, expressed juice and the ratio of expressed juice to total moisture minus expressed juice, for wheat and barley over a 16 day period. The data show that little or no increase in total moisture and expressed juice took place during the first 24 hours in the greenhouse. The plants were in a hardened condition and probably required some time to become adjusted before a rapid increase in the moisture content could take place. Total moisture reached its maximum after about seven days in the greenhouse, while the expressed juice reached a maximum after nine days. The longer time required for the expressed juice to reach its maximum may have been due to slow changes taking place in the hydrophilic colloids after the maximum amount of moisture was taken up.

Changes in the total solids, which take place in the plant fluids when plants lose hardiness may be shown by a re-

Table XVII. Total Moisture and Expressed Juice in Wheat and Barley over a 16 day Period when kept at Greenhouse Temperatures

Days in greenhouse	Crop and date tested							
	Wheat		Barley	Wheat				
	2-28	3-3	3-1	3-16	3-17	3-19	Av.	Ratio
0	72.4						72.4	.57
1	72.1						72.1	.57
2	76.4						76.4	.54
3								
4	79.3						79.3	.60
5	81.0	82.4	81.1				81.5	.72
6	81.2	86.3	82.7				83.4	.70
7	81.9	86.0	83.3				83.7	.80
8		83.9	84.1				84.0	.87
9		83.5	84.4	83.3	83.7		83.8	.93
10		84.4	84.9	83.4	84.2	82.5	83.9	.94
11		84.0	84.3	83.3	84.3	84.6	84.1	1.01
12		82.7	83.4	82.6	84.0	85.5	83.7	.93
13				83.0	84.0	83.3	83.4	1.00
14				83.7	82.6	83.1	83.1	1.10
15				83.3	83.3	82.3	83.0	1.00
16				82.0	83.7	82.4	82.7	.99
				Expressed juice				
0	26.4						26.4	
1	26.1						26.1	
2	26.9						26.9	
3								
4	29.8						29.8	
5	31.6	37.5	33.8				34.3	
6	30.5	37.3	35.8				34.5	
7	34.3	39.6	37.9				37.3	
8		40.6	37.8				39.2	
9		43.2	40.1	41.4	41.5		41.5	
10		43.0	40.1	40.6	40.8	38.7	40.7	
11		43.3	40.4	43.6	41.3	43.4	42.4	
12		41.7	39.6	39.9	39.2	41.4	40.4	
13				41.5	40.9	43.1	41.8	
14				45.3	41.3	44.3	43.6	
15				43.0	39.6	42.2	41.6	
16				39.3	39.3	44.6	41.1	

fractive index as is indicated in Table XVIII. Refraction was determined on five drops of juice expressed from duplicated four gram samples of leaves, by means of an Abbe-Zeiss refractometer. Considerable pressure was applied to the hardened plants in order to obtain the specified quantity of juice while plants which had remained in the greenhouse for six days or over did not require so much pressure due to their higher moisture content. This allowed the increase of total moisture to become a factor in the changes which occurred in the total solids.

The increase in total moisture was not an important factor affecting the refractive index during the first 24 hours in the greenhouse. The largest decrease in the refraction occurred between the zero and the one day periods which was only partially due to an increase in total moisture. The major portion of the decrease probably was due to the reduction in total solids by the respiration processes. Newton and Anderson (30) reported a rapid increase in respiration due to an increased temperature which materially lowered the food reserves or total solids in the plant. The plants in this test were not in an extremely hardened or dormant condition due to the mild winter, thus when taken into the greenhouse immediate and rapid changes took place

Table XVIII. Refractive Index* of Plant Juice at 15° C. Determined on Wheat and Barley over a 12 day Period of Greenhouse Temperatures

Days in greenhouse	Crop and date of test					
	Wheat	Barley		Wheat		
	2-19	2-22	2-23	2-26	3-3	Average
0	440	490	460			463
1	400	415	427			414
2	402	415	415			411
3	401	415	393			403
4	395	395	382			391
5	390	364	367	403	377	380
6	386	371	365	383	365	374
7	389	378	365	369	360	372
8	400	370	365	367	371	375
9				372	349	361
10				372	362	367
11				368	355	362
12				370	353	362

* All values should be preceded by 1.3 in order to read in specific refraction.

in the plant tissue. As much change in refraction occurred during the first 24 hours as during the next 11 days.

The index of refraction decreased more slowly after one day in the greenhouse, which may indicate that when the plant was capable of absorbing moisture and manufacturing its own plant food through photosynthesis, the total moisture became the major factor.

It was interesting to note that the refractive index reached the minimum after nine days which was the same time at which the expressed juice reached a maximum.

A close relation therefore was indicated between the total solids in the plant juice and the expressed juice, with the exception of the first 24 hours during which increased respiration was the predominating factor.

Relative Cold Resistance of Cereals. Tests of total moisture, expressed juice, refraction, the quantity of plant fluids exosmosed from frozen tissue as measured by electrical conductivity and refrigeration of plants were made on the same day with comparable material thus permitting a direct comparison of the results.

Physico-chemical tests. Data in Table XIX show the relative percentage of total moisture, expressed juice, and the ratio of expressed juice to total moisture minus expressed juice for rye, wheat, barley, and oats when they were subjected to different periods of greenhouse temperatures. The date of the test, the number of days in the greenhouse and the average mean temperatures preceding the test are also included in the table. The temperature for the zero day freezing was the average mean temperature for five days preceding the test. The other temperatures represent the average mean temperature for the period the plants were in the greenhouse. Data in this table are comparable for the crops and for the different periods in the greenhouse.

Most of the increase in total moisture occurred during the first six days although the crops showed some increase up to the nine day period. Oats and barley were relatively high in total moisture in the hardened condition or at zero days and the increase during the nine days was small when compared to the increase in wheat and rye.

The high moisture content of barley and oats as compared to rye and wheat at zero days indicated a low resistance to cold which was also indicated by the percentage injury as determined by artificial refrigeration. After the nine day period the total moisture showed considerable variation in all the crops.

In some cases the quantity of expressed juice showed a different relationship for the crops than was expected. Newton (28) and Dexter (7) are of the opinion that the hardy crops retain moisture with a greater force than the less hardy crops, however, they worked entirely with hardened plants. All the crops gave an increase in the quantity of juice expressed as the days in the greenhouse increased. At zero days the four crops ranked in the order of the known hardiness as one would normally expect. Wheat and rye gave

Table XIX. The Relative Percentage of Total Moisture and Expressed Juice and the Ratio of Expressed Juice to Total Moisture Minus Expressed Juice of Four Crops Subjected to Temperatures Favorable to Growth for Various Periods of Time

Days in green-house before testing	Date of test 1934	Av. mean temp.*	Per cent total moisture				Per cent expressed juice (1666 lbs. pressure per sq. in.)				Ratio: Expressed Juice to total moisture minus expressed juice			
			Rye	Wheat	Barley	Oats	Rye	Wheat	Barley	Oats	Rye	Wheat	Barley	Oats
0	1-24	40	74.7	74.6	81.7	77.2	25.8	23.9	32.4	18.2	.53	.47	.66	.58
6	1-30	64	82.5	81.6	83.4	84.4	30.6	28.7	31.6	33.8	.59	.54	.61	.67
9	2-2	68	83.8	86.0	83.9	85.7	35.9	32.3	33.9	36.2	.75	.60	.68	.73
12	2-5	66	84.1	83.1	84.1	84.6	34.1	32.7	38.1	37.4	.68	.65	.83	.79
15	2-8	65	85.3	84.1	85.1	85.5	43.1	38.9	39.8	42.2	1.02	.86	.88	.97

* Av. mean temperature to which plants were subjected preceding the test.

up less moisture than oats and barley. It was interesting to find that wheat gave less expressed juice than rye at every period tested. Baroulina (5) cited rye as being more cold resistant than wheat, even though the osmotic pressure was lower than for wheat. In wheats alone he found a direct correlation between cold resistance and osmotic pressure. It seems evident that the forces which influence osmotic pressure are closely related to those which enable the plant to retain its moisture against mechanical pressure. This being the case it is not out of line for rye to give up a larger proportion of its juice than wheat, which was found to be true under every condition tested.

According to Sayre (39) "bound water does not exist in a definite proportion relative to the solid material, but as a ratio between bound water and free water." Sayre gives a very elastic and indefinite definition of bound water.

Perhaps the relationship of the four crops may be more clearly shown by a calculated ratio of expressed juice to total moisture minus expressed juice.

Since expressed juice contains more or less solid material, this ratio does not show the exact relationship of water expressed to water held. The evidence indicates that expressed juice from hardy plants contains more solids than that from similar plants which had lost hardiness. Due to the method of calculating the ratio the difference in the relation of water expressed to water held is greater than is indicated by the expressed juice - moisture ratio. Therefore it seems safe to assume that the changes in hardiness are greater than the calculated ratios indicate. The ratio aids in comparing the crops and the different stages of hardiness of each crop by giving comparable values for plants that differ in moisture content.

The ratios reported in Table XIX indicate that the quantity of juice expressed increased in a greater proportion than total moisture as the plants lost hardiness.

The ratio for each crop tends to narrow with an increased period in the greenhouse up to nine days in the case of barley and 12 days in the case of the other crops. When tested at the 15 day period a similar change in the ratio was observed but at an increased rate. This sharp decrease in the ratio was possibly due to the fact that total moisture had reached its maximum and that a continued liberation of bound water took place due to the reaction of the hydrophilic colloids to the physiological activities in the plant.

The different crops did not show a close relationship between the quantity of juice expressed and the hardiness as measured by artificial refrigeration. However, when the loss of hardiness in one crop was considered, a good index to cold resistance was observed.

Data from a second series of tests presented in Table XX show a general increase in the total moisture and the expressed juice of the different crops with an increasing length of time in the greenhouse.

Total moisture and expressed juice determinations made January 24 on hardened plants were relatively lower than was shown by hardened plants tested February 21 in the first series of tests. The maximum total moisture and the maximum expressed juice were reached in approximately three days less time in the latter group of tests, which indicated that the plants had lost some hardiness outdoors. Wheat and rye showed little or no increase in total moisture after the six day period while a slight increase was found in barley and oats.

The calculated ratio indicated that a smaller proportion of juice was expressed from oats than from any of the other crops at the three, six and nine day period. The large decrease in the ratio for all the crops at the nine day period compares favorably with the decrease shown in Table XIX at the 12 day period indicating that the plants at this time were less hardy than comparable plants in the earlier test.

Table XX. The Relative Percentage of Total Moisture and Expressed Juice, and the Ratio of Expressed Juice to Total Moisture Minus Expressed Juice of Crops Kept in the Greenhouse for Different Periods of Time

Days in green-house	Date of test	Av. mean temp.*	Per cent total moisture				Per cent expressed juice (1666 lbs. pressure per sq. in.)				Ratio: Expressed juice to total moisture minus expressed juice			
			Rye	Wheat	Barley	Oats	Rye	Wheat	Barley	Oats	Rye	Wheat	Barley	Oats
0	2-21	34	78.2	77.2	78.3	75.0	28.8	25.1	27.0	24.8	.58	.48	.53	.50
3	2-24	63	81.6	81.2	82.9	79.1	33.4	31.7	33.3	30.0	.69	.64	.67	.61
6	2-27	62	84.3	83.5	84.6	80.4	34.3	34.8	36.4	32.0	.69	..71	.76	.66
9	3-2	64	83.6	84.1	85.4	86.1	37.8	38.4	43.8	37.7	.83	..84	1.05	.78
12	3-5	65	83.2	84.6	86.1	83.8	41.3	45.4	49.2	44.4	.99	1.16	1.33	1.13

* Av. mean temperature to which plants were subjected preceding the test.

The ratios for the different crops were not closely correlated with the relative cold resistance of the crops. However, for any one crop the hardiness at different times during the 12 day period in the greenhouse was correlated with changes indicated by the ratio.

Total solids in the plant juice, as measured by the refractometer, decreased as the plants lost hardiness. The explanation as previously suggested was the rapid respiration for the first 24 hours in the greenhouse which caused a material decrease in total solids along with a dilution of the remaining solids caused by an increase of moisture in the plant. After the first 24 hours the increase in total moisture was the main factor causing a reduction in the refractive index.

Table XXI gives the refractive index of the plant juices of the crops at intervals after having been placed in the greenhouse. The table includes the date of testing and the number of days in the greenhouse previous to the test.

The general behavior of the refractive indices of the different crops in Series 1 agree closely with those in Series 2. The decrease in the total solids between the zero and the three day periods was the largest decrease noted. In both series of tests the minimum refractive index was reached at the nine day period after which the total solids varied indirectly with a variation in the total moisture.

It is interesting to note that the total moisture, the expressed juice, the electrical conductivity, the refractive index, and the artificial refrigoration have all indicated that the four crops most nearly approach the same condition and show the least difference in cold resistance at the nine day period.

The variations in total solids between the different crops were found to vary indirectly with total moisture. The crops containing the higher moisture gave the lower refractive index.

Unreported tests made in midwinter on hardened plants showed higher indices of refraction than the hardened plants reported in Table XXI. In these cases the total moisture was considerably lower which again shows the close relation of moisture and the refractive value of the plant juice.

The test made January 24 on hardened plants showed a higher refractive index for rye and wheat than the test made February 21 which was clearly the result of an increase in total moisture.

The specific conductivity of a solution containing plant fluids exosmosed from frozen tissue of rye, wheat, barley and oats kept in the greenhouse for different periods of

Table XXI. Refractive Index* of Plant Juice at 15° C. of Rye, Wheat, Barley, and Oats, when Subjected to Greenhouse Temperatures for Different Periods of time in 1934

Days in green-house	Date of test	Series I				Date of test	Series II			
		Rye	Wheat	Barley	Oats		Rye	Wheat	Barley	Oats
0	1-24	549	513	443	528	2-21	450	482	472	533
3	----	---	---	---	---	2-24	400	380	375	407
6	1-30	388	419	385	398	2-27	404	395	380	402
9	2-2	366	366	355	355	3-2	369	367	356	364
12	2-5	396	[illegible]	370	371	3-5	358	351	354	349
15	2-8	360	362	364	360	----	---	---	---	---

* All values should be preceded by 1.3 in order to read in specific refraction.

time are given in Table XXII.

Table XXII. Specific Conductivity on Frozen Tissue of Four Crops (a) when grown at Greenhouse Temperatures for Different Periods of Time. (Exosmosed 2 hrs. at 20° C.)

Days in green-house	Date of test	Leaves frozen 2 hrs. at	Specific conductivity in reciprocal ohms ($x10^6$) at 20° C.			
			Rye	Wheat	Barley	Oats
0	1-24	5- 7° F	600	786	1543	906
6	1-30	16-18° F	301	489	348(b)	667(b)
9	2-2	17-19° F	384	628	247(b)	350(b)
12	2-5	18-20° F	429	794	529	533
15	2-8	17-19° F	494	557	453	770
Checks not frozen, av. of five samples			76	76	85	80

(a) Data for the different dates of testing are not comparable since the freezing temperature differed.

(b) Some leaves did not freeze.

Duplicate, three gram samples of leaves of the crops were frozen at a temperature that would produce some injury to all the samples. The length of the freezing period and the temperature for most of the tests were as reported in the table. It was necessary to vary the temperature and length of freezing period in some cases in order to obtain a maximum range in injury to the crops. Therefore, the rate of loss of hardiness could not be determined by this test, but merely the relationship of the crops at the different periods.

In several instances a peculiar phenomenon occurred which could not be explained. Leaf samples of barley and oats did not freeze solid in some cases, but contained some leaves which showed no evidence of ice formation within the cells. Tests proved that it was not the super-cooling phenomenon that has been described by some investigators. It occurred in the barley and the oats after the material had been in the greenhouse for three or more days. The same peculiar phenomenon was manifested by the potted plants in the artificial refrigeration tests. Leaves of barley, in particular, in many instances had little or no actual injury, however, considerable injury occurred to the stems of the plants which later caused death.

Hardiness as shown by specific conductivity in Table XXII was in accordance with the artificial refrigeration data with the exception of a few instances.

The hardened plants (0 days) indicated the usual wide range in resistance. Rye and wheat according to conductivity tests were more hardy than oats or barley. Rye was more resistant to cold than wheat at every period tested. After six days in the greenhouse barley gave a lower conductivity than either wheat or oats which probably was due to the phenomenon previously mentioned. At the nine day period wheat gave a higher conductivity than either barley or oats, here again the barley and oat leaves were able to withstand the low temperatures without the formation of ice crystals in their tissues. Wheat at the 12 day period gave a higher conductance than either barley or oats which was an indication of less resistance to cold.

The samples which were used as checks were not frozen but were allowed to exosmose two hours and then handled in the same manner as the other samples. The conductivity of the check samples of the different crops did not show a wide difference, however, barley and oats gave slightly higher readings than the wheat and rye.

A second series of conductivity tests started on February 21 are presented in Table XXIII. It was previously mentioned that the material at this time probably had passed the peak in hardiness attained during the winter.

Variations which cannot be explained occurred in the specific conductivity determinations in this table. Rye was found by this test to be more hardy than wheat at the three,

Table XXIII. Relative Specific Conductivity on Frozen Tissue of Four Crops (a) Subjected to Varying Periods of Warm Temperatures. (Exosmosed 2 hrs. at 20° C.)

Days in green-house	Date of test	Leaves frozen 2 hrs. at	Specific conductivity in reciprocal ohms ($x10^6$) at 20° C.			
			Rye	Wheat	Barley	Oats
0	2-21	6- 8° F	339	299	794	485
3	2-24	10-12° F	583	646	643(b)	540(b)
6	2-27	14-16° F	708	841	720(b)	1071
9	3-2	14-16° F	657	965	843	576
12	3-5	16-18° F	461	339	768	409
Checks not frozen, av. of five samples				72		

(a) Data for the different dates of testing are not comparable since the freezing temperatures differed.
(b) Some leaves did not freeze.

six, and nine day periods but less hardy at the zero and 12 day periods, which does not agree with results of other tests.

Oats had a lower specific conductivity than barley for each period, except the six day period when some of the barley leaves failed to freeze. The specific conductivity of oats was higher than wheat indicating less resistance at the zero, six and 12 day periods. This is not in line with the refrigeration data for the 6 and 12 day periods. According to conductivity determinations barley was more resistant to cold than wheat at the three, six and nine day periods and was less resistant at zero and 12 days.

It was clear that specific conductivity is not always a reliable test of the cold resistance. As indicated in the table several samples contained some leaves which were not frozen, which accounts for some of the variation.

Freezing data. During the winter of 1933-34 the artificial refrigeration experiment included a total of 3100 pots or 15,500 plants of the four crops.

Since a wide difference in cold resistance occurred between the crops used it was necessary at a single freezing to include plants of the different crops only when they were at about the same stage in hardiness. That is, plants that had lost some hardiness and plants which were hardened were not frozen at the same time. The relative hardiness of the four crops could be measured more accurately at each of the different periods in the greenhouse by this method than when plants of different stages of hardiness were frozen at the same time as was done in 1932-33.

This plan also made it possible to compare the relative cold resistance of the crops after the plants had been exposed to long periods of temperatures favorable to growth. In freezing the different lots it was intended so far as possible to subject the plants to a temperature that would produce some injury in the most hardy crop without completely killing the less resistant crops.

A large unit of the four crops (160 pots) was moved into the greenhouse at one time, and portions of it were tested at intervals. Eight pots of each crop (32 pots) were moved directly to the refrigerator and frozen. Three days later a similar group of the pots from the greenhouse were frozen. Thus, five freezings at intervals of three days were made from the 160 pot unit.

It was possible to compare the injury to the different crops from freezing at any one of the periods, but the injury to one crop for different periods in the greenhouse was not comparable, since they were frozen at different temperatures.

The data reported in Table XXIV give the relative injury by artificial refrigeration to rye, wheat, barley and oats, after the plants were expösed for different lengths of time to temperatures that were favorable for growth.

Table XXIV. Relative Injury to Rye, Wheat, Barley, and Oats by Artificial Refrigeration after being Subjected to Different Periods of Favorable Growth Temperatures, 1933-34

Days in green-house	Av. mean temp. (b)	Free-zing temp.	No. of free-zing units	Average percentage of injury (a)					
				Rye	Wheat	Barley	Oats	Av.P.E.	L.S.D.
0	36	4- 8° F	10	9±0.4	35±0.7	84±0.6	81±0.5	.57	2.43
3	61	9-16° F	6	33±0.9	46±0.7	84±1.1	80±0.9	.87	3.69
6	63	12-18° F	9	24±0.9	57±1.8	66±1.5	43±1.0	1.04	4.41
9	63	15-20° F	9	34±1.2	40±1.2	47±1.5	43±1.0	1.18	5.01
12	64	14-22° F	9	39±1.2	50±1.3	59±1.5	53±1.0	1.22	6.19
15	66	19-22° F	4	35±1.6	44±2.6	68±2.1	51±1.7	1.98	8.40

(a) The data are not comparable for the different periods in the greenhouse since the freezing temperatures were different.

(b) Av. mean temperature of greenhouse, except at 0 days, preceding freezing.

The table also gives the average mean temperatures to which the plants were subjected before freezing, the freezing temperatures, the number of freezing units represented, the average probable error, and the least significant difference (L.S.D.) of the four crops at each period.

The temperature preceding the zero day freezing test was the average mean temperature for the five days preceding the test. The temperatures for the other periods represent the average mean temperature for the period during which they were in the greenhouse.

Changes in the crop relationships were observed with the increasing length of time in the greenhouse. The usual wide range in hardiness was found when the crops were frozen in a hardened condition (0 days in the greenhouse). The range in injury was from $8 \pm .43$ per cent for rye to $84 \pm .59$ per cent for barley.

The difference in injury between the crops decreased until a difference of only 13 per cent was found between rye, the most hardy crop, and barley, the least hardy crop after they had been in the greenhouse nine days. The plants when kept in the greenhouse for three days showed a decided loss in hardiness as is indicated by the higher temperature at which they were frozen and the greater injury they sustained as compared with the zero day test.

The plants frozen after six, nine, twelve and fifteen days in the greenhouse indicated that the crops continued to lose hardiness and that the rate of loss decreased. A significant difference in injury among the crops was shown at the six day period and a decided change in the crop relationship had taken place. Oats were more resistant to cold than wheat at this period, while the other crops remained in the same order as to cold resistance that was indicated at the earlier periods. Thus, the crops in order of resistance to freezing after six days in the greenhouse were rye, oats, wheat and barley.

At the nine day period the percentage injury was nearly the same for all the crops. Rye was still the most resistant crop, while wheat showed more resistance than barley. A significant difference in injury was not found between wheat and oats nor between barley and oats at that time.

Plants of the four crops had retained some hardiness at the 12 day period. At this stage rye was the most hardy crop. Wheat was more resistant to cold than barley but the difference in injury between wheat and oats was not significant. After 15 days in the greenhouse the crops showed the same relationship that they did at the 12 day period.

A brief summarization of the relationships of the crops brings out the fact that rye was the most resistant crop at every period tested. Wheat was more resistant than barley at each test. Oats was also more resistant than barley except perhaps at the nine day period. The greatest change in relationship occurred between wheat and oats. Wheat was mo more resistant than oats at the three day period but at the six day period oats showed more cold resistance than wheat. After that time the injury to wheat and oats was not significantly different. In general the data show that rye and wheat lost hardiness more rapidly than barley or oats and therefore approached the same degree of resistance.

The injury data in Table XXIV has been plotted on the curve representing the average rate of loss of hardiness in four crops shown in figure 1. The relationship of the crops at three day intervals during a 15 day period was shown by deviations from the average crop injury, which were plotted on the loss of hardiness curve.

Thus, figure 2 not only shows the relative injury of the crops after certain periods in the greenhouse but also indicates the relative rate of loss of hardiness over the 15 day period.

Previous work at this station has given a good correlation between injury and survival data, especially when a large number of readings were represented.

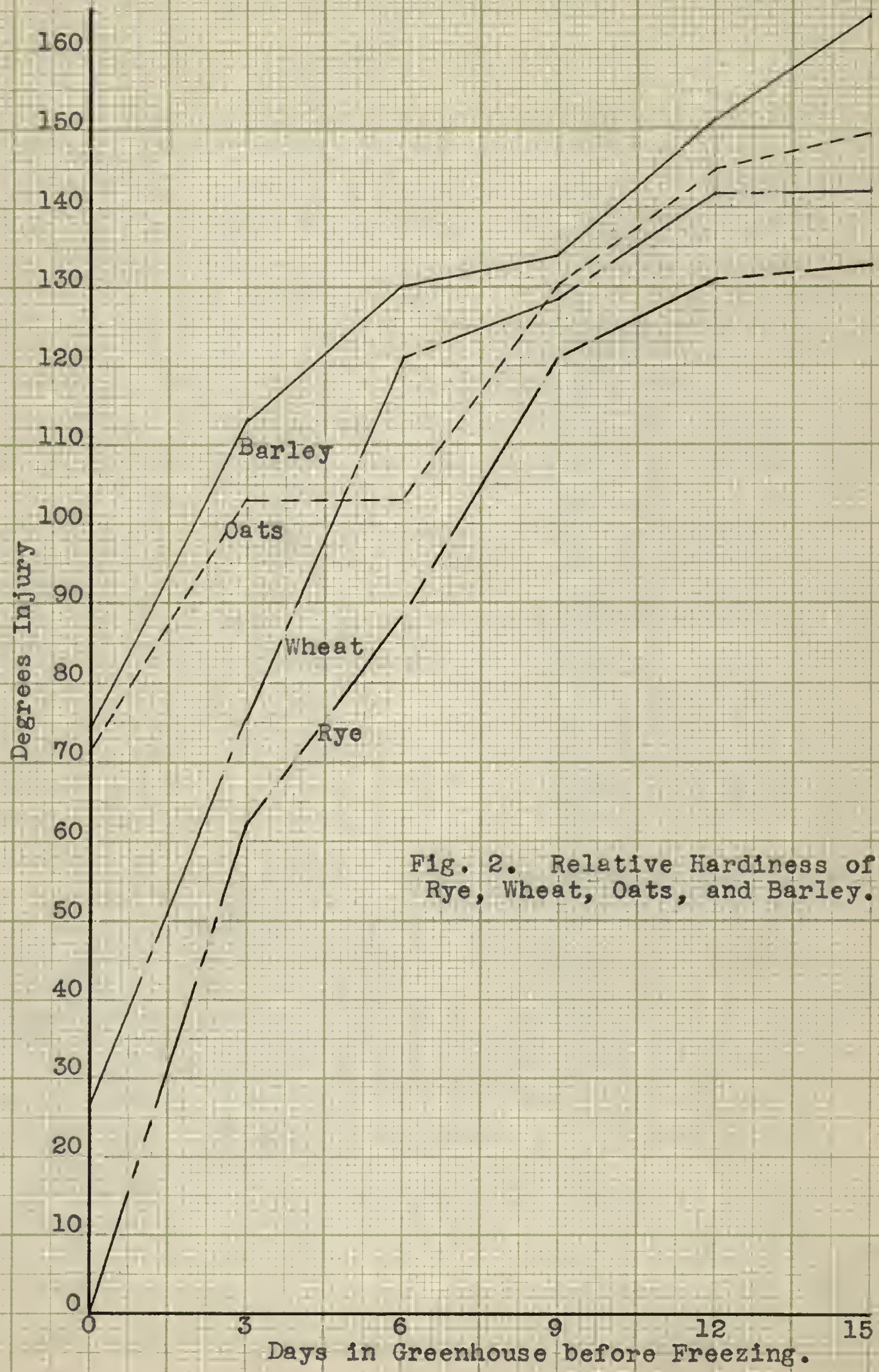


Fig. 2. Relative Hardiness of Rye, Wheat, Oats, and Barley.

Plant survival data were obtained on all the freezing lots on which injury notes were taken, except one lot representing the three day period which was disposed of by mistake before plant counts were made. The probable errors appear to be higher when calculated on the percentage survival than on injury. This may be accounted for by greater variability in survival than in injury and by the fact that a limited range in injury may represent the full range, 0 to 100 per cent, in survival. Plants injured less than 40 per cent are seldom killed while those injured 80 per cent usually die. Thus a range of 40 per cent in injury is comparable to a range of about 100 per cent in survival.

Survival data and the probable errors have been summarized and are presented in Table XXV. The table includes the temperatures at which the lots were frozen, the number of replications, the number of days in the greenhouse, the average probable error of the four crops, and the least significant difference of the crops for any one period.

In general, the survival data indicated the same crop relationship at the different periods in the greenhouse that was shown by the injury data. Therefore, only a general discussion of the data will be included here.

Rye gave the highest percentage survival of the crops for the different periods in the greenhouse. Wheat accord-

Table XXV. Relative Survival of Rye, Wheat, Barley and Oats when Artificially Frozen after being Subjected to Different Periods of Greenhouse Temperatures, 1933-34

Days in green-house	Free-zing temp.	No. of free-zing units	Average percentage survival of plants (a)					
			Rye	Wheat	Barley	Oats	Av. P.E.	L.S.D.
0	4-8° F.	10	94±0.5	44±1.6	.5±0.1	.3±0.1	.56	2.37
3	9-16° F.	5	69±1.0	61±0.2	27±1.7	28±1.8	1.16	4.95
6	12-18° F.	9	92±0.6	46±2.5	29±2.1	55±2.3	1.86	7.92
9	15-20° F.	9	82±1.2	72±1.4	52±1.4	51±2.0	1.49	6.33
12	14-22° F.	9	78±1.1	51±1.9	32±1.9	41±1.7	1.65	7.02
15	19-22° F.	4	95±0.9	76±2.1	37±2.7	59±3.1	2.20	9.33

(a) The data are not comparable for the different periods in the greenhouse since they were frozen at different temperatures.

ing to the survival data was more resistant to cold than was barley. Oats and barley appeared to have a significant difference in percentage survival at the six, twelve and fifteen day periods, when oats were more resistant to cold than barley.

The survival of wheat and oats showed a change in relationship at the six day period where oats proved to be more hardy than wheat which agrees with the injury data. A significant difference in survival between wheat and oats was found at every period tested while the injury data did not show a significant difference after the six day period.

Field Results

In the fall of 1932, triplicate eight foot rows of Rosen rye, Kanred wheat, Tennessee Winter barley, and Sporen Gray Winter oats were planted in two locations in the Kansas State College cereal breeding nursery. An estimate of the winterkilling made January 8, 1933, revealed a trace of injury in wheat and rye, while the injury in barley was estimated at ten per cent and in oats at 75 per cent. An extremely cold period February 4 to February 10 when the temperature reached -12° F. followed a period having a high average mean temperature and resulted in 100 per cent killing of all the crops. Total killing may have been partially due to the late planting of the crops.

In 1933, Dakold rye, Kanred wheat, Tennessee Winter barley and Sporen Gray Winter oats were planted at one place in the nursery in triplicate eight foot rows and at another in a single eight foot row. Notes taken January 10, 1934, showed a trace of injury in rye, five per cent in wheat, 35 per cent in oats and 35 per cent in barley. The winter was mild with the exception of one extremely cold spell late in February when the minimum temperature reached -16° F., during which the ground was covered with snow.

An estimate of the plant survival made April 26, 1934, showed that rye and wheat survived 100 per cent, oats 90 per cent and barley 72 per cent. This relationship was the same as was found by artificial refrigeration in 1933-34.

The results verify the supposition that winter-hardiness can not be accurately measured by field tests unless conducted for a number of years.

Salmon (37) has shown by means of inter-class correlation coefficients that artificial refrigeration was more reliable for predicting winter-hardiness in the Great Plains than was the survival of a single winter-hardiness nursery selected at random.

SUMMARY AND CONCLUSIONS

Injury and survival data were determined on more than 4800 four inch pots, or 24,000 plants of winter rye, winter wheat, winter oats, and winter barley which were artificially frozen during a period of two years. Eighty flats containing approximately 125 plants each also were used to measure total moisture, expressed juice, refraction of juice, and electrical conductance to supplement the artificial refrigeration in determining relative hardiness. These tests materially aided in studying some of the factors responsible for cold resistance of plants at different stages of hardiness.

The crops were tested in an effort to determine their relative hardiness and the rate of loss of hardiness when subjected to different periods of temperatures favorable for growth.

The average mean temperature over a relatively short period of time was found to be the main factor influencing the hardening processes in cereals through the winter months. Hardening was apparently the result of the inactivity of the plant and a decrease in total moisture which brought about an increasing concentration of the cell sap which was capable of retaining its moisture with a corresponding increasing force.

The rate of loss of hardiness depended primarily on the average mean temperature and on the length of time the plants were exposed to the temperature.

Wheat and rye lost hardiness more rapidly than either barley or oats and wheat more rapidly than rye during the first six days in the greenhouse as was shown by artificial refrigeration.

Total moisture increased for approximately seven days during the loss of hardiness, after which a maximum was reached. It was observed that expressed juice increased for several days after total moisture had reached a maximum, which probably was due to the slow adjustment of hydrophilic colloids and the consequent influence on the relation of free and bound water.

Martin and Newton (24) reported that the concentration, quality and state of dispersion or coagulation of colloids are factors affecting the degree of water binding.

The refractive index of the plant fluids, which is a measure of the total solids, decreased rapidly during the first 24 hours that plants were in the greenhouse after which the change was slower. The total solids reached a minimum after about ten days of greenhouse temperatures which was in accordance with the expressed juice determinations. The rapid decrease in the total solids during the first 24 hours was attributed to respiration processes, since the increase in total moisture was of little significance during that period. However, total moisture became the predominating factor in the decrease of total solids by dilution after the first 24 hours.

According to artificial refrigeration tests the loss of hardiness, although slow, was still taking place after 16 days of greenhouse temperatures. The total moisture, the expressed juice, and the refractive index did not consistently show a change in hardiness after being in the greenhouse nine days.

A calculated ratio representing the relation of the expressed juice to the total moisture minus expressed juice, aided materially in studying the moisture relations in the plant by placing the data on a comparable basis. A rapid decrease in the ratio after total moisture had reached its maximum was possibly due to the fact that a continued liberation of bound water took place due to the reaction of the hydrophilic colloids to the physiological activities in the plant.

Specific conductivity was found to be a fairly reliable test for cold resistance when used on hardened plants and also for detecting changes in the hardiness of a crop. It was not reliable for comparing different crops nor for plants having a small difference in cold resistance.

As the plants lost hardiness the differences in the cold resistance of the crops became smaller and the results of the various tests became more variable. However, the results with artificial refrigeration proved to be the least variable and the method was the most reliable test employed.

A significant change in the relative hardiness of rye, wheat, oats, and barley was shown. Wheat was more resistant than oats in the hardened condition but somewhat less resistant when the plants had been in the greenhouse for six days. This change in relationship was the result of wheat losing its hardiness more rapidly than oats.

In most cases it was less desirable to use survival of plants than injury as a criterion of hardiness, since larger numbers were necessary to obtain the same degree of accuracy.

The measurement of total moisture, expressed juice, refraction of expressed juice, and specific conductivity afforded important information on changes which took place in the plants of the four crops during the first nine days in the greenhouse. After the nine day period changes in cold resistance which were not indicated by these tests were shown by means of artificial refrigeration.

Due to relatively mild winter conditions that often prevail in this section of the country it was difficult to obtain dependable winter-hardiness information from field tests over a two year period.

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Professor H. H. Laude for his invaluable advice and assistance in planning and conducting the experiment and also for his help in the preparation and presentation of the material in the manuscript.

Thanks are also due Dr. John H. Parker for the planting of nursery plots and for helpful suggestions regarding literature.

The valuable assistance of Dr. E. B. Working in providing electrical equipment and the instruction on its use are gratefully acknowledged.

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