ARTIFICIAL REFRIGERATION AS A MEANS OF DETERMINING RESISTANCE OF CERTAIN SPRING WHEATS TO FROST

by

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INTRODUCTION

The average yield of wheat from fall sowing in the Columbia Basin of Oregon is about four bushels greater than from that seeded in the spring. This also applies to varieties capable of maturing a crop from either fall or spring seeding.

A large acreage in eastern Oregon is devoted to the growing, from fall sowing, of wheat varieties with spring growth habits. According to Hill (16),* Federation, a white spring wheat, is more uniformly distributed over the dry land section of Oregon than any other variety. This variety, when fall sown, generally heads from five to seven days earlier than most varieties of the Turkey type of winter wheat. Varieties with spring growth habits have an advantage in that their early maturity often enables them to escape hot winds and severe summer drouth at a critical period of development.

Field results indicate that there is a considerable difference in winterhardiness among the spring varieties grown in this section. As a rule, the snow covering is sufficient to prevent severe injury even when the winter temperatures fall below zero. Sometimes, however, considerable winter-

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*Numbers in parentheses refer to literature cited.

killing results, and re-seeding is necessary. Consequently, great importance must be attached to relative winterhardiness.

The experiments herein reported were conducted with the following objectives: (a) to determine the relative resistance of eleven varieties of spring wheat, sometimes fall sown in the Columbia Basin of Oregon, to low temperatures in the seedling stage, (b) to observe the value of artificial freezing as a means of determining resistance in relatively non-hardy varieties, as measured by the correlation with field results, (c) to note whether relative resistance remains constant at different periods of growth, and (d) to observe the effect of hardening on the frost resistance of spring wheat varieties.

REVIEW OF LITERATURE

The review of literature in this paper is divided into sections to enable the reader to get a clear picture of the possible causes and effects of low temperatures and other factors on winter survival. Some work with plants other than wheat is also cited. Previous work is reviewed under the following headings: (a) causes of winterkilling, (b) hardening, and (c) measures of hardiness.

Causes of Winterkilling

Akerman (3) stated that "as yet it does not seem possible to form a definite conception of the immediate causes of death from cold."

There are many possible causes of winterkilling, most of which relate directly or indirectly to low temperatures. It is with these that we are concerned here. Salmon (29) lists them as follows: (a) heaving, caused by alternate freezing and thawing, (b) smothering, in which frozen snow or an ice covering keeps out the air, (c) physiological drouth, where the plants cannot obtain moisture because of the frozen soil, and (d) the direct effects of low temperature. Akerman (2) observed these conditions, and noted that varieties frosted during the winter are less likely to survive spring drouth. Janssen (19) observed the influence of date of seeding winter wheat on winterkilling, and concluded that many late-seeded plants are killed by "heaving" in the spring. He believes that the injury is due primarily to the separation of the plant from the ground, and the consequential rapid drying of the crown.

Although, as pointed out by Maximov (25) and others, freezing to death is seldom the only cause of winterkilling, the varieties investigated by Akerman (3) all reacted in a similar manner toward the factors accounting for winterhardiness. Thus it seems that varieties which are capable of withstanding the direct effects of low temperatures generally are those most resistant to winterkilling.

The discussion which follows deals specifically with the direct results of low temperatures on plants. According to Wiegand (41), Sachs in 1860 and Nageli in 1861 showed that the expansion of water in the cell is not sufficient to rupture the cell wall, contrary to the theory of the early Greek philosophers. Wiegand states, as originally noted by Muller-Thurgau in 1880, that ice forms within the cells only on very rapid freezing. He believes that death is due to water withdrawal from the cells to form ice, and not to the cold as such. In the opinion of Maximov (25), however, there is a mechanical deformation of the protoplasm as a result of being compressed by ice. Wiegand (42) lists two theories to explain the movement of water from the cells to the intercellular spaces during ice formation. They are: (a) expulsion, due to actual contraction of the protoplasm, or more likely to a sudden change in permeability of the protoplasmic membrane to the solute, and (b) attraction, or the pulling action of the ice crystals already formed. Wiegand agrees with Abbe (1) and Chandler (6) in that the injury is caused by rapid evaporation rather than rapid thawing. Abbe considers that as the ice crystals in the intercellular spaces melt, the water is lost by transpiration, and the plant wilts. However, Akerman (2) found that death occurs when the plant is frozen, and not when it is thawed.

Harvey (13) considers that the term "hardiness" should be applied to the ability to survive ice formation within the tissues.

Several authors go a step further and list some of the chemical-physical effects of water withdrawal upon the protoplasm. Newton (24, 25) states that disorganization of the protoplasm, i. e., precipitation of the proteins by the increasing salt concentration or acidity of the cell sap, is a probable result of desiccation. He stresses the importance of intercellular adaptations to resist drying out. Martin (24) believes that the plant is killed because low temperatures coagulate and dehydrate the protoplasm to such an extent that it cannot take up water. Rosa (32) and Akerman (3) agree with Newton in listing the effects of freezing on plant tissue. Akerman considers that "every organism has a fixed minimum temperature below which it cannot live for long." The general consensus of opinion seems to have been summed up by Salmon (33) when he stated that desiccation of the protoplasm, mechanical injury caused by ice, chemical changes such as precipitation of the proteins, and suspension of metabolism are all direct effects of low temperature.

Hardening

Previous investigators have shown that even rather sensitive plants can withstand relatively low temperatures if first subjected to a period of hardening. Several methods of hardening have been tried, as measured by various physical and chemical tests.

In 1913, Chandler (6) published data which showed that slow wilting or partial withholding of water over a long period increased resistance to cold. Salmon and Fleming (34) and Harvey (13) obtained similar results. Work done by Janssen (20) demonstrated that plants are less succulent and consequently harden more when grown in a soil of low (10 per cent) moisture content. Newton and Brown (30) observed a greater reduction of moisture content in hardy varieties due to hardening.

After extensive trials, Hill (15) showed that hardening is very important in a winter hardiness test by artificial freezing. He induced hardening by subjecting the plants to temperatures near the freezing point, and found periods of from six to ten days to be satisfactory. Hill and Salmon (17) noted a marked difference in the amount different varieties can harden when exposed to the same conditions. Martin (24) states that "the hardier the variety the more it can harden."

Chandler (6) found that resistance to frost is increased by previous exposure to low temperatures. Bayles and Salmon (5) stated that a long exposure to medium low temperatures is necessary to build up maximum hardiness. They observed that the temperature necessary to secure marked injury gradually had to be increased as the season progressed. Akerman (2) discovered that wheat plants are more resistant during the coldest months, but become less so as the temperature rises in the spring. Steinmetz (37) arrived at the same conclusion with alfalfa plants. Akerman (3) found that careful technic is needed to detect individual differences between varieties. Plants should be of the same age and stage of development, and must have been exposed to low temperatures some time before being frozen.

Harvey (14) suggested that "hardening is a cold shock response, not correlated with the product of temperature and time exposure." Working with Jersey Wakefield cabbage, he found that plants exposed to 0°C for four hours a day were injured no more at -5°C than plants which had been continuously subjected to 0°C.

Sellschop and Salmon (36) increased the vegetative growth of certain plants by means of nitrate fertilizers, and found that greater injury was apparent.

Experiments reported by McCool (23) showed that fertilization of corn plants may decrease or prevent frost injury, because the freezing point of the sap is lowered by increasing the osmotic pressure.

Harvey (13) carried on extensive investigations with cabbage in relation to hardening. According to him, the growth rate is decreased so that the plants are smaller and more mature, and the leaves are considerably thicker. He watered plants with a solution of N/10 sodium chloride and found that growth was retarded and cold resistance increased. Harvey also noted that protein was less easily precipitated from the juice of hardened plants.

Newton (28), working with winter wheat, observed no relation between the volume of press-juice or the imbibition pressure of fresh leaves and resistance to cold with unhardened plants. However, there was a definite association when the plants previously had been exposed to hardening conditions. He also noted an increase in the quantity of sugar and hydorphylic colloids in hardened tissue.

Rosa (32), experimenting with certain vegetables, found that hardened plants retained more unfrozen water and had a greater total pentosan content. His work showed that hardened plants lose less moisture per unit of leaf area, because of a lower transpiration rate.

Martin (24) sums up the effects of hardening. He states that "during the hardening of wheats there is a decrease in moisture content, and an increase in total solids in the sap, freezing point depression of the sap, and imbibition pressure of the cell colloids." He adds that there are not always observable differences between varieties, as all the points mentioned fluctuate widely during the fall and winter.

According to Harvey (13), hardiness is acquired or lost in a short time. He noted that young cabbage leaves are less hardy than the old ones. In regard to age of plants, Klages (22) found that older wheat seedlings are more easily injured by low temperatures, and emphasizes the danger of too rank a growth in the fall. Klages exposed plants one, two, three and four weeks old to a temperature of -15.6°C for thirty minutes. All were killed excepting those one week old, which were not injured. Exposure to the same temperature for fifteen minutes gave the following results: wheat seedlings one, two, three and four weeks old were 0, 40, 20 and 100 per cent killed, respectively. Experiments by Bayles and Salmon (5) showed that hardiness decreased rapidly when the plants were taken into the warm greenhouse from the outside. The greater part of the loss occurred within twenty-four hours. Suneson (38) obtained similar results, and noted that the loss of resistance increased up to ninety-six hours of exposure to the warm temperatures. According to Chandler (6), Schaffnit found it much easier to precipitate the proteins of greenhouse grown rye than of that grown in the open.

Rosa (32) concluded that cold resistance will be increased by any form of treatment which materially checks growth.

Measures of Winterhardiness

Many investigators have attempted to associate some definite morphological structure with winterhardiness, but with little practical success. Certain characters may indicate hardiness, but there are always enough exceptions to make recommendations for their use unjustifiable. Thus, Baroulina (4) found that although the average length of the stomata tended to be greater with hardy varieties, the relation was not definite. Similarly, Govorov (11) could find no strict correlation between any morphological or anatomical character and hardiness. Govorov (12), however, did observe a connection between resistance and the distance from the seed at which the tillering node is developed. Resistant varieties develop the node lower when exposed to low temperatures, whereas the reverse is true for non-hardy wheats. Martin (24) and others have not been able to note a definite relation between dark color of leaves, spreading growth habit and hardiness.

Regardless of this, it is true that hardy varieties are more apt than not to possess certain of these characters.

Harvey (13) noted that the more resistant plants of tomato and cabbage are characterized by a heavier bloom on the surface of the leaves. Akerman (3) considers that undercooling is more probable in tissues with small cells. Consequently, they should be more resistant to ice formation.

Akerman of Sweden has been the leading exponent of the determination of sugar content as a criterion of cold resistance. This author (2) found a parallelism between winterhardiness and sugar content in hybrid segregates. He (3) states, however, that the samples must be taken when the weather is stable and the temperature low. Steinmetz (37) noted that a hardy variety of alfalfa contains more sugar, expressed in terms of total carbohydrates. According to Maximov (25), Lidforss was the first to publish work in regard to the protective role of soluble carbohydrates. Maximov considers that the protective action is physico-chemical, based on the concentration of the sugars. This increases the power of the cells to retain unfrozen water, and thus decreases the formation of ice. Meyer (26) believes that the protective action which sugars exert against the precipitation of proteins is important. This view also is held by Newton and Brown (30) and others. Coville (7) observed that the transformation of starch to sugar is a result of the protoplasmic membranes being rendered permeable to the amylytic enzyme by chilling. Newton (28) suggests that greater dormancy and a slower respiration rate may account for the lower rate of sugar loss from the leaves of hardy varieties in the late fall. Experiments conducted by Janssen (19) indicate a positive correlation between total soluble carbohydrates and optimum dates of seeding as measured by winterhardiness. The work of Newton (27), Meyer (26) and of Govorov (11, 12) has not shown a very close agreement between sugar content and hardiness.

Dexter et al. (9) determined that the frost resistance of plants may be measured by the diffusion of electrolytes and other substances from chilled or frozen tissues into water after the tissues have thawed. These authors obtained a fair correlation between hardiness and the degree of retention of electrolytes by alfalfa roots. Newton (27) concluded that there is no constant parallelism between the specific conductivity of the cell sap and relative frost resistance.

Dunn (10) attempted the use of the dye absorption test to determine hardiness in apples, but found the results too inconsistent. He considers this test just as accurate as any, however, and of value when used along with other methods. Dunn concludes from his results that other factors in addition to hydrophylic colloids are responsible for hardiness. In this regard, Maximov (25) maintains that changes other than the accumulation of sugars take place, as resistance to cold proceeds more rapidly than can be accounted for by this factor.

Govorov (11, 12) definitely states that there is no correlation between dry matter in the leaves and resistance to cold. Steinmetz (37) also found this to be the case. Govorov could find no difference in the percentage of dry matter of winter and spring forms.

On the other hand, Tumanov and Borodin (39) consider the determination of the dry matter in the expressed sap to be the best of the indirect methods. They believe that in many cases the resistance of a variety may be correctly estimated by this method. Akerman (2) found a close relation between dry matter content and hardiness only with wide differences in hardiness. Newton (27) observed no constant relation between these factors. Martin (24) considers that hardy wheats are characterized by a high percentage of total solids in the juice.

It has been observed by Tumanov and Borodin (39), Steinmetz (37), Salmon and Fleming (34), and Martin (24) that the determination of the freezing point depression of the expressed sap gives no absolute parallelism with winter-hardiness under normal field conditions. However, Salmon and Fleming (34) and Martin (24) noted a definite relation in the case of relatively non-hardy wheats when they were actively growing, as in periods of mild weather in the field, or in the greenhouse.

Observations made by Govorov (11, 12), Baroulina (4), Meyer (26) and others indicate that the osmotic pressure of the sap may be only a minor factor in cold resistance. In fact, Baroulina found that rye had a lower osmotic pressure than many varieties of wheat.

Newton (28, 29) found that the imbibition pressure of fresh leaves in the hardened condition generally was directly related to hardiness. The same author showed that the volume of press juice of hardened leaves is inversely proportional to hardiness. He emphasizes the importance of hardening before making either of these determinations. According to the results of Tysdal (40), however, hardiness cannot be reliably measured by determining the quantity of press juice.

The results of work by Newton (28), Martin (24), and Tysdal (40) and others showed that moisture content of the tissues was a fairly consistent index, being inversely proportional to hardiness.

The quantity of hydrophylic colloids in the press juice of hardened tissues, as measured by the bound water, is directly proportional to hardiness, as determined by Newton (28, 29) and Newton and Brown (30).

Newton and Brown (30) state that lipoids are of little importance in cold resistance.

Janssen (19) observed that wheat plants from the most favorable dates of seeding change the protein nitrogen to a form which is not precipitated by cold. Newton and Brown (30) found that the higher concentration of sugars in hardy varieties acts as a protection against protein precipitation. However, they were not able to find a correlation between the degree of protein splitting and relative hardiness.

Tysdal (40) used the viscosity of the cell sap as a measure of hardiness. He obtained a correlation of $.9000 \pm .0386$ between known rank and viscosity, and considers this property to be a consistent index of hardiness.

The use of artificial refrigeration as a measure of cold resistance is becoming increasingly important. Almost without exception, a high correlation has been obtained between results of this method and field data, provided the plants have had the proper environment before freezing. Akerman (2), Bayles and Salmon (5), Harvey (14), Hill and Salmon (17), Martin (24), Quisenberry (31), Steinmetz (37) and others have used this method successfully. These results indicate that artificial refrigeration is the most satisfactory test yet devised as a substitute or supplement for field data on winterhardiness.

MATERIALS AND METHODS

The experiments herein reported were conducted during the winter of 1930-31 at the Kansas Agricultural Experiment Station. The artificial freezing equipment used consists of a thick-walled chamber of sheet cork protected on the outside by a layer of concrete, and a direct expansion carbon dioxide refrigeration plant thermostatically controlled. The inside dimensions of the chamber are 4 by 10 feet with a capacity inside the coils of 43 cubic feet. The thermostat regulates the temperature within a range of approximately 6°F, although this range is somewhat less when temperatures as high as 20°F are being used. A minimum of about minus 30°F may be attained.

An electric fan placed near one end of the freezing chamber was fairly effective in promoting uniform temperatures throughout if the plants were not too tall. Wheat plants nearest the coils, however, when frozen in the boot or heading stage, were more severely injured than those farther removed.

The following varieties and strains of wheat were included: Federation, C. I. 4734; Hard Federation x Martin No. 995; Galgalos No. 39; Pacific Bluestem, C. I. 4067; Hybrid 143, C. I. 4513; Red Chaff Sel. W I; Hybrid 63, C. I. 4510; Hybrid 123, C. I. 4511; Little Club, C. I. 4065; Jenkin, C. I. 5177; Jenkin Sel. 160; Marquis, C. I. 4158; Hard Federation, C. I. 4733; and Hybrid 128, C. I. 4512. Marquis was included only in those trials in which the varieties were allowed to grow to the boot stage before freezing, as it is seldom if ever fall sown in Oregon. Hard Federation, a strictly spring wheat, was used as a non-hardy check, and Hybrid 128, a variety with winter growth habit, was used as a hardy check.

Wooden flats, 3 by 12 by 24 inches in dimensions, and adequately drained, were used for the bulk of the trials. Flats of this size are easily handled, and the conditions more nearly approximate those found in the field than can be obtained in pots. As suggested by Hill (15), flats take longer to freeze because of greater soil insulation, and the effect of this slower drop in temperature may show up in the rate of killing as compared to that in pots. However, this condition is similar to that found in the field and is a point in favor of flats. Competition between varieties in a flat did not appear to be a factor when freezing plants in the seedling stage, but becomes increasingly important in the later states of growth. Care must be used to get reasonably uniform stands, because thin rows tend to be more severely injured.

Border rows were placed one inch from each end of the flat. The varieties were grown 2 inches apart across the flats, giving rows 12 inches long and containing approximately 12 plants each.

The soil was kept moist by watering every other day.

Particular care was taken to have a uniform moisture supply in all the flats at the time of freezing.

The experiment was divided into three parts which hereafter will be referred to as classes I, II, and III. The varieties in class I were seeded in flats on September 22 and allowed to remain in the greenhouse until October 11. At this time they were taken outdoors and placed on the ground, thus permitting the varieties to harden off under more natural conditions. The plants were clipped before being moved outside, because of the excess of foliage produced by the high greenhouse temperatures. Half of the varieties were grown in one group of flats, and the other half in another. The two flats of each series were always treated alike. Twenty flats of each group were grown, each flat containing but one row of a variety, excepting checks, making a total of about 240 plants of each variety in the test.

Five flats of each group in class I were taken into the warmer temperatures of the greenhouse on November 13 and kept there for four or five days before freezing, to test the effect of loss of hardiness on relative rank.

Two check rows each of Hybrid 128 and Hard Federation were sown in each flat of class I. The results of one row each, however, were discarded because of being next to the border rows and showing some "border effect." It seems probable that if an electric fan had been used to keep the air in the chamber circulating, the effect on the plants of being near the edge of the flat and close to the coils would not have been so pronounced.

The wheat plants plainly showed the effects of close proximity to the coils for about three inches in from the ends of the flats. To test for differences in soil temperature, one thermometer was placed, with the bulb one inch deep in the soil, two inches from the end of the flat, one in the center and another half way between the two. Readings were taken at intervals all day beginning one hour after putting the flats in the freezing chamber. The temperature at the end of the flat at first dropped to about 0.5°C below that farther in, but after two hours the readings of all the thermometers were the same. Probably the variations causing "border effect" were confined to air temperatures in these experiments.

The plants in class I were frozen approximately eight weeks after emergence. The percentage of leaf injury was estimated about a week after freezing. The percentage of plants killed could not be determined accurately until about two weeks had elapsed.

The varieties used in class II were identical with those in class I except that Marquis was added, and Galgalos was included as the hardy check in place of Hybrid 128. Ten flats of each group, or approximately 120 plants of each variety, were seeded October 3 and moved into the outside enclosure on October 11. They were taken into the greenhouse December 12 where they remained until several varieties were in the boot and Hard Federation had partially headed, at which time the varieties were frozen. Outdoor and greenhouse temperatures to which the plants were subjected during this period are listed in Table 1 and 2.

Hard Federation, Pacific Bluestem and Galgalos, representing three degrees of relative hardiness, were seeded in 4-inch flower pots at times calculated to bring all three to the heading stage together. Pacific Bluestem and Galgalos head at approximately the same time under field conditions, but Hard Federation is ten to twelve days earlier. To obviate this difficulty, seedings of Hard Federation were made on the same date as that of Galgalos (October 16), and also at 5, 10, 15 and 20-day intervals later. Seed of Pacific Bluestem was planted October 16, and 5 days before and 5 days after as well. Twenty pots of each variety were sown on each date. Care was exercised to get the seed uniformly distributed around the pots about one inch from the edge. Each pot later was thinned to five plants.

More water is necessary to keep the outside pots moist where a number are grouped together, a fact which may be attributed to lower humidity along the border.

A uniform freezing period of 12 hours, beginning and ending at seven o'clock, was used throughout the experiments. This period was adopted because, as shown by Suneson (38), considerable time is required for soil temperature to approach that of the air. This is particularly true in the case of flats, where the insulation caused by soil is particularly obvious.

All coefficients of correlation were calculated from varietal ranks, as there was less fluctuation, for practical purposes, in ranking than in percentage injury.

Spearman's formula for rank correlation was used, and is as follows: $p = 1 - \frac{6 \Sigma d^2}{n\ (n^2 - 1)}$ in which p = the rank correlation coefficient; n = the number of cases; and d = the difference between ranks of paired measurements. The coefficient calculated by the above formula, according to Kelly (21), differs but slightly from that obtained directly from the scores, if ranks constitute the scores. However, if many individuals are given the same rank, as in the case of ties, considerable error may be present. The probable error formula for p as worked out by Pearson is $P.E._p = .7063 \frac{1-p^2}{\sqrt{n}}$, which Kelly states gives a figure about 5 per cent greater than the probable error of r.

Daily maximum and minimum temperatures for the period October 11 to December 11, inclusive, are listed in Table I. This period includes all the time that any of the plants in these experiments were subjected to outdoor hardening.

The average maximum and minimum greenhouse temperatures by weeks, for the periods November 15, 1930 to January 31, 1931, and February 11 to March 2, 1931 are given in Table II. The temperatures were sufficiently high at all times for the plants to continue growing.

Table I. Daily Maximum and Minimum Temperatures for Manhattan, Kansas, 1930

Date	October		November		December	
	Max.	Min.	Max.	Min.	Max.	Min.
1			68	33	39	25
2			67	30	52	19
3			69	26	36	24
4			71	29	36	32
5			57	29	34	31
6			52	19	37	29
7			61	23	51	24
8			68	41	55	26
9			71	47	57	33
10			72	37	57	40
11	87	56	72	43	53	28
12	76	65	72	41		
13	65	53	71	46		
14	65	54	65	55		
15	73	57	70	52		
16	69	40	54	29		
17	55	28	68	31		
18	50	28	74	39		
19	45	34	70	54		
20	37	25	63	37		
21	39	30	45	30		
22	43	36	50	28		
23	61	26	52	29		
24	70	27	40	29		
25	74	35	55	27		
26	67	50	36	20		
27	71	37	39	22		
28	59	42	43	20		
29	59	32	48	35		
30	47	28	49	30		
31	57	17				

Table II. Greenhouse Temperatures for the Periods November 15, 1930 to January 31, 1931, and February 11 to March 2, 1931

Week ending		Maximum	Minimum
November	21	62.5	47.6
	28	60.6	45.9
December	5	59.1	42.7
	12	62.1	47.0
	19	58.1	44.7
	26	57.4	37.3
January	2	45.3	38.0
	9	60.1	44.9
	16	58.1	41.1
	23	60.9	48.1
	30	66.1	48.6
February	16	69.0	51.4
	23	65.9	52.4
March	2	67.0	47.6

EXPERIMENTAL RESULTS

Field Experiments

Cereal nurseries were established at various locations throughout the Columbia Basin of Oregon in 1924 and 1925. Each variety has been grown in triplicate rod-row plots in three different series at each nursery at some time during the experiment. Climatic conditions vary considerably over this area, so killing temperatures sometimes were encountered in one section but not in another. The available information on percentage of winterkilling of varieties in these nurseries is presented in Table III. Field data on winterkilling are too limited to state definitely the relative ranks assumed by all varieties in this test. The data are summarized on the basis of results with Federation, since it was the only variety grown every year at all locations listed. The plus or minus percentage of winterkilling of each variety in comparison with Federation for comparable years is given. Although open to criticism, this method probably is the most satisfactory available, considering the limited data. For example, the strain of Hard Federation x Martin is given a relative rank of fourth, although apparently it was a little more hardy than the other varieties in the trial at Moro in 1930. No other data on this variety were obtainable. Hybrid 143 is placed ahead of Red Chaff, although the average injury of Red Chaff at Pendleton in 1927 and at Condon in 1930 was slightly below that of Hybrid 143. Results at Moro in 1928 are the cause of the latter reversal in ranking.

Probably these discrepancies are apparent rather than real, as general observations indicate that the first four or possibly six of the varieties listed in Table III are, for practical purposes, nearly identical in winterhardiness.

Table III. Percentage of Winter Injury of Fall-Sown Spring Wheat Varieties in the Columbia Basin of Oregon

Variety	Percentage of Winterkilling										± Federation for comparable years	Rank
	1924	1925		1927*	1928	1930						
							Condon					
	Moro	Pendleton	Dufur	Pendleton	Moro	Moro	1	2	3	Average		
Galgalos No. 39					1	4	10	5	15	10.0	+ 82.1	1
Hybrid 123					2	4					+ 81.0	2
Hybrid 63				5	5		7	10	20	12.3	+ 72.0	3
Hd. Fed. x Martin						2					+ 68.0	4
Hybrid 143				8	3	6	12	10	50	24.0	+ 66.8	5
Red Chaff W I				5		6	20	15	35	23.3	+ 58.7	6
Little Club							15	35	90	46.7	+ 46.6	7
Jenkin Sel. 160				15	12	75	50	80	96	75.3	+ 32.8	8
Jenkin	1	100	100	18	20		80	90	98	89.3	+ 28.4	9
Pacific Bluestem	15			30	60		70	97	99	88.7	+ 26.2	10
Federation	60	100	100	47	98	70	90	98	92	93.3	0	11
Hard Federation	93										- 33.0	12

*Frost 4/20/27

Cold Resistance of Hardened Plants

Fifteen flats of hardened plants of each group in class I were frozen at minimum temperatures of from 14° to 18°F during the period November 13 to 16. With the exception of lot 3, none of the lots was sufficiently injured to show differences between varieties. Repeated trials are necessary to find temperatures which will give satisfactory results with varieties of unknown cold resistance.

The conditions encountered by the varieties previous to artificial refrigeration, as noted by several investigators, also are of prime importance in determining lethal temperatures. There was a period of mild weather, as shown in Table I, just previous to the artificial freezing, but the varieties possessed considerable hardiness in spite of this.

As soon as it was apparent that the exposure in the freezing chamber had not been sufficiently severe, the plants again were moved outdoors.

All the hardened lots in class I were refrozen at 11°F for 12 hour periods from November 22 to 24. Results are summarized in Table IV.

The rank of Hybrid 128 is not included in the column of ranks, because these experiments were conducted primarily to determine cold resistance in spring wheats. The column of averages was calculated from a weighted average of each freezing lot, as the number of flats was not the same in every lot. It was thought not essential to keep the number constant, since results for each variety are in every case strictly comparable.

Percentages of leaf injury fluctuate considerably from one lot to another, but varietal ranks remain fairly constant among those varieties which differ significantly in hardiness. Hybrid 128, the check variety with winter growth habit, was consistently more cold resistant than any of the others. An important inconsistency of the artificial freezing results compared to field data was the rather high percentage of injury sustained by Galgalos. This variety has shown marked winterhardiness in the field, being injured very little more than true winter wheats in most seasons. Possibly Galgalos owes its relatively high winterhardiness to factors other than cold resistance, as Salmon and others have shown that these factors are sometimes of considerable importance.

Hybrid 63 and Hybrid 123 appear to be distinctly more resistant to cold, on the average, than the other spring wheats. The percentage of leaf injury of Hard Federation x Martin fluctuated from 10 to 95 per cent in the different freezing lots. This variety probably should not be grown where low temperature is likely to be a factor.

Table IV. Average Percentage of Leaf Injury, by Freezing Lots, of Wheat Varieties Grown Outdoors until Frozen

Variety	Nov. 15 16°F	Nov. 22 11°F		Nov. 23 11°F		Nov. 24 11°F	Average (a)	Rank
	N*	D	N	D	N	D		
Galgalos No. 39	42.5	53.3	34.3	10.0	13.7	12.7	29.1	6
Hybrid 123	5.5	19.7	4.3	8.0	9.0	4.7	8.8	2
Hybrid 63	4.0	17.0	5.3	6.0	5.7	3.3	7.2	1
Hd. Fed. x Martin	10.0	70.0	19.7	95.0	24.3	13.3	33.1	8
Hybrid 143	17.5	71.7	13.3	10.0	4.0	7.7	22.3	3
Red Chaff W I	20.0	91.0	21.7	10.0	9.3	26.0	32.9	7
Little Club	9.0	11.7	26.7	20.0	6.0	56.7	22.7	4
Jenkin Sel. 160	9.0	20.0	11.7	50.0	32.0	53.3	27.9	5
Jenkin	32.5	40.0	42.7	75.0	46.0	78.3	50.7	9
Pacific Bluestem	60.0	89.3	44.7	80.0	51.7	80.0	66.5	10
Federation	70.0	99.0	94.7	99.0	96.0	96.0	93.1	11
Hard Federation	93.8	100.0	99.2	100.0	99.7	100.0	96.0	12
Hybrid 128	3.5	5.5	2.5	3.5	2.2	2.2	3.3	

* Symbols N and D represent night and day freezing respectively.
(a) Weighted Average.

The spring varieties fit fairly well into four groups on the basis of resistance to cold as measured by leaf injury in this trial. Hybrid 63 and Hybrid 123 constitute the most hardy group, with Hybrid 143, Red Chaff, Jenkin Sel. 160, Little Club, Galgalos and Hard Federation x Martin next, Jenkin and Pacific Bluestem following, and with Federation and Hard Federation as the least hardy. However, if the percentages from the lot frozen during the day of November 22 were not considered, Hybrid 143 and Red Chaff would be more nearly where field results placed them. The average leaf injury of the hardened lots is significantly correlated with field results, as evidenced by a coefficient of .762 ± .085. Considering that factors other than cold resistance may influence winterhardiness, this figure is perhaps as high as would be expected.

A second note was taken several days after the one on leaf injury to determine the percentages of plants actually killed. The results are given in Table V. A rank correlation of .740 ± .092 was obtained between the average of these data and field results.

Table V. Average Percentage of Plants Killed, by Freezing Lots, of Wheat Varieties Grown Outdoors until Frozen

Variety	Nov. 22 11°F D*	Nov. 22 11°F N	Nov. 23 11°F D	Nov. 23 11°F N	Nov. 24 11°F D	Average	Rank
Galgalos No. 39	5.3	10.3	5.0	2.0	2.0	4.9	7.
Hybrid 123	1.3	0	0	1.7	0.7	0.8	2
Hybrid 63	1.0	0	0	0.7	0	0.4	1
Hd. Fed. x Martin	2.7	0	20.0	4.7	1.3	3.5	5.5
Hybrid 143	8.3	1.0	0	0	0.7	2.3	3
Red Chaff W I	30.0	1.0	0	2.0	2.0	8.1	9
Little Club	3.7	1.3	1	1.7	8.3	3.5	5.5
Jenkin Sel. 160	2.0	0	1	7.3	3.3	3.0	4
Jenkin	6.7	1.3	3	14.0	6.7	6.8	8
Pacific Bluestem	55.0	33.7	60	29.0	41.7	41.4	10
Federation	86.7	56.7	75	48.3	46.7	60.8	11
Hard Federation	97.5	95.8	100.0	85.8	91.5	93.4	12
Hybrid 128	0	0	0	0	0	0	

* Symbols D and N represent day and night freezing, respectively.

Recovery was practically complete with the lot frozen at 16°F, so no notes on percentage of killing were taken. Hybrid 63 and Hybrid 123 again appeared to have an advantage over the others, but if it were not for the first lot, Hybrid 143 and Red Chaff could be classed in the hardy group. Jenkin apparently has considerably more inherent resistance to cold than Pacific Bluestem, although the note on leaf injury showed them to be similar. The leaves, when succulent, of even the most hardy varieties may be nipped by frost, but the crowns and roots are more difficult to injure. Federation and Hard Federation also are much more sharply separated on the basis of plants killed. The average killing of Federation was 60.8 per cent, whereas leaf injury averaged 93.1 per cent. A comparison of these figures with those obtained from Hard Federation demonstrates the relatively superior resistance of Federation.

It is concluded that where spring wheat varieties are relatively rather widely separated in regard to hardiness, notes on leaf injury may be satisfactory. If the varieties are similar in reaction, a few more days must have elapsed after refrigeration so the percentage of plants killed may be determined. It also may be necessary to conduct freezing trials at several different temperatures to sharply delineate the place each variety occupies in relative cold resistance. However, a highly significant correlation coefficient of .946 $\pm$ .021 was obtained between ranks based on average leaf injury and percentage of plants killed of the varieties in a hardened condition.

Cold Resistance of Unhardened Plants

Five flats of plants from each group in class I were taken into the greenhouse on November 13 and kept there under warm conditions. Maximum and minimum weekly temperatures in the greenhouse during most of this period are reported in Table II. Lots 7 and 8 were frozen on November 17 and 18, respectively, at a minimum temperature of 21°F. With the exception of Hard Federation, very little injury was noticeable. As the plants doubtlessly had acquired some hardiness from the exposure to the low temperatures of the refrigeration chamber, they were again placed in the warm greenhouse for a few days. These lots were refrozen at 15°F on November 24 and 25, after which considerable injury was discernible, as shown in Table VI.

It readily may be seen that much more leaf injury resulted from freezing unhardened plants, compared to those hardened, even though the minimum temperature used was, with one exception, 4°F lower for the latter condition. In every case the average injury of the unhardened plants is higher. For example, the injury recorded for Hybrid 63 and Hybrid 123 was four times as great for the hardened as for the unhardened lots. However, the difference decreases from the hardy to the least hardy varieties, the percentages of injury for the least hardy wheats being practically the same under the two conditions. This tends to support the theory proposed by Martin (24) and others that the hardier the variety the more it can harden.

Table VI. Percentage of Leaf Injury, by Flats, of Wheat Varieties Frozen in an Unhardened Condition

Variety	Nov. 24 15°F N			Nov. 25 15°F D		Average	Rank
Galgalos No. 39	70	15	60	100	90	67.0	9
Hybrid 123	40	30	40	60	6	35.2	3
Hybrid 63	10	20	30	85	5	30.0	2
Hd. Fed. x Martin	60	50	40	65	50	53.0	6
Hybrid 143	40	5	25	60	10	28.0	1
Red Chaff W 1	50	8	10	90	20	35.6	4
Little Club	50	50	95	90	30	63.0	8
Jenkin Sel. 160	50	60	50	80	15	51.0	5
Jenkin	80	40	60	80	50	62.0	7
Pacific Bluestem	80	50	80	90	90	78.0	10
Federation	98	98	90	99	95	96.0	11
Hard Federation	100	99	100	100	100	99.8	12
Hybrid 128	7.5	17.5	12.5	20	8.5	13.2	

A study of the data in Tables IV and VI indicates that while fluctuations in percentage injury of different lots are very little greater with hardened than with unhardened plants in this experiment, the differences in hardiness between varieties are more sharply defined with hardened plants. A correlation of .636 ± .121 was found to exist between field results and leaf injury to unhardened plants artificially frozen. While this figure is somewhat lower than that calculated for the hardened lots, it nevertheless demonstrates a rather close relationship.

The percentage, by flats, of plants killed in lots 7 and 8 are shown in Table VII.

Table VII. Percentage of Plants Killed, by Flats, of Wheat Varieties Frozen in an Unhardened Condition

Variety	Nov. 24 15°F N.			Nov. 25 15°F D		Average	Rank
Galgalos No. 39	5	1	8	50	10	14.8	6
Hybrid 123	15	10	15	15	2	11.4	5
Hybrid 63	3	5	15	15	0	7.6	2
Hd. Fed. x Martin	5	5	20	10	15	11.0	4
Hybrid 143	1	1	5	10	1	3.6	1
Red Chaff W 1	1	2	2	30	5	8.0	3
Little Club	10	20	60	70	10	34.0	9.5
Jenkin Sel. 160	30	15	10	60	5	24.0	7
Jenkin	20	15	20	70	30	31.0	8
Pacific Bluestem	20	10	30	60	50	34.0	9.5
Federation	75	90	90	90	80	85.0	11
Hard Federation	100	95	100	99	100	98.8	12
Hybrid 128	1.5	3	4	5	1	2.9	

As was the case with hardened lots, the percentage of killing was, in general, proportional to the leaf injury first noted. In fact, the records taken on the two dates are very similar for the least hardy varieties.

A small percentage only of injury or killing may differentiate the hardiness of one variety from that of another, but the relation remains reasonably constant. The data recorded in Tables VI and VII for Federation and Hard Federation illustrate this point. Where first one variety and then the other leads in cold resistance, there probably is no practical difference.

The correlation between field data and the average percentage of unhardened plants killed by artificial refrigeration is .761 ± .086. This coefficient is slightly higher than that obtained between field results and leaf injury of the unhardened plants. The difference may be significant, because unhardened plants have succulent leaves which naturally exhibit considerable injury before the crowns and roots are greatly effected. However, a reasonably close association exists between leaf injury and plants killed, as shown by the high correlation coefficient of .911 ± .035.

Inter-lot Varietal Ranking Comparison

Data on the ranking of varieties based on the percentages of leaf injury in all freezing lots of hardened and unhardened plants are presented in Table VIII. It was deemed practical to consider the hardened and unhardened lots together, since a correlation of .888 ± .043 was obtained from the results of the two methods of handling. The ranks indicate that Hybrid 63 is more resistant to low temperatures in the seedling stage, as measured by leaf injury, than any other spring variety in the experiment. It ranks first four times out of a possible eight, and also was injured less than Hybrid 123 in four of the lots. The same line of reasoning places Hybrid 123 second and Hybrid 143 third. The next five varieties are difficult to classify into average ranks. However, on the basis of the number of lots in which one variety excels another, the ranking is as follows: Red Chaff, Jenkin Sel. 160, Little Club, Galgalos and Hard Federation x Martin. This differs slightly from the relative ranks as measured by the sum of the ranks from each lot, but is considered to more nearly approximate a true ranking of the varieties. Jenkin, Pacific Bluestem, Federation and Hard Federation occupy the ninth, tenth, eleventh and twelfth places, respectively. Sufficient difference in cold resistance exists between these four varieties so that they rather consistently make manifest their relative positions. A distinct difference also exists between Jenkin and the more hardy group of varieties.

Table VIII. Ranks of Varieties Based on Percentage of Injury

	Lot Number								
	1	2	3	4	5	6	7*	8*	Total of Ranks
	Number of Flats								
Variety	3	3	2	1	3	3	3	2	
Galgalos No. 39	6	8	9	4	6	4	5	10	52
Hybrid 123	3	1	2	2	4	2	4	1	19
Hybrid 63	2	2	1	1	2	1	1	3	13
Hd. Fed. x Martin	7	5	5	10	7	5	6	6	51
Hybrid 143	8	4	6	4	1	3	3	2	31
Red Chaff W 1	10	6	7	4	5	6	2	5	45
Little Club	1	7	3.5	6	3	8	9	7	44.5
Jenkin Sel. 160	4	3	3.5	7	8	7	7	4	43.5
Jenkin	5	9	8	8	9	9	8	8	64
Pacific Bluestem	9	10	10	9	10	10	10	9	77
Federation	11	11	11	11	11	11	11	11	88
Hard Federation	12	12	12	12	12	12	12	12	96

Inter-lot correlations based on leaf injury in each freezing lot are shown in Table IX. For some unknown reason, the data obtained from lot 1 are out of line both with field and artificial freezing results. However, in the whole group, there is only one coefficient which is not significant in relation to its probable error, this being between lots 1 and 7. The others range from .553 ± .142 to .951 ± .019, the majority being above .700. The constancy of the association between lots is due, to a considerable extent,

*Lots 7 and 8 were unhardened when frozen.

to the consistent behavior of Hybrid 63, Hybrid 123, Jenkin, Pacific Bluestem, Federation and Hard Federation. The relative ranks of the other varieties fluctuated much more, probably because of a similarity in cold resistance.

Table IX. Inter-lot Correlations Between Ranks Based on Percentage of Injury

Lots	2	3	4	5	6	7*	8*
1	.657±.116	.845±.058	.615±.127	.608±.128	.560±.140	.385±.174	.553±.142
2	---	.925±.029	.769±.083	.762±.085	.874±.048	.797±.074	.951±.019
3		---	.712±.101	.761±.086	.761±.086	.659±.115	.858±.054
4			---	.860±.053	.874±.048	.871±.049	.748±.090
5				---	.846±.058	.811±.070	.783±.079
6					---	.916±.033	.804±.072
7						---	.783±.079

*Lots 7 and 8 were unhardened when frozen.

Relative ranks from the percentage of plants killed are reported in Table X. The same difficulty, i. e., that of ranking the varieties occupying the medium hardy groups, was encountered with this data as with that concerning percentage of leaf injury. Differential killing might have been more pronounced between these varieties if lower refrigeration temperatures had been used. However, more intense cold would have completely killed the less hardy wheats, and probably the leaf growth of the medium resistant varieties would have been destroyed. The first three and the last four ranks were filled by the same varieties as were noted in connection with leaf injury.

Table X. Ranks of Varieties Based on Percentage of Plants Killed

	Lot Number							
	1	2	4	5	6	7*	8*	Total of Ranks
	Number of Flats							
Variety	3	3	1	3	3	3	2	
Galgalos No. 39	6	9	8	5.5	5.5	3	5	42
Hybrid 123	2	2.5	2.5	3.5	2.5	6	3	22
Hybrid 63	1	2.5	2.5	2	1	4	2	15
Hd. Fed. x Martin	4	2.5	9	7	4	5	4	35.5
Hybrid 143	8	5.5	2.5	1	2.5	2	1	22.5
Red Chaff W 1	9	5.5	2.5	5.5	5.5	1	9	38
Little Club	5	7.5	5.5	3.5	9	10	7	32.2
Jenkin Sel. 160	3	2.5	5.5	8	7	7.5	6	39.5
Jenkin	7	7.5	7	9	8	7.5	8	54
Pacific Bluestem	10	10	10	10	10	9	10	69
Federation	11	11	11	11	11	11	11	77
Hard Federation	12	12	12	12	12	12	12	84

*Lots 7 and 8 were unhardened when frozen.

Correlation coefficients between rankings in freezing lots, based on percentage of plants killed, are listed in Table XI. A reasonably close relationship is shown, as only one coefficient is below the limit set by three times its probable error.

Ranks from percentage of plants killed when unhardened compare favorably with those obtained with hardened lots, as evidenced by a correlation of .726 ± .097. The association, however, is not quite so close as that existing between rankings from leaf injury under the two conditions.

Table XI. Inter-lot Correlations Between Ranks Based on Percentage of Plants Killed

Lots	2	4	5	6	7*	8*
1	.857±.054	.614±.127	.633±.122	.734±.094	.452±.162	.769±.083
2	---	.740±.092	.642±.120	.831±.063	.663±.114	.776±.081
4		---	.871±.049	.797±.074	.708±.102	.712±.101
5			---	.841±.060	.694±.106	.864±.052
6				---	.820±.067	.927±.029
7					---	.666±.113

*Lots 7 and 8 were unhardened when frozen.

The correlation of leaf injury and actual killing is shown in Table XII. There is a certain amount of spurious correlation because the data from each lot are included in the average, but this should not be sufficient to render the results of doubtful value. Evidently considerable faith may be placed in the data from an individual lot, under the conditions of this experiment, regardless of whether leaf injury or percentage of plants killed is considered. The results of each lot tended to place the varieties into about three groups, viz., relatively hardy, medium and non-hardy, and the combined data permit of little further classification.

Table XII. Correlation of Results from Individual Freezing Lots with the Average Injury and Killing of All Lots

	Correlation Coefficient	
Lot	Injury	Killing
1	.699±.104	.734±.094
2	.909±.035	.804±.072
3	.911±.035	---
4	.881±.046	.785±.078
5	.937±.025	.885±.044
6	.867±.051	.990±.004
7*	.825±.065	.806±.071
8*	.909±.035	.951±.019

*Lots 7 and 8 were unhardened when frozen.

Data from individual freezing lots are compared with the ranking of the varieties under field conditions in Table XIII. The association is fairly close in each case, although some fluctuation is evidenced. The average correlation of either leaf injury or killing of the plants with field data is above .7.

Table XIII. Correlation of Field Results with Injury and Killing, by Lots, of Artificially Frozen Plants

	Correlation Coefficient	
Lot	Injury	Killing
1	.546±.143	.720±.098
2	.727±.096	.650±.118
3	.635±.122	. ---
4	.797±.074	.631±.123
5	.748±.090	.787±.078
6	.930±.027	.854±.055
7*	.811±.070	.761±.086
8*	.601±.130	.832±.063

Time of Heading and Cold Resistance

An association is generally considered to exist between time of heading and winterhardiness, particularly with varieties having a winter growth habit. Suneson (38) obtained a correlation of -.452 ± .0015 between cold resistance and

*Lots 7 and 8 were unhardened when frozen.

time of heading of 13 winter wheats. The relationship is not so constant with the spring varieties used in these experiments, as is shown in Table XIV. The more hardy varieties tend to be late, whereas Hard Federation, Federation and Pacific Bluestem, the least hardy of the wheats in the test, rank first, second and fourth, respectively, in earliness of heading. However, the variety Jenkin is one of the latest wheats in the test, and also one of the least hardy.

Table XIV. Ranks of Varieties Based on Time of Heading, Winterkilling in the Field, and Injury and Killing from Artificial Freezing

Variety	Ranks Based on			
	Time of Heading	Cold Resistance		
		Field	Artificial Freezing	
			Injury	Killing
Galgalos No. 39	5	1	8	5
Hybrid 123	9	2	2	3
Hybrid 63	9	3	1	1
Hd. Fed. x Martin	3	4	7	4
Hybrid 143	6	5	3	2
Red Chaff W 1	7	6	5	6
Little Club	9	7	4	8
Jenkin Sel. 160	12	8	6	7
Jenkin	11	9	9	9
Pacific Bluestem	4	10	10	10
Federation	2	11	11	11
Hard Federation	1	12	12	12

A coefficient of $-.280 \pm .188$ was procured between time of heading and winterkilling under field conditions. The correlation of heading with leaf injury and plants killed from artificial refrigeration was -.587 ± .133 and -.350 ± .179, respectively. None of these coefficients is highly significant in relation to its probable error. The fact that several of the varieties head at about the same time and also exhibit similar degrees of cold resistance tends to obscure the relation. It is concluded that while late heading indicates resistance to cold in many varieties, the association is not sufficiently constant to permit time of heading to be considered an accurate index of hardiness in spring wheats.

Comparison of Day and Night Freezing

Davis (8) observed that, in his experiments, plants frozen during the day were injured more severely than those at night. His explanation is that photosynthetic activity builds up the resistance of the plant during the day by increasing cell sap concentration, whereas at night this concentration drops. Hubbard (18) procured analogous results. However, Suneson (38) could find no constant association between injury and time of refrigeration.

Comparable lots from day and night freezing trials were compared as to percentage of leaf injury and plants killed. In every case the average leaf injury was less for plants frozen at night, as shown in Table XV. The average injury of all varieties was 50.6 per cent for day freezing compared

Table XV. Comparison of Leaf Injury from Day and Night Freezing of Hardened Lots Frozen at 11°F

Variety	Day: Injury (Per cent) Lot 1	Lot 6	Average	Rank	Night: Injury (Per cent) Lot 2	Lot 5	Average	Rank
Galgalos No. 39	53.3	12.7	33.0	3	34.3	13.7	24.0	8
Hybrid 123	19.7	4.7	12.2	2	4.3	9.0	6.7	2
Hybrid 63	17.0	3.3	10.2	1	5.3	5.7	5.5	1
Hd. Fed. x Martin	70.0	13.3	41.7	7	19.7	24.3	22.0	7
Hybrid 143	71.7	7.7	39.7	6	13.3	4.0	8.7	3
Red Chaff W 1	91.0	26.0	58.5	8	21.7	9.3	15.5	4
Little Club	11.7	56.7	34.2	4	26.7	6.0	16.4	5
Jenkin Sel. 160	20.0	53.3	36.7	5	11.7	32.0	21.9	6
Jenkin	40.0	78.3	59.2	9	42.7	46.0	44.4	9
Pacific Bluestem	89.3	80.0	84.7	10	44.7	51.7	48.2	10
Federation	99.0	96.0	97.5	11	94.7	96.0	95.4	11
Hard Federation	100.0	100.0	100.0	12	99.2	99.7	99.5	12
			Ave. 50.6				Ave. 34.0	

to 34.0 per cent for plants frozen at night. However, with the exception of two or three varieties, the ranks remained about the same, the correlation being .818 ± .071.

The difference in percentage of plants killed was not so pronounced, the average for all varieties being only 4.5 per cent more for day than for night freezing. Data showing this are presented in Table XVI. Possibly the cell sap concentration of the crowns does not fluctuate to the extent it does in leaves, in which case the number of plants killed should be less. A correlation coefficient of .750 ± .089 was obtained between varietal rankings from day and night freezing.

Table XVI. Comparison of Killing from Day and Night Freezing of Hardened Lots Frozen at 11°F

Variety	Day: Injury (Per cent) Lot 1	Lot 6	Average	Rank	Night: Injury (Per cent) Lot 2	Lot 5	Average	Rank
Galgalos No. 39	5.3	2.0	3.7	5	10.3	2	6.2	8
Hybrid 123	1.3	0.7	1.0	2	0	1.7	0.9	3
Hybrid 63	1.0	0	0.5	1	0	0.7	0.4	1
Hd. Fed. x Martin	2.7	1.3	2.0	3	0	4.7	2.4	6
Hybrid 143	8.3	0.7	4.5	6	1.0	0	0.5	2
Red Chaff W 1	30.0	2.0	16.0	9	1.0	2.0	1.5	4.5
Little Club	3.7	8.3	6.0	7	1.3	1.7	1.5	4.5
Jenkin Sel. 160	2.0	3.3	2.7	4	0	7.3	3.7	7
Jenkin	6.7	6.7	6.7	8	1.3	14.0	7.7	9
Pacific Bluestem	55.0	41.7	48.4	10	33.7	29.0	31.4	10
Federation	86.7	46.7	66.7	11	56.7	48.3	52.5	11
Hard Federation	97.5	91.5	94.5	12	95.9	85.8	90.9	12
		Ave.	21.1			Ave.	16.6	

Effect of Location in Refrigerator on Freezing Injury

Table XVII shows an average of the data procured from flats frozen in the end, intermediate and center positions in the refrigeration chamber. Results from four freezing lots are included. With two exceptions, all the varieties were injured more severely when directly exposed to the end coils in addition to those on the sides. This point also holds to a certain extent with percentage of plants killed, although the difference is not so pronounced. Evidently the air circulation in the chamber was not sufficient to counteract the effects of close proximity to the coils. If an electric fan had been run in the chamber, the differences probably would not have been noticeable.

Table XVII. Effect of Location in the Freezing Chamber on Percentage of Injury and Killing of Wheat Varieties

Variety	Injury			Killing		
	End	Intermediate	Center	End	Intermediate	Center
Federation	97.8	96.3	94.8	81.3	67.5	58.8
Hd. Fed. x Martin	52.0	43.3	27.8	2.8	3.5	6.8
Hybrid 128	7.0	5.5	6.5	0.5	0.3	1.3
Galgalos No. 39	70.0	11.3	31.0	12.5	0.5	3.8
Pacific Bluestem	91.8	42.5	57.5	63.8	7.0	32.5
Hard Federation	100.0	99.0	100.0	99.8	85.0	100.0
Hybrid 143	37.5	15.5	31.3	4.5	1.5	2.8
Red Chaff W 1	52.0	24.5	32.0	12.0	5.5	8.5
Hybrid 63	16.0	8.5	11.5	1.8	1.3	4.0
Hybrid 123	27.5	11.5	13.3	5.8	2.5	4.0
Hybrid 128	5.3	6.3	3.5	0.3	1.3	0.8
Little Club	21.3	18.3	31.3	7.0	5.3	15.3
Hard Federation	100.0	100.0	98.8	98.8	91.3	91.3
Jenkin	72.5	16.5	52.5	18.3	3.8	8.3
Jenkin Sel. 160	40.0	20.3	27.5	13.8	3.8	3.3

Varietal Resistance in or Near the Boot Stage

Late spring frosts are an important factor in spring wheat production in high altitudes, as they often occur just as the wheat heads begin to form. The varieties in class II were frozen when near the boot stage to test their reactions at this period. Although all the varieties were not in the same state of growth at freezing time (Table XVIII), the relations in growth habit nevertheless were similar to those encountered in the field.

Table XVIII. Stage of Growth of Each Variety at Time of Freezing, and the Average Percentage of Injury Resulting

Variety	Injury (Per cent)	Stage of Growth
Hard Federation	73.4	Headed - 90%
Federation	63.8	Early boot - 60%
Hd. Fed. x Martin	55.0	Very early boot - 5%
Galgalos No. 39	51.9	Very early boot - less than 1%
Pacific Bluestem	72.5	Very early boot - less than 1%
Marquis	67.3	No swelling
Hybrid 63	57.5	Leaf
Hybrid 123	47.3	Leaf
Hybrid 143	55.0	Leaf
Little Club	55.0	Leaf
Jenkin Sel. 160	66.3	Leaf
Jenkin	60.0	Leaf

Two freezing lots were subjected for 12 hours to a minimum temperature of 16°F, and all plants were killed.

Two other lots were frozen at a minimum of 22°F, and no injury was apparent. The latter two lots, hereafter designated lots 9 and 10, were refrozen about 5 days later, beginning March 7, at a minimum of 19°F, and a considerable amount of injury resulted. A difference of only 3°F was sufficient to change the reaction from no apparent injury to one in which the killing produced was very severe. This point in itself is enough to render results from freezing in the boot stage unsatisfactory, because of the difficulty of keeping the temperature within such narrow limits.

The injury recorded 5 days after freezing was final, as no recovery was apparent at any time. In some instances, as with Hard Federation, the leaves and heads would be killed without the culms showing injury for some time, demonstrating that the crowns and roots were still in fair condition. After several days the culms died, probably because a supply of carbohydrates was no longer available. Hard Federation, because of earliness and consequently thicker culms, exhibited less injury immediately after freezing than some of the other varieties, but the end result was the same.

The percentages of injury and the average ranks of varieties are given in Table XIX. By way of comparison, the ranking from field data is also included. The available data are too meagre to be the basis for many conclusions,

Table XIX. Comparison of Ranks from Field Winterkilling in the Seedling Stage with Those Based on Artificial Freezing in the Boot Stage

Variety	Field Rank	Artificial Freezing Injury					
		Lot 9		Lot 10		Average	
		Per cent	Rank	Per cent	Rank	Per cent	Rank
Galgalos No. 39	1	71.3	3	32.5	7	51.9	3
Hybrid 123	2	84.5	5	10.0	1	47.3	1
Hybrid 63	3	90.0	7	25.0	5	57.5	7
Hd. Fed. x Martin	4	65.0	2	45.0	9	55.0	5
Hybrid 143	5	90.0	7	20.0	2.5	55.0	5
Red Chaff W 1	6	75.0	4	27.6	6	51.3	2
Little Club	7	90.0	7	20.0	2.5	55.0	5
Jenkin Sel. 160	8	95.0	10.5	37.5	8	66.3	10
Jenkin	9	97.5	12	22.5	4	60.0	8
Pacific Bluestem	10	95.0	10.5	50.0	10	72.5	11
Federation	11	45.0	1	82.5	12	63.8	9
Hard Federation	12	94.7	9	52.1	11	73.4	12
Marquis	--	89.5	-	45.0	-	67.3	-
				Average*........		59.1	

*Excluding Marquis

but they at least show the wide diversity found between results of different freezing lots. No measurable correlation exists between varietal ranks in lots 9 and 10, as demonstrated by the coefficient .004 ± .204. Neither is there any very significant relation between individual lots frozen in the boot stage and field or artificial refrigeration results with seedlings. Although the average of lots 9 and 10 agrees surprisingly well with field data, the coefficient of correlation being .818 ± .067, it is probable that the figures merit very little confidence. In a general way, the varieties normally considered to be more hardy are towards the top in rank, but little faith can be placed in the data. Federation, for example, ranked first in one lot and last in the other.

However, a comparison of averages of lots 9 and 10 with data from leaf injury and plants killed in the seedling stage gave correlation coefficients of .643 ± 0.119 and 0.671 ± .112, respectively, indicating a definite relation. The average percentage of injury in the boot stage fluctuated from 47.3 to 73.4 per cent, with a mean of 59.1 per cent. These figures indicate that varieties of spring wheat differ relatively little in cold resistance at this stage. Bayles and Salmon (5) froze Galgalos, Jenkin, Pacific Bluestem, Federation, Marquis and Hard Federation in both the boot and seedling stages. Their data showed rather close agreement with that procured under field conditions in Oregon, particularly when the average of the boot and seedling stages was considered. They conclude, however, that the same relation between varieties may not be held at different periods of growth.

The relative injury of Marquis appears to be about where it has been placed from field observations, i. e., between Federation and Hard Federation. The leaves of Marquis retained their green color for some time after thawing, in contrast to Galgalos whose leaves immediately changed color. Differences in appearance after freezing also exist between other varieties.

The stages of growth in which the varieties happened to be when frozen doubtlessly influenced the results to some extent. There was no constant relation exhibited between hardiness and stage of development in this test, however. Hard Federation, for example, ranked twelfth in hardiness and about 90 per cent of the plants were fully headed. In contrast to this, Jenkin Sel. 160 ranked tenth in hardiness and was still in the leaf stage. However, Hybrid 123 was first in average ranking and had not yet grown beyond the leaf stage.

Cold Resistance in the Heading Stage

As a further check on relative resistance to cold of wheat in different stages of development, the varieties Galgalos, Pacific Bluestem and Hard Federation were grown to the heading stage before being frozen. They were sown in 4-inch pots, later thinned to 5 plants per pot, at times calculated to bring them to the heading stage together. This material was frozen by lots, beginning March 8 for 12 hours at 19°F. The data procured are presented in Table XX. The temperature used was a little too severe for most satisfactory results, as no pots of Hard Federation, and only one of Pacific Bluestem and three of Galgalos showed signs of life five days after freezing. There was evidence of differential mortality after about two weeks. From one to five new tillers appeared in each pot of Galgalos. New growth developed in some instances in Pacific Bluestem, but all the plants of Hard Federation were dead. The results are entirely in accord with winterkilling of the varieties under field conditions, and also agree with artificial freezing data from the varieties when frozen in the seedling and boot stages. These three varieties differ noticeably in cold resistance at all stages of growth. It is probable, however, that with varieties of nearly corresponding hardiness, the range of temperature between perfect survival and entire killing is too small to permit of varietal differentiation.

Table XX. Percentage of Leaf, Culm and Head Injury, and Number of Plants Killed When Frozen in the Heading Stage

Percentage Injury (By Pots)			Plants Killed (By Pots)		
Galgalos	Pacific Bluestem	Hard Federation	Galgalos	Pacific Bluestem	Hard Federation
100	100	100	3	5	5
100	100	100	0	5	5
100	100	100	0	5	5
100	100	100	2	5	5
100	100	100	4	5	5
100	100	100	0	1	5
100	100	100	4	5	5
100	100	100	0	5	5
95	100	100	0	5	5
100	100	100	4	5	5
100	100	100	4	5	5
100	100	100	4	5	5
100	100	100	4	5	5
100	100	100	3	5	5
100	100	100	0	4	5
85	100	100	0	4	5
60	85	100	0	3	5
100	100	100	0	5	5
100	100	100	0	5	5
100	100	100	0	1	5

SUMMARY

The experiments herein reported were conducted with the following objectives: (a) to determine the relative resistance of eleven varieties of spring wheat, sometimes fall sown in the Columbia Basin of Oregon, to low temperatures in the seedling stage, (b) to observe the value of artificial freezing as a means of determining resistance in relatively non-hardy varieties, as measured by the correlation with field results, (c) to note whether relative resistance remains constant at different periods of growth, and (d) to observe the effect of hardening on the frost resistance of spring wheat varieties.

The following varieties of spring wheat, in order of winterhardiness based on field data, were subjected to artificial refrigeration during the seedling stage and at or near the boot stage: Galgalos, Hybrid 123, Hybrid 63, Hard Federation x Martin, Hybrid 143, Red Chaff W 1, Little Club, Jenkin Sel. 160, Jenkin, Pacific Bluestem, Federation and Hard Federation. Marquis was included in the boot stage trials. Galgalos, Pacific Bluestem, and Hard Federation were also frozen after heading.

A correlation coefficient of .762 ± .085 was computed from data on winterhardiness under field conditions and leaf injury of hardened plants artificially frozen in the seedling stage. The coefficient between field data and percentage of hardened plants killed was .740 ± .092.

Where spring wheat varieties are relatively rather widely separated in regard to cold resistance, notes on leaf injury of hardened plants are satisfactory. If the varieties are similar in reaction, the percentage of plants killed must be determined to delineate the position each one occupies.

Correlation coefficients of .636 ± .121 and .761 ± .086 were obtained between field results and leaf injury and plants killed, respectively, of unhardened varieties frozen in the seedling stage.

Differences in hardiness between varieties were more sharply defined when the plants have been hardened. However, a high correlation existed between results from hardened and unhardened lots.

The correlation of leaf injury in hardened with that in unhardened lots was high, as evidenced by the coefficient of .888 ± .043. A coefficient of .726 ± .097 was obtained from percentages of plants killed under the two conditions.

Data from individual freezing lots are about as accurate, on the whole, as the average results from all lots, when taken in the seedling stage.

The association between time of heading and resistance to cold is not sufficiently constant to permit its use as an index of hardiness in spring wheat.

The average leaf injury was greater for plants frozen during the day than during the night. The difference based on percentage of plants killed was not very pronounced.

There was a "place effect" from location in the refrigeration chamber. Leaf injury was most severe for plants directly exposed to the end coils in addition to those on the sides.

There is a definite association between relative amount of injury from freezing in the seedling and in the boot stages. However, the fluctuation between data from different lots in the boot stage trials is too great to permit much confidence being placed in the results. A difference of only 3°F in the minimum temperature of the refrigerator chamber was sufficient to change varietal reaction from no apparent injury to one in which the killing produced was very severe.

Results from freezing varieties in the heading stage were in accord with winterkilling under field conditions and with cold resistance in the seedling and boot stages. It is probable, however, that the range of temperature between perfect survival and entire killing of the plants is too small to permit of differentiation between varieties of nearly corresponding hardiness.

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