EFFECTS OF VARIOUS SOAKING AND FREEZING TREATMENTS AS FACTORS IN DETERMINING WINTER HARDINESS OF SELECTED WHEAT VARIETIES

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EFFECTS OF VARIOUS SOAKING AND FREEZING TREATMENTS AS FACTORS IN DETERMINING WINTER HARDINESS OF SELECTED WHEAT VARIETIES

INTRODUCTION

Winter-hardiness is a major problem in much of the winter wheat growing area of the United States. Winter wheat varieties generally outyield spring wheat varieties when they survive the winters in good condition. Much work has been done to develop winter-hardy varieties, especially in the north central part of the United States where the winters are usually very severe. Here in the Northwest, work on winter-hardiness has not kept pace with other developments in wheat improvement. In this area the winters are generally rather mild compared with the northern Great Plains, but occasionally, severe winters take a heavy toll of the wheat crop. The varieties here do not need the same degree of winter-hardiness as those in the northern Great Plains, but they should be sufficiently hardy to withstand the more severe winters. Because the severe winters are rather infrequent it is difficult to obtain reliable tests of winter-hardiness in the field within the space of a few years.

It is very difficult for the wheat breeder to carry

on very many different lines from any one wheat cross because of the limitations of time and space to care for them. Because of the lack of sufficient seed the selections have not been tested for winter-hardiness until they were far enough along to be tested for yield at many locations and this is usually after most selections are discarded. Therefore, there is a high probability that some selections with more winter-hardiness and just as good in other agronomic factors are discarded, when the plant breeder depends upon field conditions for testing.

Considerable research had been done on the factors involved in winter-hardiness and a number of attempts have been made to devise a rapid and easy method to measure the winter-hardiness in plants. Controlled freezing of wheat seedlings in laboratory cold chambers was tried and has proven quite successful. Quisenberry (20, p. 45) pointed out that the high correlation of differences in varietal resistance to laboratory freezing with varietal differences of winter-hardiness in the field indicates resistance to freezing damage is the major factor in winter killing. Controlled freezing of wheat seedlings in laboratory cold chambers is frequently used to determine resistance to freezing damage of varieties. Although the freezing of seedlings in cold chambers has been a major

improvement in the time and space required to test varieties for freezing tolerance, it still requires considerable time, labor and space to grow the seedlings and test them in cold chambers. A more rapid test requiring less space would be desirable to facilitate handling of large populations in breeding wheat for winter-hardiness.

Recently Ivanoff (12, pp. 90-94) has reported that controlled soaking and freezing of oat seeds, of varieties known to differ in cold resistance in the field, caused differences in their germination which were highly correlated with their winter-hardiness. The report by Ivanoff prompted this study. The purpose of these experiments was to determine the feasibility of using soaking and freezing treatments on wheat seeds similar to those used by Ivanoff, as a means of determining varietal differences in winter-hardiness.

LITERATURE REVIEW

Winter-hardiness in plants is a complex, physiological condition, having many ramifications. Investigations have been made attacking the problem from different angles. Considerable knowledge has been gained concerning some of the mechanisms involved in winter-hardiness which has been of help in developing defenses against winter-killing and in breeding winter-hardy plant varieties. All of the investigations point up the fact that there are many factors involved in winter-hardiness and that many genes may be involved in its inheritance. Harvey (9, pp. 223), Luyet (17, pp. 341), Levitt (16, pp. 211) and others have compiled comprehensive bibliographies and reviews of much of the work done on the winter-hardiness of plants. Their purpose was to summarize the information on winter-hardiness and make it available, thus enabling investigators to make better use of other research on winter-hardiness, when attacking the problem of reducing or preventing winter injury to our useful plants. Levitt (16) has prepared an excellent review of much of the work that has been carried out on winter-hardiness prior to 1941 and organized it under various phases of winter-hardiness along with some of the

theories concerning the factors involved in it and presented the evidence both for and against these theories.

The Search for Methods to Determine Relative Winter-Hardiness

When it was discovered that many of the factors for winter-hardiness in plants are heritable, considerable effort was made to find quick reliable methods of determining differences in winter-hardiness among varieties of the same species. Attempts have been made to relate certain morphological, physiological and physico-chemical characteristics of plants with their relative winter-hardiness. The use of electrical conductivity of expressed juice from leaves and stems of plants, as an estimation of winter-hardiness was proposed by Dexter and his co-workers (7, pp. 215-223; 8, pp. 63-78; 6, pp. 721-726) and by Megee (19, pp. 685-697). Tysdal (26, pp. 219-240) suggested that the enzymatic activity of alfalfa varieties could be used as an index of their winter-hardiness. Van Doren (27, pp. 392-401) found a high correlation between the amount of bound water, electrical conductivity of expressed juice and winter-hardiness.

Different chemical and physical techniques reported to give good results in testing winter-hardiness were tried by Martin (18, pp. 493-535), who found they were

not satisfactory in selecting hardy segregates from his hybrid wheat material. He concluded that the best laboratory determinations of winter-hardiness could be made by artificially freezing plants. Hill and Salmon (10, pp. 933-937); Quisenberry (20, pp. 45); Suneson and Peltier (23, pp. 50-58) and Weibel and Quisenberry (30, pp. 336-343) have used the artificial freezing technique and reported that it is satisfactory, at least in preliminary testing for winter-hardiness, as a selection tool in obtaining relatively hardy varieties of wheat. Clark (4, pp. 64) studied various factors involved in artificially freezing eight wheat varieties which varied from very winter-hardy to non winter-hardy varieties. The factors included stage of growth, hardening temperature, hardening time, effect of light during the hardening process and different freezing temperatures. He presented data to show that under properly controlled conditions the eight varieties when frozen artificially had a survival ranking which closely paralleled that found for the same varieties under winter conditions in the field.

Freezing of Seeds

In 1950 and 1951 Ivanoff (11, pp. 13-14; 12, pp. 90-94) reported on a new approach for testing and

selecting for winter-hardiness in oats. He subjected oat seeds, of several oat varieties having known winter-hardiness, to soaking and freezing treatments. He presented data to show that there was a high correlation between the germination percentage of the seeds following treatment and their known winter-hardiness in the field. Because this technique appeared to offer promise as a quick reliable test for winter-hardiness in oat varieties, this study was initiated to determine its usefulness in testing wheat varieties for winter-hardiness. Although Ivanoff did not mention it, a review of the literature revealed that in Russia Ivanov (13, abst. 929) reported in 1936 that he subjected soaked wheat kernels from several varieties to frost and then attempted to germinate them. He concluded that the germination percentages of the different varieties used corresponded closely to their frost and drought resistance.

Through the efforts of Brown and Escombe (3, pp. 1); Thiselton-Dyer (25, pp. 1-2) and Becquerel (2, pp. 2) it was learned near the beginning of the twentieth century, that air dry, (below 12 percent moisture), seeds of cereals and of many other plants could be subjected to the very low temperatures of liquid air and liquid hydrogen, -185°C. to -250°C. and lower, without injuring their germinating

ability. Wright (31, pp. 337-344); Adams (1, abst. 653); Steinbauer (21, pp. 281-286) and others froze moist and swollen seeds at various temperatures and found a rapid decrease in germination at the higher moisture levels. Jensen (14, pp. 630-631) reported that the length of soaking time affected germination, much more in proportion than did the length of freezing time. He also reported that, when the seeds had above 25 percent moisture, sudden freezing and thawing at any temperature and for any length of time greatly reduced germination and few wheat kernels germinated when frozen after soaking for more than four hours. Soaking periods of ten and thirty minutes and of one and two hours did not materially decrease germination.

Three investigators, Wright (31) working with wheat, and Kiesselbach and Ratcliff (15, pp. 96) working with corn, claimed their experiments showed that the longer moist kernels were exposed to freezing, the more serious was the injury to them.

Tautphous cited by Wright (31, pp. 338) and Wright (31), reported that rapid thawing of moist seeds after freezing greatly reduced germination as compared with the germination after slow thawing.

Quality, source and the condition of seed were

shown by Suneson and Peltier (24, pp. 687, 693), to have an important bearing on the winter-hardiness of plants arising from them. De Candolle (5, pp. 258) remarked that few seeds will react to environment in the same way or at the same time. The reasons he gave were differences in the heredity and differences in physical and chemical make up caused by environmental differences even when the seeds come from the same plant and from the same seed pod. At the optimum conditions many seeds will germinate at approximately the same time but as temperature and other factors tend toward the extremes fewer seeds will germinate.

Jensen (14) stated that freezing of seeds containing over 25 percent moisture delayed germination of those seeds that survived. Wright (31) also noted that freezing caused slower germination of surviving seeds.

Stucky and Curtis (22, pp. 815-833) stated that the seed coats of wheat did not interfere with the rapid absorption of water. Their line graph representing the percent germination for every percent increase in moisture of Minhardi, a very winter-hardy variety, and Leap, a non-hardy variety indicated the same general pattern but with rather wide fluctuations. However their graph also shows that Leap approached zero in germination percentage

at 32 percent moisture while Minhardi fluctuated between 20 and 50 percent germination until about 34.5 percent moisture then went rapidly down to zero by 35 or 36 percent moisture.

Considerable work has been done on winter-hardiness in plants. Numerous attempts have been made, without success, to develop a rapid and reliable test for winter-hardiness. The most successful laboratory test of winter-hardiness in wheat has been to grow the seedlings in the greenhouse, harden them off at temperatures slightly above or below freezing, then artificially freezing them in refrigeration units. The effects of freezing on seeds of high and low moisture contents have been reported by several workers, however, little has been reported on varietal differences in the reaction of seeds of the same species to soaking and freezing.

MATERIALS AND METHODS

The objective of this research was to ascertain the feasibility of using the germination percent of wheat varieties following soaking and freezing treatments as indices of their relative winter-hardiness. The work was initiated in April 1955 and terminated in February 1956 and was conducted in the Farm Crops Department, Oregon State College. The importance of the following factors were considered in planning the experiments:

1. Kernel size.
2. Soaking temperature with or without freezing.
3. The length of soaking time.
4. Freezing temperature.
5. The length of time frozen.
6. The rate of thawing after soaking and freezing.
7. The source, age and condition of kernels and their reaction to soaking and freezing.
8. Different combinations of the various soaking, freezing and thawing treatments on kernels and their relationship to winter-hardiness.

Two varieties of wheat were used in the initial experiments. They were Minter, a very winter hardy, hard red winter wheat and Federation, a non winter-hardy, soft, white spring wheat. In later experiments Hymar, a

semi-hard, white club, winter wheat was added. Hymar is one of the most hardy white wheats grown in the Northwest but it is well below Minter in winter-hardiness. Hymar was added to facilitate finding a treatment that would not only separate extremes, but also distinguish the intermediates from the extremes. Finally fifteen different wheat varieties which ranged in scale of winter-hardiness from that of Minter to that of Federation were used. The range of varieties provided a final criterion of cold tolerance as indicated by the germination following soaking and freezing. Summaries of winter-hardiness data (28, pp. 58) and 29, pp. 2), obtained from field trials over several years and several locations were supplied by Dr. O. A. Vogel, coordinator of wheat research in the Pacific Northwest, and these results were used in ranking the wheat varieties used in these experiments.

The following procedure was used in preparing the samples of seed. Several samples of each kernel size and sample size used from each of the three varieties involved in eleven of the experiments were counted out and then weighed on a torsion balance. These weights were then averaged for each of the kernel sizes and each size sample from each variety so that the many samples needed could be weighed rather than counted out. In two of the

experiments involving moisture percentage, a chainomatic balance, accurate to five decimal places, was used. However, all of the samples in the final three experiments were counted out by hand. The seed samples were stapled in cheesecloth bags and tagged with the variety name and the experiment and treatment numbers for identification.

In the early experiments galvanized steel buckets of three gallon capacity were filled with water of the desired temperature and the water from the tap was allowed to run into the bucket constantly to maintain the temperature of the water and to prevent injurious effects of stale water on seeds. The samples of seed were placed in the buckets and left to soak for a specified time. This method was not entirely satisfactory because of fluctuations in the temperature of the tap water which necessitated almost constant checking and adjustment to maintain a constant soaking temperature. It was soon found that a soaking time of less than 20 hours was sufficient to cause a high absorption of water by the kernels even at low soaking temperatures. Information was obtained that indicated that wheat kernels could be soaked in still water for as high as 24 hours without harming their germinating ability.[1] In the remaining experiments the

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[1] This information was obtained by personal communication from Dr. M. C. Parker, Oregon State College Seed Testing Laboratory.

kernels were soaked in buckets of water that were placed in constant temperature germinators. Experiments were run to determine the effect of soaking temperature and soaking time alone, without freezing, on the germination of the kernels used.

A walk-in cold chamber and the freezing compartment of a home size refrigerator were used to freeze the kernels in the first three experiments. Those kernels frozen at temperatures slightly below freezing were placed in the walk-in cold chamber and those frozen at the lower temperatures were frozen in the refrigerator. In the later experiments only the large cold chamber was used because the refrigerator had wide temperature fluctuations and unsatisfactory controls.

In most of the experiments, when the kernels were removed from the cold chamber they were thawed in running, tap water of 10°C. for 15 or 20 minutes. Later, a rate of thawing experiment was conducted and as a result the last three experiments had different methods of thawing.

The procedure used to determine the germination after treatment conformed to the official seed testing rules for wheat. The kernels were removed from their cheesecloth bags and rolled up in wet paper toweling and placed into a germinator. They were kept at a constant temperature of 20°C. Counts were made of the number

germinating on the 4th, 6th, and 8th or 9th days after they were placed in the germinator. The kernels were not considered germinated unless the coleoptile and the roots were normal and unless they and the plumule inside the coleoptile were twice the length of the kernel. This was selected as a measure of germination because many kernels after treatment germinated only enough to break the seedcoat and then died. Some were abnormal in that only the roots would develop or only the coleoptile, and in others only the roots and the coleoptile emerged from the kernel, without the inner leaf. In some of the rest, the coleoptile split or broke down near the kernel and the plumule emerged from the break.

Except for a complex factorial design used in experiment III the type of experimental design used in all the experiments was a completely randomized factorial. A condensed summary of the treatments used in the experiments is found in Table 1. For detailed information of the factors used in these experiments see Appendix tables 1, 2, 3 and 4 and tables 2 through 17 in the section on experimental results.

TABLE 1

Summary of the variables used in Experiments 1 to 14

Experiments	Factors								
	Varieties	Seed Size	Seed Source	Soaking Temp.	Soaking Time	Freezing Temp.	Freezing Time	Rate of Thawing	Table No.
1	x	x		x					2
2	x	x		x		x			3
3	x	x		x	x	x	x		4
4	x			x	x		x		5
5	x			x	x				6
6	x			x	x				7
7	x			x	x				8
8	x			x	x		x		9,10
9	x			x	x				11
10	x				x			x	12
11	x								13
12	x								14,15
13	x								14,15,17
14	x		x						16,17

EXPERIMENTAL RESULTS

The first three experiments were designed to gain preliminary information on the effect of soaking and freezing treatments on the germination of wheat in an attempt to relate the germination percentage to the known winter-hardiness of the varieties. Three kernel sizes, two varieties and two ranges of soaking temperatures were the variables studied in Experiment I. The results are given in Table 2. A comparison of the three kernel sizes indicated that following these treatments the smaller sizes had a somewhat lower germination percentage. The 16°-21°C. soaking temperature resulted in about one-fourth as many kernels of both varieties germinating after freezing as germinated when frozen following soaking at 9° to 10° C. Minter had a somewhat better average survival than did Federation.

The effect of seed size was studied again in Experiment II using the same two varieties, and two ranges of soaking temperature. Two ranges of freezing temperature were also used. As shown in Table 3, the -13° to -30°C. temperature was too severe for determining differences between seed sizes and varieties.

All three kernel sizes of Federation had a higher germination percentage than any of the kernel sizes of

TABLE 2

Germination percentage of two wheat varieties as affected by differences in kernel size and soaking temperature when soaked for 4 hours and frozen at -4°C. for 24 hours. One sample of 500 kernels used per treatment. (Experiment I)

Kernel Size	Soaking Temperature					
	9°-10°C.			16°-21°C.		
	Federation	Minter	Average	Federation	Minter	Average
over 7/64"	99	96	98	25	19	22
6/64" 6.5/64"	28	99	64	6	31	19
under 5/64"	32	58	45	7	25	16
Average	53	84	69	13	25	19

Germination percent of the untreated checks. Minter size over 7/64" 87%; 6/64"-6.5/64" 87%; under 5/64" 85%. Federation (composite sample) 97%.

Minter when frozen at -2 to -4°C. irrespective of the soaking treatment used. The differences in germination among the three seed sizes were not the same as those found in Experiment I. A possible explanation for the greater germination of Federation over that of Minter is that the moist kernels of Federation were not injured seriously when frozen at temperatures slightly below the

freezing point and the fact that the Minter seed used, although the check had 87 percent germination in Experiment II, was later found to have been in poor condition and was deteriorating rapidly in viability.

TABLE 3

Germination percentage of two wheat varieties as affected by differences in kernel size, soaking temperature and freezing temperature when soaked for 4 hours and frozen for 72 hours. One sample of 500 kernels used per treatment. (Experiment II)

Freezing Temperature	Seed Size	Soaking Temperature					
		10°-11°C.			19°-22°C.		
		Federation	Minter	Average	Federation	Minter	Average
-2° to -4°C.	over 7/64"	88	50	69	69	55	62
	6/64" 6.5/64"	82	47	65	74	41	57
	under 5/64"	88	49	69	59	41	50
Average		86	49	68	67	46	56
-13° to -30°C.	over 7/64"	0	0	0	0	0	0
	6/64" 6.5/64"	0	0	0	0	0	0
	under 5/64"	0	0	0	0	0	0
Average		0	0	0	0	0	0

The germinating percentages obtained in Experiment III are presented in Table 4 along with the averages for the three kernel sizes. Very little information could be gained from data obtained from this experiment because of the nature of the experimental design. The only reliable comparisons are among the three kernel sizes. The small kernels had nearly a third lower average germination than that of the large kernels. The kernels of medium size germinated nearly as well as the large kernels following treatment.

The data from Experiment IV are summarized in Table 5. The germination percentage for the three-day freezing time was obtained from one observation, but the germination percentages of the seven and eleven day freezing periods were averages of two observations. The germination percentage was very low for all three varieties when frozen at -10°C. for three, seven and eleven days following soaking at 30°C. for four, eight and sixteen hours. It seems evident from these data that a ten degree rise in soaking temperature reduced germination approximately the same extent as did a doubling of the soaking time. For all varieties the germination percentage was reduced more when frozen for three days than it was after seven days and was highest at the end of eleven days freezing time. These differences were greatest when the

TABLE 4

Germination percentage of Federation as affected by differences in seed size, soaking time and temperature and freezing time and temperature. One sample of 500 kernels used per treatment. (Experiment III)

Soaking Temperature (°C.)	11				21		
Soaking Time (hours)	16				4		
Freezing Temperature (°C.)	-4		-29		-4	-29	
Freezing Time (hours)	24	96	24	96	96	96	Ave.
Kernel Size over 7/64"	77	60	0	0	90	0	38
6/64"-6.5/64"	71	63	0	0	80	0	36
under 5/64"	44	47	0	0	64	0	29

TABLE 4a

Germination percentage of Minter as affected by differences in seed size, soaking time and temperature and freezing time and temperature. One sample of 500 kernels used per treatment. (Experiment III)

Soaking Temperature (°C.)	11		21				
Soaking Time (hours)	16		4				
Freezing Temperature (°C.)	-4	-29	-4		-29		
Freezing Time (hours)	96	96	24	96	24	96	Ave.
Kernel Size over 7/64"	73	20	24	37	0	0	26
6/64"-6.5/64"	68	15	16	43	0	0	24
under 5/64"	64	11	12	30	0	0	20

seed was soaked at 10°C. for four hours prior to freezing. Federation slightly surpassed Minter in germination percentage in many of the treatments used. This, as it was mentioned previously, may have been due to the poor quality seed of Minter that was used. Hymar germinated much better than either Federation or Minter for most of the treatments applied. Because Minter was much lower in germination than Hymar, Experiment V was set up to study the effect of soaking treatments alone on the germinating ability.

A study of the effect of soaking times and soaking temperatures without freezing on the germination of three wheat varieties was made in Experiment V. These results are shown in Table 6 and they are an average of two observations for each of the two longest soaking periods but only one observation for the shortest soaking period. There was no reduction in germination percentage of the Federation and Hymar varieties at any of the three soaking temperatures and soaking periods tested. The variety Minter showed a slight decrease in germination when soaked at the lower temperatures and also for the shortest soaking periods. At the highest soaking temperature and longer soaking periods the germination of Minter was reduced about 20 percent less than that of the untreated check. The germination of the untreated check was 83 percent; this

TABLE 5

Germination percentage of three wheat varieties after freezing at -10°C. as influenced by differences in soaking temperature and time and freezing time. One sample of 1000 kernels was used in each of the three day freezing treatments and two samples of 1000 kernels each were used in each of the seven and eleven day freezing treatments. (Experiment IV)

Varieties		Federation			Hymar			Minter			Grand Average
Freezing times (days)		3	7	11	3	7	11	3	7	11	
Soaking Temperature	Soaking Times										
10°C.	4 hrs.	16	28	54	49	67	69	30	29	48	43
	8 hrs.	4	9	18	23	24	40	4	9	13	16
	16 hrs.	1	2	7		3	8	3	3	5	
20°C.	4 hrs.	2	7	17	17	23	37	5	12	14	15
	8 hrs.	1	2	2	1	1	5	0	0	5	2
	16 hrs.	0	0	1	0	1	4	0	2	5	1
30°C.	4 hrs.	0	4	12	2	5	14	0	3	8	5
	8 hrs.	0	1	5	0	4	5	0	1	4	2
	16 hrs.	0	0	1	0	0	3	0	0	2	1
Grand Average		3	6	13		14	21	5	7	12	

was four percent lower than it had been when checked six months earlier.

The information obtained in Experiment V indicated that the Minter seed being used in the experiments was weak and easily injured by soaking treatments so a new lot of Minter seed was obtained from the Minnesota Agricultural Experiment Station, St. Paul, Minnesota. This new lot of Minter seed was subjected to the same treatments used in Experiment V and like Hymar and Federation the germination percentage was not affected by the soaking treatments. The germination percentage of the untreated check was 93 percent.

Federation, Hymar and Minter were tested for differences in the amount of water absorbed during various soaking treatments in Experiments VI and VIII. The purpose of these two experiments was to see if differences in the germination among the three varieties of wheat following soaking and freezing treatments could be related to differences in moisture absorbed during the soaking treatments. The moisture percentages by variety and treatment are presented in Table 7 and 8. The total percent moisture recorded for each treatment was based on the oven dry weight and was an average of two observations. When

TABLE 6

Germination percentage of three wheat varieties as influenced by different soaking temperatures and times. One sample of 200 kernels was used in each of the 4 hour soaking treatments while the 8 and 16 hour treatments had two samples of 200 kernels each. (Experiment V)

Varieties	Federation			Aver-age	Hymar			Aver-age	Minter			Aver-age
Soaking Temperature °C.	10	20	30		10	20	30		10	20	30	
Soaking Time (hrs.)												
4	98	98	98	98	95	96	94	95	76	80	74	70
8	98	98	98	98	96	96	98	97	80	78	64	74
16	97	98	98	98	96	97	96	96	78	80	62	73
Average	98	98	98	98	96	96	96	96	78	79	67	75

Germination percent of untreated seed

Federation	98.0
Hymar	94.7
Minter	83.0

TABLE 7

Total percent moisture based on oven dry weight of the samples of three wheat varieties following soaking treatments at three temperature levels and three lengths of soaking time. Two samples of 200 kernels each used in all treatments. (Experiment VI)

Varieties		Federation			Average	Hymar			Average	Minter			Average
Soaking Temperature °C.		10	20	30		10	20	30		10	20	30	
Soaking Time (hrs.)	4	33	44	54	44	34	43	50	42	32	40	48	40
	8	42	56	67	55	40	52	62	51	36	50	61	49
	16	56	65	74	65	52	61	69	61	49	58	68	58
Average		44	55	65	55	42	52	60	51	39	49	59	49

TABLE 8

Total moisture percentage, based on oven dry weight, of the samples of three wheat varieties following soaking treatments at two temperature levels and two lengths of soaking time. Two samples of 200 kernels each used in each treatment. (Experiment VII)

Varieties	Federation		Average	Hymar		Average	Minter		Average
Soaking Temperature °C.	10	20		10	20		10	20	
Soaking Time (hrs.) 1	28	30	29	26	30	28	24	27	28
2	31	36	34	31	35	33	28	32	32
Average	30	33	32	28	32	30	26	30	30

soaking the wheat seeds, the average percent moisture in the kernels increased approximately 10 percent with every ten degrees increase in temperature for any given period of time. A doubling of the soaking time at any given temperature also increased the moisture in the kernels by 10 percent. Federation, Hymar and Minter each differed in the amount of moisture absorbed. Federation had the highest percent absorption followed by Hymar and then Minter. An analysis of variance was calculated and these results are presented in Table 9.

Experiment VIII was run to determine what the effect

TABLE 9

Analysis of variance of the percent moisture contained in three wheat varieties following various soaking treatments. (Experiment VI)

Source of Variation	Degrees of Freedom	Mean Squares	F
Varieties	2	135.89	31.46**
Soaking temperatures	2	1,764.66	408.49**
Soaking times	2	1,679.49	388.77**
Soaking temperature x varieties	4	2.98	
Soaking time x varieties	4	4.59	
Soaking temperature x soaking time	4	20.80	
Experimental error	8	15.42	
Sampling error	27	2.78	
Total	53		

of the lengths of freezing time at intervals up to nineteen days had on the germination of two varieties frozen at one temperature following a soaking treatment. The germination percentages shown in Table 10 are averages of two observations. The reduction in germination percentage resulting from all freezing treatments was consistently greater for Federation than for Hymar. The germination percentages for both varieties were somewhat reduced at the three and five

TABLE 10

Germination percentage of two wheat varieties following soaking at 20°C. for four hours and freezing at -10°C., as affected by eight different lengths of freezing time. Two samples of 200 kernels each were used in each treatment. (Experiment VIII)

Varieties	Federation	Hymar	Average
Days frozen			
1	4	17	10
3	4	16	10
5	2	12	7
7	3	19	11
10	9	20	14
13	14	22	18
16	31	40	36
19	23	39	31
Average	11	23	17

day freezing period below that observed after one day of freezing. By the seventh day the germination percentages were the same as that found on the first day of freezing. The germination at the end of eleven days was higher than at the end of seven days of freezing. This trend continued through the thirteen days of freezing. Finally at the end of sixteen days the germination was 23 to 27 percent higher

than at the end of one day of freezing. When the freezing time was extended to nineteen days the reduction in germination percentage became greater again. Federation took the greatest drop which was eight percent lower than at the sixteen day freezing time. Hymar had but a one percent drop in germination percentage. The freezing periods providing the widest spread between varieties were the seven and the nineteen day periods. The thirteen and sixteen day freezing periods provided the least differences between Hymar and Federation.

A new lot of Minter was used in Experiment IX along with seed of Hymar and Federation. The effect of the one and two hour soaking periods at both the 10° and 20°C. soaking temperature was compared with the effect of the four hour soaking period at the same temperatures on the germination of the wheat kernels when frozen at -10°C. for three days following the soaking treatments. There was only a slight reduction in germination of all three varieties when soaked for one hour at 10°C. There was a marked reduction in germination of all three varieties when soaked for one hour at 20°C. The treatment which seemed to place the three varieties best in their respective winter-hardiness ranking was the soaking treatment for two hours at 20°C. The germination of Federation was only nine

percent, Hymar 32 percent and 35 percent for Minter. The data are presented in Table 11 and are averages of two observations for each treatment.

TABLE 11

Germination percentage of three wheat varieties soaked at 10°C. and 20°C. for one, two and four hours, then frozen at -10°C. for 3 days. Two samples of 200 kernels each were used in each treatment. (Experiment IX)

Varieties	Federation		Average	Hymar		Average	Minter		Average
Soaking Temperature °C.	10	20		10	20		10	20	
Soaking Time (hrs.) 1	88	37	62	91	54	72	84	44	64
2	68	9	38	83	32	58	66	35	50
4	21	2	12	40	14	27	36	13	24
Average	59	16	38	71	33	52	62	31	46

Only the 20°C. soaking temperature and the two and four hour soaking times were used in Experiment X. Four observations were taken for each treatment. A comparison of the data presented in Table 12 with that in Table 11 shows that Federation had a 23 percent higher germination for the two hour soaking period at 20°C. in this experiment

than it did for the same treatment in Experiment IX. Hymar was nine percent higher while Minter had a one percent higher germination percentage. The germination after freezing following the four hour soaking period at 20°C. was somewhat higher than for the same treatment in Experiment IX with Minter having a five percent higher germination percentage, Federation two percent and Hymar a two percent higher germination percentage. The reason for such fluctuations in germination percentage between experiments for the same treatment was not known. Such variability would have to be controlled before such treatments could be used in a test of winter-hardiness.

TABLE 12

Germination percentage of three wheat varieties soaked at 20°C. for two and four hours, then frozen at -10°C. for 3 days. Four samples of 200 kernels each were used in each treatment. (Experiment X)

Varieties	Federation	Hymar	Minter	Average
Soaking time (hrs.)				
2	32	41	36	36
4	14	16	18	13
Average	18	28	27	24

Experiment XI was designed to test the effect of four different thawing treatments on three varieties of wheat. The data, presented in Table 13 which are averages of four observations for each treatment, show about a 10 percent difference in the percent germination, for all three varieties, between the treatment producing the highest germination percentage and that producing the lowest germination percentage. A surprising observation made was that all three varieties had slightly less reduction in germination percentage when thawed in water at 30°C. for twenty minutes than when thawed at 10°C. for an hour. The two treatments which ranked Minter, Hymar and Federation best, in accordance with their known winter-hardiness, were thawing in water at 30°C. for twenty minutes and thawing in air at room temperature for one to two hours.

Seeds of fifteen varieties of wheat, that are known to range widely in winter-hardiness, were used in Experiments XII and XIII. The treatments included two combinations of soaking, freezing and thawing procedures. These were selected from treatments which in the earlier experiments seemed to give the most consistent differences in the germination percentages of Minter, Hymar and Federation. The fifteen varieties were ranked in order of decreasing germination percentage after treatment. A

TABLE 13

Germination percentage of three wheat varieties as affected by different thawing treatments following soaking at 20°C. for 2 hours, and freezing at -10°C. for 3 days. (Experiment XI)

Thawing Procedure	Varieties			
	Federation	Hymar	Minter	Average
1. In air at 23°C. to 24°C. for 1 to 2 hours	22	37	38	32
2. In wet paper towels at 21°C.	16	38	34	29
3. In water at 10°C. for 1 hour	13	28	26	22
4. In water at 30°C. for 20 minutes	16	30	33	26
Average	17	33	33	28

correlation of rank was calculated from differences between the treatment ranking and the known winter-hardiness ranking of the fifteen varieties for both Experiment XII and XIII. These data are presented in Table 14. These data show that the correlation coefficients indicated little and no correlation between the treatment ranking the varieties in the two experiments and their winter-hardiness ranking. The correlation coefficients are +0.31 for Experiment XII and zero for Experiment XIII.

TABLE 14

The varieties used in Experiments 12 and 13 with their known winter-hardiness ranking. Also their survival ranking by experiment and the correlation of rank for each experiment to the known field rank. (Experiments XII, XIII)

Varieties	C.I. No.	Field Rank	Experiment XII Survival Rank	Experiment XIII Survival Rank
Minter	12138	1	2	5
Columbia	12928	2	12	15
Kharkof	1442	3	7	1
Rio	10061	4	6	4
Wasatch	11925	5	1	14
Hymar	11605	6	4	3
Orf. x Hyb. 50-3	12692	7	5	6
Rex	10065	8	15	13
Triplet	5408	9	9	11
27-15 x RR #41	12696	10	13	10
RR x A P8-1	12694	11	11	12
Brevor	12385	12	14	9
Elmar	12392	13	10	7
Orfed	11913	14	3	8
Federation	4734	15	8	2
Correlation of Rank		0.31	0.0	

Seed of eleven of the fifteen varieties used in Experiments XII and XIII came from a varietal trial grown near Corvallis. Because these seeds should be more uniform than seeds from several locations, a correlation of rank was run on these eleven varieties. These data are presented in Table 15. The correlation was better as it increased from +0.31 to +0.50 for the eleven varieties in Experiment XII. This, however, was barely significant at the five percent level of significance. It changed from zero to 0.29 in Experiment XIII. The latter correlation coefficient was still not significant at the five percent level.

Experiment XIV was designed to see if seed of wheat varieties from different sources would have approximately the same germination percentages when subjected to the same soaking, freezing and thawing treatments. There were great differences in germination percentages between sources of the same variety as shown in Table 16. Orfed from Moro, Oregon, which was hand threshed had about 95 percent germination following treatment. This is considerably higher than the highest of the other seed lots. The other source of Orfed and the other varieties used were machine threshed in addition to coming from other locations.

Experiment XIV was run as part of Experiment XIII so that a comparison between the germination percentages of

TABLE 15

The varieties used in Experiments 12 and 13 which came from the same source, a varietal yield nursery at Corvallis. Also their known winter-hardiness rank and their rank as determined by their survival percent in each of the two experiments. The correlation of rank is included. (Experiments XII, XIII)

Varieties	C.I. No.	Field Rank	Experiment XII Survival Rank	Experiment XIII Survival Rank
Kharkof	1442	1	4	1
Rio	10061	2	3	4
Hymar	11605	3	1	3
Orf. x Hyb. 50-3	12692	4	2	5
Rex	10065	5	11	11
Triplet	5408	6	6	9
27-15 x RR #41	12696	7	9	8
RR x A P8-1	12694	8	8	10
Brevor	12385	9	10	7
Elmar	12392	10	7	6
Federation	4734	11	5	2
Correlation of Rank			0.53	0.29

TABLE 16

Two sources of three wheat varieties used in Experiment XIV. The germination percentage of the check samples and their germination percentage after soaking, freezing and thawing treatments. (Experiment XIV)

Varieties	C.I. No.	Source	Age (Months)	How Threshed	Percent Germination	Adjusted Germination Percent
Orfed	11913	Moro,Ore.	6	Hand	100.0	92.9
Orfed	11913	Varietal Trial Corvallis,Ore.	6	Machine	97.5	26.6
Hymar	11605	Pendleton,Ore.	18	Machine	95.5	26.2
Hymar	11605	Varietal Trial Corvallis,Ore.	6	Machine	97.5	32.2
Federation	4734	Pendleton,Ore.	18	Machine	99.0	16.3
Federation	4734	Varietal Trial Corvallis,Ore.	6	Machine	98.0	33.0

both sources of three of the fifteen varieties used in Experiments XII and XIII and the germination percentages of the other twelve varieties could be made. The data are presented in Table 17. The hand threshed seed of Orfed from Moro, Oregon, had nearly 93 percent germination following treatment while Kharkof, the next highest ranking variety in percent germination, had only 38 percent. The seed of Kharkof and the other varieties used in the experiments were all machine threshed. Federation and Hymar seed from Corvallis was third and fourth highest respectively in germination percentage in Experiment XII. The seed of the same two varieties from Pendleton, Oregon, had a lower germination percentage with Hymar ranking tenth and Federation last. The variety Minter ranked in sixth place, as a result of the treatments in Experiment XIII, so the relative rank of Minter compared to that of the Hymar and Federation from Pendleton, Oregon, was similar to their relative positions in winter-hardiness rankings. It is obvious from these observations that the environment in which the wheat seeds were grown and harvested greatly affected the percent of the seeds which germinated following soaking, freezing and thawing treatments.

The results from Experiments XII, XIII and XIV showed that there were wide differences in the germination percentages of various wheat varieties following soaking,

freezing and thawing treatments. The differences in germination of seed of the same wheat variety grown at different locations were very large. The treatments, used in Experiments XII and XIII on the wheat varieties grown and threshed under the same comparable conditions, did not reduce their germination percentage in direct proportion to their winter-hardiness.

TABLE 17

The varieties used in Experiment XIII including two sources of three varieties which were included for Experiment XIV. Rank in germination following treatment, germination percentage and winter-hardiness rank are also included. (Experiments XIII, XIV)

Variety	C.I. No.	Rank in Germination	Germination Percentage	Winter-hardiness Rank
Orfed*	11913	1	92.9	14
Kharkof	1442	2	37.9	3
Federation	4734	3	33.0	15
Hymar	11605	4	32.2	6
Rio	10061	5	32.1	4
Minter	12138	6	30.6	1
Orf. x Hyb.50-3	12692	7	29.6	7
Elmar	12392	8	28.0	13
Orfed	11913	9	26.6	14
Hymar*	11605	10	26.2	6
Brevor	12385	11	25.8	12
27-15 x RR #41	12696	12	24.6	10
Triplet	5408	13	23.8	9
RR x A P8-1	12694	14	23.5	11
Rex	10065	15	22.0	8
Wasatch	11925	16	21.8	5
Columbia	12928	17	21.6	2
Federation*	4734	18	16.3	15

* Included for Experiment XIV

DISCUSSION

A published report by Ivanoff (12, pp. 90-94) describing a new laboratory technique for obtaining winter-hardy strains of oats prompted this study. The seeds of several oat varieties that ranged in winter-hardiness from non-hardy to hardy were soaked and frozen. The data showed that the germination percentage of the oat varieties following treatment had a direct relationship to their winter-hardiness. Such a technique, if it could successfully be employed in testing winter-hardiness of wheat varieties, would be a much faster and easier method of selecting the winter-hardy lines from segregating wheat hybrids.

The results of these studies indicate that it is apparently more difficult to develop a laboratory test for winter-hardiness involving soaking and freezing wheat seeds than it is with oat seeds.

It was apparent during the preliminary experiments that the seeds of various wheat varieties do not germinate equally well following soaking and freezing treatments. However, these differences did not always relate to the winter-hardiness of the varieties involved. The lower freezing temperatures seemed to best provide a variation in germination percentage between the winter-hardy

varieties and the non-winter-hardy variety. This may indicate that there is some relationship between the expression of winter-hardiness of a variety and the ability of its seeds to germinate after soaking and freezing.

It was thought that the size of the wheat kernels might be important in the ability of a variety to withstand soaking and freezing treatments. The results showed that the intermediate and large size kernels did not vary widely in germination percentage following treatment but the small size seed averaged somewhat lower. This relationship held for both the non-winter-hardy and the winter-hardy varieties involved. The fact that small seeds were affected adversely by the soaking treatment whereas the larger seeds were not would indicate somewhat less vigor in the smaller seeds. This would be a natural assumption. In view of the small differences obtained, it would not be necessary for wheat seed to be sized when testing for varietal differences in germination following treatment, unless there were large differences between the average kernel size of the varieties used.

Soaking treatments alone did not lower the germination of wheat when the seed was in good viable condition.

The total percent moisture contained in wheat kernels following various soaking treatments differed

significantly among the three varieties tested. Following any one soaking treatment the non-winter-hardy variety, Federation, contained the highest percent moisture. Hymar, the intermediate variety, was second in moisture absorbed and the most winter-hardy variety, Minter, absorbed the least water. These differences in moisture absorbed were not great but they were constant. Although it was demonstrated that higher moisture contents tend to lower germination following freezing, there were times when the germination of Federation exceeded that of Minter and Hymar after treatment and many times that Hymar exceeded Minter in percent germination. Evidently within small limits the varieties which absorbed more water could in some instances, withstand freezing better than the variety with a lower moisture content. The above results and observations illustrate some of the many factors involved which modify the effects of soaking and freezing treatments on the germination of wheat seeds.

Seed of the same wheat varieties obtained from different sources varied widely in germination percentage following soaking and freezing treatments despite close agreement in the germination percentages of the untreated check samples. In some instances the variation in germination between different seed sources of the same

varieties was nearly as great as the extremes in germination between varieties. It is well known that the environmental conditions to which a seed is exposed during its development may affect its value as seed, i.e., its ability to grow. Some variation within varieties, therefore, would normally be anticipated.

The coefficient of correlation that was calculated on the treatment rankings from Experiments XII and XIII was found to be only +0.31 from Experiment XII, and the correlation was zero for Experiment XIII. These data indicate little relationship between the treatment ranking and winter-hardiness ranking in either experiment. Eleven of the fifteen varieties used in Experiments XII and XIII came from uniform nursery varietal yield trial. The seed of these varieties should have been very uniform since the varieties were grown and threshed under the same conditions. The correlation coefficient for the eleven varieties was +0.50 in Experiment XII which was barely significant at the five percent level. The ranking of the eleven varieties in Experiment XIII had a correlation coefficient of only +0.29. These results indicate that the seed treatment rank of the wheat varieties correspond more closely to their winter-hardiness rank when the seed of all the varieties is grown and threshed under the same conditions. However, the

relationship of the treatment rank to the winter-hardiness rank was still not very close. The differences between treatment rank and winter-hardiness rank may be due to small injuries to the wheat kernel inflicted by a threshing machine. Some wheat varieties may be more easily damaged than others in the threshing operation. These injuries may be so slight that they have little or no effect on the germination when the seeds are not subjected to freezing after soaking. One such injury may be a cracking or rupturing of the seedcoat of the kernel which renders it more permeable to moisture. Such kernels would absorb more moisture when soaked within a given time than kernels which do not have their seed coats broken. There is evidence from Experiments XIII and XIV that machine threshed wheat seed will not germinate as well as hand threshed wheat seed following soaking and freezing treatments. The germination of the hand threshed seed of Orfed from Moro, Oregon, was reduced little by the treatment. The germination of the machine threshed seed of Orfed from Corvallis and also the seed of other varieties from Corvallis and other places which were machine threshed had a big reduction in germination percent as a result of soaking and freezing treatments. There is ample evidence from this study that the environment in which a wheat variety is grown and the harvesting methods, including the method of threshing,

greatly influences the ability of the seed to germinate following treatments used in these experiments.

A very interesting phenomenon was observed in two experiments which was apparently in conflict with conclusions of some other workers. Wright (31, pp. 337-344), Kiesselbach and Ratcliff (15, p. 96) and others have claimed that the longer moist seeds are frozen the greater the injury. In Experiment IV wheat seeds of three varieties were soaked in water for several hours and then frozen at -10°C. for three, seven, and eleven days. The germination percentages were quite low after three days of freezing. The percentage was higher at the end of the seven days and considerably higher after eleven days of freezing. This applied to all three varieties. Because of this unexpected phenomenon Experiment VIII was planned to test this further. Two varieties, Hymar and Federation, were soaked for four hours at 20°C. and then frozen at -10°C. for one, three, seven, ten, thirteen, sixteen and nineteen days. The germination of both varieties was down considerably after one day of freezing. At the end of three days the germination was somewhat lower. The five day soaking period had a somewhat higher germination percentage than the three day period. By the end of seven days the percent germination was about the same as

it had been after one day of freezing. The germination percentage rose still higher until it reached a peak after sixteen days of freezing, which was considerably higher for both of the varieties involved than it was at the shorter freezing periods. Federation had a sharp drop in germination percentage from the sixteenth to the nineteenth day while Hymar had but a slight drop. Possibly the reason the other investigators claimed that the longer the freezing the greater the injury was because they had frozen the seeds for no more than one or two days and had obtained the decreases in percent germination up to as far as they had gone.

The reason for the greater survival may be due to evaporation of the ice from the kernels over the longer freezing periods. It is well known that frozen foods lose moisture in freezing chambers unless they are placed in moisture proof containers. Some theories of freezing injury are that frozen tissues are injured during the thawing process by ice rupturing walls or a bursting of the plasma membrane by too rapid an expansion of cell membranes by the water coming from the ice and re-entering rapidly into the cell. If either or both of these theories are right or partly right then evaporating the ice from frozen materials would reduce the freezing injury. The

drop in germination from the sixteen to nineteen day period may be due to so much moisture being evaporated that there is a drought caused by prolonged freezing.

The use of the seeds of wheat in determining varietal differences can be used only if the environmental factors which cause much of the variation found within and between varieties can be largely controlled. A soaking and freezing test involving hand threshed seed of several wheat varieties grown in one location and ranging in winter-hardiness should then provide a better test on whether or not such treatments could be used to discover new winter hardy wheat varieties.

SUMMARY AND CONCLUSIONS

The object of this study was to determine the feasibility of using the germination of the seeds of wheat varieties following different soaking and freezing treatments as a measure of the winter-hardiness of the wheat varieties tested.

Fifteen varieties of wheat from different sources were subjected to various combinations of soaking, freezing, and thawing treatments. Related studies such as differences in absorption rates among different varieties of wheat, effect of soaking treatments without freezing on seed germination and differences within varieties due to the source of seed were also investigated.

The data presented here show that if a reliable test for winter-hardiness in wheat is perfected using the technique of soaking and freezing wheat seeds, it will be much more difficult to accomplish than Ivanoff (12, pp. 90-94) apparently found it in oats. Although there are definite differences among certain wheat varieties in their reaction to soaking and freezing, they do not, under the conditions with which these experiments were conducted, correspond very closely to their known winter-hardiness. The principal results and

conclusions derived from this study may be summarized briefly as follows:

1. There were differences in the ability to germinate following soaking and freezing between the small and the large seed sizes of the two wheat varieties studied.

2. The higher the soaking temperature the fewer wheat seeds that germinated when frozen following soaking.

3. The longer the soaking time the fewer wheat seeds that germinated when frozen after soaking.

4. The lower the temperature the seeds were frozen after soaking, the fewer the wheat seeds that germinated.

5. Of the three varieties tested none of them showed any reduction in germination percentage after soaking in water even at 30°C. for 16 hours. At first the variety Minter appeared to be an exception. When checked, the viability of the seeds before soaking had dropped four percent in six months. A new lot of Minter having a good germination percentage before treatment was subjected to the same soaking treatments and the germination percentage was not affected.

6. There were differences among the varieties checked, in the amount of water absorbed in a given amount of seed for the same soaking temperatures and lengths of soaking time. These differences among the varieties tested did not correspond closely to the germination when frozen after soaking.

7. The germination percentage of moist wheat seeds when frozen was decreased the most at the end of three days freezing time. Evidently increased length of freezing time had less injurious effects up to the end of the sixteen day period, because the germination percentage rose to a higher level for each

period of increasing length after the third day of freezing. There was a drop in germination percentage when frozen for nineteen days.

8. Lower temperatures than -4°C. was necessary to insure that Minter and Hymar could be differentiated from Federation.

9. Thawing in water at 10°C. or higher resulted in lower germination of wheat seed than when the seeds were thawed in air or in wet paper toweling at room temperature.

10. Thawing in water at 10°C. for one hour was somewhat more injurious to the varieties used than thawing in water at 30°C. for 20 minutes.

11. There were differences in the germination percentages of different wheat varieties following soaking and freezing treatments.

12. Under the conditions with which these experiments were run, the germination of wheat seeds of different varieties following soaking and freezing treatments did not correspond closely to their known winter-hardiness.

13. The hand threshed seed of Orfed, grown at Moro, Oregon had a 55 percent germination following soaking and freezing. This was higher than the highest germination percentage of any of the machine threshed varieties. The machine threshed seed of Federation and Hymar, grown at Corvallis, Oregon germinated better than machine threshed seed of the same varieties grown at Pendleton, Oregon. The seed from Pendleton was a year older but the germination of the untreated checks were nearly the same from both sources.

BIBLIOGRAPHY

1. Adams, J. The effect of very low temperatures on moist seeds. Scientific proceedings of the Royal Dublin society, n. s. 2;1-6. 1905. (Abstracted in Experiment station record 17:653. 1906)

2. Becquerel, P. Action of liquid air on the life of seeds. Comptes rendu hebdomadaire des seances de l'Academie des sciences. (Paris). 140:1652-1964. 1950. (Abstracted in Experiment station record 17:653:654. 1906)

3. Brown, H. T. and F. Escombe. The influence of very low temperatures on the germinative power of seeds. In J. Levitt's Frost killing and hardiness of plants. Minneapolis, Minn., Burgess, 1941. p.1.

4. Clark, G. W. Winter hardiness in common wheat as measured by cold chamber tests. Master's thesis. Corvallis, Oregon state college, 1955. 64 numb. leaves.

5. DeCondolle, A. On germination at different degrees of constant temperature. Annals and magazine of natural history, ser. 3, 17:241-265. 1866.

6. Dexter A. T. Studies of the hardiness of plants: A modification of the Newton Pressure Method for small samples. Plant physiology 7:721-726. 1932.

7. Dexter A. T., W. E. Tottingham, and L. F. Graber. Preliminary results in measuring the hardiness of plants. Plant physiology 5:215-223. 1930.

8. _____. Investigation of the hardiness of plants by measurement of electrical conductivity. Plant physiology 7:63-78. 1932.

9. Harvey, R. B. An annotated bibliography of the low temperature relations of plants. Minneapolis, Minn., Burgess, 1935. 223 p.

10. Hill, D. D. and S. C. Salmon. The resistance of certain varieties of wheat to artificially produced low temperature. Journal of agricultural research 35:933-937. 1927.

11. Ivanoff, S. S. A Laboratory technique for testing cold resistance in oats. (Abstr.) Phytopathology 40:13-14. 1950.

12. _____. The use of activated oat seeds in the study of winter-hardiness. Botanical gazette 113:90-94. 1951.

13. Ivanov, P. K. On the diagnostics of frost-and-heat resistance by their seeds. Bulletin, Academy of science U. S. S., ser. biol., 1:89-110. 1936.

14. Jensen, I. J. Winter wheat studies in Montana with special reference to winter killing. Journal of American society of agronomy 17:630-631. 1925.

15. Kiesselbach, T. A. and J. A. Ratcliff. Freezing injury of seed corn. Lincoln, Nebraska, Agricultural experiment station, 1920. 96 p. (Its Research bulletin no. 16)

16. Levitt, J. Frost killing and hardiness of plants. Minneapolis, Minn., Burgess, 1941. 211 p.

17. Luyet, B. J. Life and death at low temperatures. Biodynamica monograph no 1: 1940. 341 p.

18. Martin, J. H. Comparative studies of winter-hardiness in wheat. Journal of agricultural research 35:493-535. 1927.

19. Megee, C. R. A search for factors determining winter-hardiness in alfalfa. Journal of the American society of agronomy 27:685-697. 1935.

20. Quisenberry, K. S. Inheritance of winter-hardiness, growth habit and stem rust reaction in crosses between Minhardi and H-44 spring wheat. Washington, U. S. Dept. of agriculture, 1931. 45p. (U. S. Department of Agriculture. Technical bulletin no. 218)

21. Steinbauer, G. Differences in resistance to low temperature shown by clover varieties. Plant physiology 1:281-286. 1926.

22. Stucky, I. H. and O. F. Curtis. Ice formation and the death of plant cells by freezing. Plant physiology 13:815-833. 1938.

23. Suneson, C. S. and G. L. Peltier. Cold resistance adjustments of field-hardened winter wheats as determined by artificial freezing. Journal of American society of agronomy 26:50-58.

24. _____. Effect of source, quality and condition of seed upon the cold resistance of winter wheats. Journal of American society of agronomy 28:687-693. 1936.

25. Thiselton-Dyer, W. The influence of the temperature of liquid hydrogen on the germinative power of seeds. In J. Levitt's Frost killing and hardiness of Plants. Minneapolis, Minn., Burgess, 1941. p. 1.

26. Tysdal, H. M. Determination of hardiness in alfalfa varieties by their enzymatic response. Journal of agricultural research 48:219-240. 1934.

27. Van Doren, C. A. Bound water and electrical conductivity as measures of cold resistance in winter wheat. Journal of American society of agronomy 29:392-401. 1937.

28. Washington State College. Agronomy department. Compilation of agronomic data from cereal varietal trials at Pullman, Pomeroy, Walla Walla, Lind, Harrington, Waterville, Prosser, Puyallup, and Enumclaw in 1951. Pullman, Washington state college, June, 1952. 58 p. (Mimeographed)

29. Washington State College. Agronomy department. Supplemental winter hardiness nursery data, 1954. Pullman, Washington state college, 1955. 2 numb. leaves. (Hectographed)

30. Weibel, R. O. and K. S. Quisenberry. Field versus controlled freezing as a measure of cold resistance of winter wheat varieties. Journal of American society of agronomy 33:33-343. 1941.

31. Wright, S. G. The relation of low temperature to the growth of wheat. Agricultural science 5:337-344. 1890.

APPENDIX

Appendix Table 1. A summary of the wheat varieties and the source, age and condition of the seed used in Experiments 1 to 14.

Varieties	C.I. No.	Seed Source	Age (Mo.)	Condition	Experiments 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Brevor	12385	Corv.[1]	6	Good												x	x	
Columbia	12928	Moro[2]	6	Good												x	x	
Elmar	12392	Corv.	6	Good												x	x	
Federation	4734	Pen.[3]	18	Good	x	x	x	x	x	x	x	x	x	x	x		x	x
Federation	4734	Corv.	6	Good												x	x	x
Hymar	11605	Pen.	18	Good				x	x	x	x	x	x	x	x		x	x
Hymar	11605	Corv.	6	Good												x	x	x
Kharkof	1442	Corv.	6	Good												x	x	
Minter	12138	St.P.[4]	?	Fair	x	x	x	x	x	x								
Minter	12138	St.P.	6	Good							x		x	x	x	x	x	
Orfed[5]	11913	Moro	6	Good													x	x
Orfed[6]	11913	Corv.	6	Good												x	x	x
Orf. x Hyb. 50-3	12692	Corv.	6	Good												x	x	
Rex	10065	Corv.	6	Good												x	x	
RR x A P8-1	12694	Corv.	6	Good												x	x	
Rio	10061	Corv.	6	Good												x	x	
Triplet	5408	Corv.	6	Fair												x	x	
27-15 x RR #41	12696	Corv.	6	Good												x	x	
Wasatch[6]	11925	Corv.	6	Good												x	x	
Table Number					2	3	4	5	6	7	8	10	11	12	13	14	14	16

1. Corvallis, Oregon
2. Moro, Oregon
3. Pendleton, Oregon
4. St. Paul, Minnesota
5. This seed was hand threshed, all other seed was machine threshed.
6. Seed from the smut project, other seed from Corvallis was from a varietal trial.

Appendix Table 2. A summary of the kernel sizes and the procedure used in preparing the samples of seed for treatment in the fourteen experiments.

Kernels screened and weighed	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Seed sizes over 7/64"	x	x	x											
between 6/64" and 6.5/64"	x	x	x											
under 5/64"	x	x	x											
between 5/64" and 7/64"				x	x									
Kernels unscreened and weighed						x	x	x	x		x			
Counted by hand												x	x	x
Table number	2	3	4	5	6	7	8	10	11	12	13	14	14	16

Appendix Table 3. Summary of the soaking temperatures and soaking times used in Experiments 1 to 14

Experiments	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Soaking Temp.														
10°C.	x	x	x	x	x	x	x	x	x					
20°C.	x	x	x	x	x	x	x	x	x	x	x	x	x	x
30°C.				x	x	x								
Soaking Times														
1 hour							x		x					
2 hours							x		x	x	x	x	x	x
4 hours	x		x	x	x	x		x	x	x				
8 hours				x	x	x								
16 hours		x	x	x	x	x								
Table No.	2	3	4	5	6	7	8	10	11	12	13	14	14	16

Appendix Table 4. Summary of the freezing temperatures and freezing times used in Experiments 1 to 14.

Experiment	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Freezing Temp.														
-4°C.	x		x											
-13°C. to -29°C.		x												
-29°C.			x											
-10°C.				x				x	x	x	x	x	x	x
Freezing Times														
24 hrs.	x		x					x						
36 hrs.			x											
3 days		x		x				x	x	x	x	x	x	x
4 days			x											
5 days								x						
7 days				x				x						
10 days								x						
11 days				x										
13 days								x						
16 days								x						
19 days								x						
Table No.	2	3	4	5	6	7	8	10	11	12	13	14	14	16