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Resistance of Varieties of Winter Wheat and Rye to Low Temperature in Relation to Winter Hardiness and Adaptation

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FIG. 1.—(Upper) Winter wheat frozen artificially. Top row, Kanred; second row, Blackhull. Frozen 6, 12, 15, 18, 21, and 24 hours, respectively.

(Lower) Hybrids and parents of the cross Dakold rye × Chinese wheat frozen artificially. Left to right: Dakold rye, hardy hybrids, semihardy hybrids, tender hybrids, Chinese wheat.

Resistance of Varieties of Winter Wheat and Rye to Low Temperature in Relation to Winter Hardiness and Adaptation[1]

S.C. SALMON[2]

INTRODUCTION

It appears to be generally conceded that injury or death of crop plants during the winter is for the most part a direct or an indirect result of freezing temperatures. The primary causes have been classified as (1) heaving, (2) smothering, (3) physiological drought, and (4) freezing of the plant tissue *(67)*.

Heaving, as is well known, occurs only when a wet, heavy soil is subjected to alternate freezing and thawing; smothering only when plants are covered by an impervious blanket of ice or snow; and physiological drought only, if at all, when the soil is frozen and the plants are unable to secure water from it to replace that lost by transpiration. Drought is then assumed to be the immediate cause of death. Freezing of the plant tissues may or may not result in death, depending upon the kind of plant, the kind of tissue, previous exposure to low temperature, the intensity and duration of the cold, etc. Bouyoucous and McCool *(9)* have shown that heaving is caused, not so much by expansion of the soil and the water contained in it, as by the formation of ice, either as solid ice or as capillary needles or columns, which push the plants upward, breaking and exposing the roots.

Plants may also be injured or killed during the winter season by fungi, drouth, and soil blowing, but since such injuries are not limited to the winter season nor, in general, determined by low temperature, they are not usually thought of as being included in the terms winter injury and winter killing.

The resistance of plants to artificially produced low temperatures offers many attractive possibilities for a study of winter hardiness. For one thing the effect of all factors, except the direct effect of the low temperature itself, may be excluded by means of carefully controlled apparatus. This affords an opportunity to determine whether differences in varietal resistance are due entirely to resistance to cold or to one or more of the indirect effects of low temperature,

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as is commonly believed. This method would also seem to be useful in a study of the relation of injury to hardening, to moisture content of the tissue and surrounding soil, to the addition of nutrients and other substances to the effects of rate of freezing and thawing, etc.

Finally, if it can be shown that resistance to low temperature is highly correlated with winter hardiness under field conditions, the fact should make it possible to determine the relative winter hardiness of new varieties and strains in a few weeks, instead of several years, as is so frequently the case. As pointed out by Salmon *et al.* *(72),* Martin *(46),* Hildreth *(31),* Quisenberry *(62)*, Maximov *(48)* and others, winter killing varies greatly from year to year, and determinations of relative winter hardiness are very uncertain without tests for many years or extensive tests over a wide range of territory in any one year. This latter consideration would seem to be of special importance from the practical viewpoint, since it is often difficult to learn anything definite about the winter hardiness of newly introduced varieties until they have been grown several years and, perhaps, on a large scale. Knowledge of any deficiency in winter hardiness is then gained at the expense of heavy losses. The plant breeder also is greatly handicapped if compelled to wait for a "test" winter to eliminate nonhardy selections from his crosses.

Newton *(51, 52, 53)*, Akerman *(3)*, Hildreth *(31),* and more recently Tumanov and Borodin *(86),* Lebedincev, Borodin and Brovcine *(40),* and Gocholashvili *(22)* have shown that certain physical and chemical determinations such as bound water, gold number, sugar content, viscosity and dry matter content of the expressed sap, etc., are related to winter hardiness. The relations, however, as intimated or stated by these authors, and as more definitely stated by Martin *(46)* and by Steinmetz *(79),* are not consistent enough as yet to be used with confidence as a measure of hardiness.

The use of artificially produced or controlled low temperatures has been suggested by a number of investigators and used in a few cases with success. The present study was inaugurated to determine the application of this method to the study of winter hardiness primarily in wheat and, to a slight extent, in rye.

REVIEW OF LITERATURE

The general subject of the effect of low temperature on plant tissue has been thoroughly reviewed by many authors, including Blackman *(8)*, Chandler *(10),* Rosa *(65),* Harvey *(29),* Akerman *(3)*, Potter *(60, 61)*, Hildreth *(31),* Newton *(51),* Maximov *(47),* Steinmetz *(79),* and Martin *(46),* and it seems unnecessary to repeat here what so well and so thoroughly has been presented by others. It is only in recent years that mechanical refrigeration has been extensively used in studies of this kind, early investigators having been obliged to depend on naturally occurring low temperatures, or on mixtures of ice or snow and salt or similar devices, with the attendant difficulties in reaching the desired temperatures and in controlling and maintaining them for any length of time. The advent of mechanical refrigeration has removed, or so greatly lessened, these difficulties that the investigator of to-day may well consider the possibilities in a new light. A brief review of recent contributions in this field should, therefore, be of interest.

As pointed out by Hildreth *(31),* the general idea of subjecting plants to low temperature artificially produced is not new, this method of study, in fact, having been used by Goeppert *(23)* at least one hundred years ago. Goeppert *(24)* also seems to have been the first to make use of mechanical refrigeration for studying the effects of low temperature on plants, for in January, 1871, he subjected twigs and seeds of plants, including wet and dry seeds of certain cereals, to temperatures as low as -36° C. by means of a Carre ice machine.

Harvey *(29)* was, perhaps, the first of the modern investigators to see the possibilities in, and to make use of, mechanical refrigeration. He was closely followed by Akerman, in Sweden. In recent years Hildreth *(31),* Steinmetz *(79),* Martin *(46)* and Quisenberry *(62),* at the Minnesota Agricultural Experiment Station; Potter *(60, 61),* at the Wisconsin and New Hampshire Stations; Salmon and associates *(16, 25, 36, 37, 73, 74, 80, 84),* at the Kansas Station; Tysdal *(87)* and Peltier and Tysdal *(58)* at the Nebraska Station; Holbert and Burlison *(33)*, at the Illinois Station; Tumanov *(85),* at the Institute of Plant Diseases in Bonn, Germany; and Tumanov and Borodin *(86),* Gocholashvili *(26),* and Lebedincev, Borodin and Brovcine *(40),* in Russia, have used mechanical refrigeration to study the reaction of plants to low temperature. The Ohio and the Indiana agricultural experiment stations have recently installed equipment for similar studies, In foreign countries provision has been made for such studies at the Institute of Applied Botany and New Culture, at Detskoje Sselo; at the Botanical Institute, at Braunschweig *(47),* and at the Centre de Recherches Agronomiques de Versailles *(15)*.

Akerman and his associates *(1, 2, 3, 4)* made an extensive study of the relative winter hardiness of varieties of wheat in Sweden, and also of their resistance to controlled low temperature. The agreement was such as to lead them to consider it the most promising method, for determining the relative hardiness of new strains, developed in the course of plant breeding work.

Martin *(46)* made an extensive physicochemical study of winter hardiness in Minnesota No. 2 rye and several varieties of winter wheat, including Minhardi, which is very winter hardy, and White Winter, which is relatively nonhardy. He concluded that "no laboratory method yet devised, except, perhaps, controlled freezing, is any more accurate for determining hardiness than is careful field study," and that "freezing under controlled temperatures offers the greatest promise in measuring the hardiness of wheat plants by laboratory methods."

Steinmetz *(79)* froze a large number of plants of Grimm and

Kansas Common alfalfa at various times during one winter, and for comparison made several physical and chemical determinations of characteristics presumed to be related to winter hardiness. These included freezing-point depression of the root tissue and of the expressed sap, total solids and viscosity of the sap, bound water, volume of press juice, soluble and insoluble carbohydrates, including sugars, total nitrogen, amino-nitrogen and pentosans. In all cases where differential injury was observed Kansas Common was the more severely injured. Excepting the reaction of the plants themselves to controlled freezing, and excepting the sugar content expressed as total carbohydrates, no definite relation between hardiness and any of the physical or chemical determinations was established. Steinmetz concludes that "as positive measures of the differences between the varieties under study, the freezing of potted plants, or roots removed from the soil, has been found to be the most practical and reliable method." Steinmetz also states that G. Nilsson-Leissner has been able to differentiate between hardy and nonhardy alfalfa by the refrigeration method.

Hildreth *(31)* found a close correlation between the results of artificial refrigeration of twigs from 17 varieties of apples and their winter hardiness as determined by field experience. On the other hand he found that, "neither moisture content, sugars, pentosans, nor amino-nitrogen offered a reliable basis for separating hardy and nonhardy varieties."

Maximov *(47),* apparently on the basis of observations in his own laboratory as well as in other European laboratories equipped with mechanical refrigeration, points out that "the application of such equipment, besides yielding purely practical results, such as the possibility of rapidly and exactly determining frost resistance of different varieties of crop plants, puts into the hands of investigators a powerful means for the further study of the problem of the physiological factors of resistance, thus bringing us nearer to the final aim of work in this field of investigation."

Tumanov and Borodin *(86)* exposed a considerable number of varieties of Russian winter wheat to low temperatures secured by mechanical refrigeration and determined the freezing point and, also, the dry matter content of the expressed sap. The latter was determined by the refractometer method. In the majority of cases the resistance to low temperature as determined by the direct-freezing method correlated well with the relative hardiness of the same varieties under field conditions, and the method was considered satisfactory for determining varietal resistance to frost. Such divergences as were observed were believed to be related to the fact that factors other than low temperatures influenced survival in the field. The refractometer method was considered useful in making preliminary selections for resistance to frost.

Lebedincev, Borodin, and Brovcine *(40)* report the dry substance in the expressed sap as determined by the Abbe refractometer, the bound water as determined by the dilatometer method, the soluble carbohydrates and, also, the results of direct freezing for 44 varieties of winter wheat, 16 of rye, and four of barley, being in part those reported by Tumanov and Borodin *(86)*, previously cited. Varieties occupying extreme positions were readily singled out by all methods; others only with difficulty. The direct-freezing method was considered the most satisfactory, but many tests were required for determining small differences.

Gocholashvili *(92)* subjected a number of varieties of the tea shrub to low temperatures produced artificially. The dilatometer method was also used to determine the bound water and the refractometer method to determine the dry matter in the expressed sap. The direct-freezing method established the relative hardiness of the different varietis as determined by field trials in 1929-'30. The results of the indirect methods (refractometer and dilatometer) in some cases correlated well with the direct freezing, and in other cases were quite opposite.

Quisenberry *(62)* subjected the parents and F_3, segregates of a cross between Minhardi winter and H-44 spring wheats to artificially produced low temperatures, and grew the same lines at St. Paul, Minn., and Moccasin, Mont., for comparison. The relative hardiness for each place or condition was expressed as a hardiness index, which took into consideration not only the percentage of plants killed, but also those which were badly injured or weakened. The correlation coefficients between the hardiness indices for the artificial-freezing test on the one hand and the field results on the other were 0.582 ± 0.041 for St. Paul, Minn., 0.629 ± 0.038 for Moccasin, Mont., and 0.713 ± 0.031 for the average of the two stations. The coefficient between indices for the field results at St. Paul and at Moccasin was 0.416 ± 0.020. Quisenberry concluded that the rather limited data seemed to show that "artificial freezing offers considerable promise in eliminating hybrid lines susceptible to cold."

Foster Martin *(45)* froze 12 varieties of spring wheat commonly grown in Eastern Oregon, ranked them according to injury and correlated these ranks with those of the same varieties arranged according to their survival under field conditions. The correlation coefficient was —0.762 ± 0.085.

Hill and Salmon *(32)* reported the survival of ten varieties of winter wheat subjected to artificially produced low temperatures, compared with the survival of the same varieties in the winter-hardiness nurseries of the United States Department of Agriculture, as reported by Clark, Martin, and Parker *(11)*. The results were found to agree very well, except that Minhardi, Buffum and Minturki, the three hardiest varieties included in the artificial-freezing tests, were relatively nonhardy when frozen without previous hardening.

Sellschop and Salmon *(74)* subjected a number of summer annuals to chilling temperatures (above freezing), secured by means of mechanical refrigeration. Many of them were severely injured or killed by relatively brief exposures, and marked differential responses were observed and reported.

Holbert and Burlison *(33)* subjected corn plants growing under natural conditions in a field to low temperatures artificially produced by means of a portable refrigeration unit. Some strains of corn were injured by above-freezing temperatures and others were markedly resistant to subfreezing temperatures.

Dexter, Tottingham and Graber *(18)* subjected several varieties of wheat and alfalfa of known hardiness to artificially produced low temperatures at the Wisconsin Station, and determined the subsequent exosmosis of electrolytes from the frozen tissue by means of conductivity measurements supplemented by colorometric tests for chlorides. A distinct correlation was noted between the known hardiness and the degree of retention of electrolytes by the frozen roots.

Peltier and Tysdal *(58)* subjected sixteen varieties and regional strains of alfalfa, mostly strains of Turkestan, but including, also, such well-known ones as Grimm, Ladak, Nebraska Common, and Arizona Common, to low temperatures produced by means of the mechanical-refrigeration equipment described by Peltier *(57)*. The survival was in accordance with expectations based on field behavior so far as the latter is known. Of the well-known varieties, Grimm was the most resistant and Arizona Common the least resistant to the low temperatures. Hardistan, Provence (S. P. I. No. 34886), Ladak and several strains of Turkestan were more resistant than Grimm.

Tysdal *(87)* used the equipment described by Peltier *(57)* for a study of the hardening process in three varieties of alfalfa; Turkestan, Grimm and Arizona Common. These varieties were found to respond very differently to hardening temperatures. When frozen after hardening, the survival was in agreement with their known hardiness as determined under field conditions.

Timmons *(84)* working with the writer, made a study of the cold resistance of fifteen varieties and regional strains of alfalfa by means of artificial freezing, the order of survival, ranging from the most resistant to the least resistant, being Hardistan, Ladak, Grimm, Colorado Common, Dakota Common, Nebraska Common, Utah Common (two strains), Kansas Common, Oklahoma Common, Idaho Common, New Mexico Common (two strains), Arizona Common and California Common. The results are almost exactly what would be expected on the basis of the geographical distribution of these varieties and their winter hardiness under field conditions so far as is known.

MATERIALS AND METHODS

The present study deals chiefly with varieties of wheat known to differ in their ability to survive severe winters. A few varieties of winter rye and one variety each of winter barley and winter oats have been included. In a number of comparisons segregates from crosses between relatively hardy and nonhardy varieties also have been studied. The experiments here reported were conducted over a period of five years, during which somewhat more than 30,000 four-inch pots or their space equivalent in flats, comprising between 125,000 and 150,000 plants, were frozen.

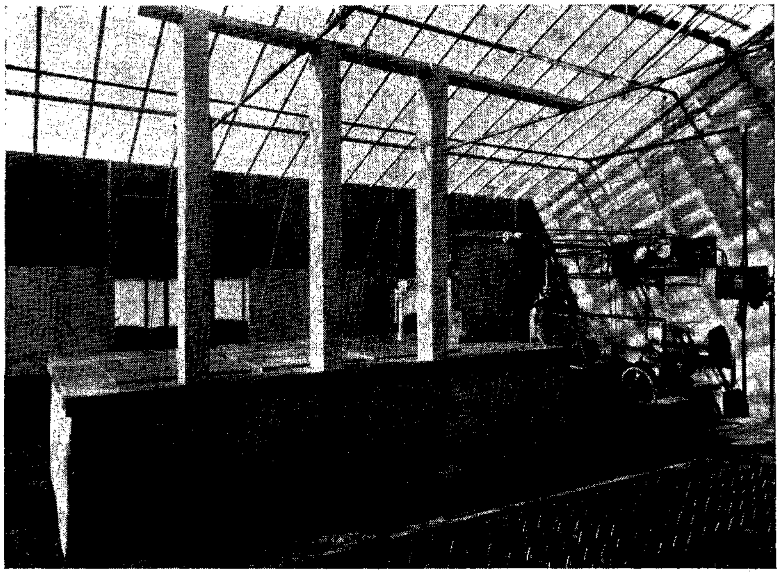

FIG. 2.—Freezing chamber and compressor used for exposing plants to low temperature.

The freezing was accomplished by means of the carbon-dioxide direct-expansion refrigeration machine described by Sellschop and Salmon *(74)*. (Fig, *2.)* In most cases the freezing period was twelve hours, the plants being placed in the freezing chamber either in the morning or evening. The twelve-hour period was chosen partly for convenience and partly because it requires nearly that period of time for the temperature of the soil in a four-inch pot (in which most of the palnts were frozen) to approach the temperature of the freezing chamber. The latter is not an essential requirement, but was considered desirable in the present case, since otherwise it would have been difficult to estimate the temperatures to which the portion of the plant surrounded by soil was subjected. Judging from a number of observations of temperature changes in the soil in four-inch pots, it is probable that before the end of the twelve-hour period the crowns and roots of the plants reached approximately the temperature of the chamber.

Plants were frozen at various ages but, unless otherwise stated, never before they had tillered nor after they had begun to shoot. Usually they were in the rosette stage of development and had from four to eight tillers per plant.

In all cases reported here the plants were frozen surrounded by soil either in four-inch clay pots or in "flats." The latter usually were twenty-four inches square and about four inches deep. The method of freezing in which the plants were removed from the soil and frozen in small bundles was tried but was considered less satisfactory with the equipment available, partly because when frozen in place in the soil the change in temperature of the plants is buffered by the soil and, consequently, accurate control of the temperature of the freezing chamber is not so necessary. Without accurate control differential injury of fully exposed plants is difficult to secure, since all or none is likely to be killed.

It was observed very early in the course of the investigations that variations in the moisture content of the soil were responsible for marked differences in injury. Consequently all lots were thoroughly watered a few hours before freezing, except in a few cases where the effect of moisture content of the soil on injury was being studied.

Two classes of material were frozen: (1) plants grown in the field and transplanted to pots or flats previous to freezing, and (2) plants grown in four-inch pots or flats in the greenhouse and frozen either with or without previous exposure to outside temperatures. The method that seemed to be the most satisfactory, and the one used for most of the later work, was to start the plants in pots in the greenhouse and transfer them outside soon after they emerged, thus exposing them to natural temperatures until frozen. In this case seeding was done the latter part of September or early in October in order to avoid, on the one hand, a too heavy luxuriant growth and, on the other, the killing of the plants, which would have almost certainly occurred with sudden exposure of greenhouse-grown plants to the low temperatures outside in the late fall or winter. Unfortunately, controlled temperatures were not available for hardening, and this method seemed as satisfactory as any that could be devised for the greenhouse-grown plants. The field-grown plants were left in the field until just preceding their transfer to the refrigerator and hence were hardened under natural conditions.

When the experimental work reported herein was inaugurated, it was expected that the percentage of plants killed or its complement, the percentage survival, would be used as the criterion of relative injury, but this was soon found to have some more or less serious limitations. One of the more serious was that in many cases the plants after freezing died as the result of a secondary effect, probably physiological after effects of injury to the roots. The plants turned yellow a few days after freezing and soon thereafter were dead. Invariably the inside crown tissue was badly discolored. Since the cause of this phenomenon is being investigated by others, no further report will be made here, other than to point out that there seems to be no relation between varieties and the degree of injury, and, further, that the injury seemed to have no definite relation to the temperatures to which the plants were subjected, provided only that they were sufficiently low to cause some injury. That is to say, plants which appeared to have been only slightly injured by low temperatures were often killed completely by this secondary effect. It is obvious that percentages of survival cannot serve as a criterion of freezing where this secondary effect occurs.

Another objection to this criterion is the fact that in many experiments no plants whatever are killed, even though there may be obvious and marked differences in injury. Thus it is not uncommon for certain susceptible varieties to be frozen almost to the ground level and yet completely survive. The survival in such cases gives no indication whatever of the degree of injury, and, consequently, when the investigator depends upon survival, no results are secured from such experiments. Since it is sometimes difficult to accurately determine beforehand the temperatures at which differential killing will be secured, dependence on survival alone means that only a part of the experiments that are conducted furnish data of value. The proportion of useful experiments in many cases is not more than 50 per cent. For these reasons the major dependence in this work has been placed upon the degree of injury.

This is, of course, somewhat arbitrary, in so far as intermediate degrees of injury are concerned, since they must be estimated. The upper and lower limits are easily established in that plants are considered as 100 per cent injured if in the judgment of the operator none of them will survive, and they are recorded as not injured at all if there is no apparent effect of the low temperature. Ordinarily there are no difficulties in establishing these limits. It is more difficult to estimate the degree of injury to plants that are injured but not killed. The tips of the leaves are the first to be injured and the amount of killed tissues increases more or less uniformly from the tip of the leaf to the crown of the plant, and, consequently, it is not usually difficult to differentiate between plants or lots according to the percentage of the total leaf tissue that is injured. In those cases where the injury is nearly 100 per cent, the turgidity of the base of the plant, as well as the percentage of the tissue that appears to be killed, is taken into consideration. Thus if all of the visible leaf tissue is killed, but the turgidity of the lower portion of the plants indicates that life exists in the inside tissue, the plants may be reported as being 90, 95 or 98 per cent injured. The method adopted here is similar to that used by Quisenberry *(68)* and analogous to that used in estimating the degree of rust infection *(13)* which has proved eminently successful. There obviously are objections to evaluating experimental results in this way, but since these appear not to be serious, and since the matter was made a subject for special investigation, the results of which are reported later, they may be dismissed at this time.

The statements by Newton and Anderson *(54),* that "the leaves are the organs which determine winter survival in wheat," and that "in this respect the cereals differ from legumes, such as clover and alfalfa," are of interest in this connection. If one includes the crown tissue and crown buds with the leaves in the wheat plant, but differentiates between the crown buds on the one hand and the leaves on the other hand in the alfalfa plant, the statements are, so far as the writer is aware, correct. But it is pertinent to note that the method of recording injury discussed here is based on the assumption, not that the leaves are the organs which determine winter survival, but rather that injury to the leaves is highly correlated with injury to that portion of the plant which determines survival whether it be leaves, some other portion of the plant or the plant as a whole. It is common knowledge in cold climates where the tops are not protected by snow that the fall-grown leaves of wheat as well as those of alfalfa are frozen to the ground, so that in the spring not a spear of green can be seen, but after a few days of warm weather the plants may start into active growth as though no injury whatever had occurred. In this respect there is no distinction between alfalfa and wheat, and in estimating the degree of injury from artificial freezing no distinction in principle seems necessary and none, in fact, has been made in the work reported here.

A general probable error was calculated for each experiment by the Fisher-Student formula suggested by Student *(81)* unless otherwise indicated. It is reported in each table as a probable error of the mean and should be multiplied by V_2 for the probable error of the difference between means.

EXPERIMENTAL RESULTS

EXPERIMENTS IN TECHNIC

Success in evaluating the results of artificial freezing and in studying resistance to cold in general, obviously depends on good technic; that is, on avoiding various sources of error which may interfere with a logical interpretation of the results. Since the inception of the work reported in this bulletin, various efforts have been made not only to improve the technic empirically but also to investigate sources of error which may be expected to lead to improvement.

DETERMINING THE DEGREE OF INJURY

There are two sources of error in estimating the degree of injury, and these sources have been investigated at various times. The first, and probably the most important, is the error resulting from mistakes in the judgment of the operator as to the amount of injury that has been sustained. This is likely to be a systematic error; that is to say, it is likely to be consistently too high or too low. In so far as the same error is present for all varieties or treatments it may have no material effect on the experimental results. If it is different for some varieties than for others, it may constitute a very serious error. Errors of the second kind are more or less random and may be expected to compensate each other where averages are based on large numbers.

The first source of error has been investigated, whenever there has been an opportunity to do so, by comparing the percentages of estimated injury with the percentages of plants killed, in those cases where there was no secondary effect as mentioned above. A considerable number of such cases have occurred from time to time, and the results, so far as they have a bearing on the relation between estimates of injury and plants killed, are presented in Table I. As will appear later, different groups of plants were frozen, some of each being hardened and some not hardened previous to freezing. It seemed desirable to retain the identity of each group, and accordingly the data have been presented separately for each.

The number of pots on which the determinations of plants killed were based is, in some cases, less than the number on which the estimates of injury were based, for the reason that the former were limited to pots in which the secondary effect of freezing did not appear, whereas this limitation did not apply to the injury estimates.

As will be seen, the two methods of evaluating the freezing results agree very well, as shown by the correlation coefficients given in the last column of Table I. No statistical tests for linearity were applied because of the small number of varieties, but there were no obvious departures from linearity. The probable errors of the means are in some cases rather high, largely because of the very small numbers involved. With less random variation the correlation coefficients probably would have been larger.

Altogether it would appear that a fairly reliable determination of the comparative effects of freezing may be secured by estimating the degree of injury. This conclusion is in agreement with the results secured by Foster Martin *(45)* who estimated the injury and counted the number of plants killed of twelve varieties of spring wheat from eastern Oregon subjected to artificial freezing. The correlation coefficient between ranks of the varieties based on artificial freezing and winter killing in the field was 0.95 ± 0.021 for unhardened plants and 0.91 ± 0.035 for hardened plants. Timmons *(84)* made a similar study in alfalfa and found a high correlation ($r = 0.97 \pm 0.010$) between estimates of injury and number of plants killed. It will be observed that the probable errors are considerably greater for plants killed than for estimated injury. But the range of variation and, hence, the difference between varieties is also greater for plants killed. One ordinarily is interested in the ratio between observed differences and the probable errors of those differences. The total range of variation for each case divided by the corresponding probable error should, therefore, constitute a fairly reliable measure of the usefulness of the two measures of injury. It

TABLE I.—RELATION BETWEEN ESTIMATED INJURY AND PLANTS KILLED FOR EVALUATING THE RESISTANCE OF VARIETIES OF WHEAT TO LOW TEMPERATURE.

Date of freezing and kind and condition of plants when frozen.	Plants killed.				Estimated injury.				Correlation between plants killed and estimated injury.	
	Number of pots.	Mean per cent.	E_m	Ratio; range to E_m	Number of pots.	Mean per cent.	E_m	Ratio; range to E_m	N.	r:
November-December, 1929: Regional varieties; hardened	31	67.5	2.9	2.9	40	90.4	1.2	2.9	21	.93±.020
November-December, 1929: Regional varieties; not hardened	16	67.0	7.8	1.1	25	84.9	2.6	1.4	25	.93±.020
December, 1930: Regional varieties; hardened	20	38.6	6.4	10.8	20	66.9	2.9	12.4	16	.77±.067
December-January, 1930-'31: Varieties from winter-hardiness nurseries; hardened	8	42.2	11.8	5.7	9	66.4	2.7	9.7	35	.76±.048
December, 1930: Varieties and time-of-seeding test; not hardened	10	62.9	3.7	8.0	10	68.5	2.8	7.6	14	.95±.017

will be seen that these ratios as given in the table do not greatly differ, although there would seem to be some difference in favor of the injury estimates.

VARIATION IN INJURY ESTIMATES

In a number of cases two independent estimates of injury were recorded in the same half day in order to determine what the personal error of judgment may be in making them. This was done only when the numbers involved were sufficient to preclude the possibility of the remembering of preceding estimates. The procedure was to estimate the injury for all pots and then, after a short period, to make new estimates. Table II gives the mean average percentage injury for two independent estimates, the number of estimates in each case and the probable error of the difference between the two estimates for one lot each of Kanred and Blackhull wheat which has been frozen for other purposes. The probable errors are expressed on the basis of a single pot.

TABLE II.—VARIATION IN INJURY ESTIMATES.

VARIETY.	Date of freezing.	Number of pots.	Average estimated percentage of injury.		Probable error of the difference (single pots).
			1st estimate.	2d estimate.	
Kanred....................	Dec., 1928....	230	76.7	76.0	2.94
Blackhull..................	Jan., 1929....	150	55.5	56.1	5.30

The data presented here are in agreement with similar observations in other tests indicating that a probable error of the difference of not to exceed 5 per cent may be expected, due to random variation in making estimates. This means that if 25 pots each of two or more varieties or lots are frozen at one time the probable error of the difference between means due to variation of this kind may be expected to be about 1½ per cent. Under favorable conditions this degree of accuracy is easily attained.

DAY VERSUS NIGHT FREEZING

It was observed in some of the earliest freezing work that a marked difference in injury occurred depending on whether the plants were frozen at night or during the day. The differences were especially marked in certain experiments reported by Hubbard *(37)* and by Davis *(16)* in which the parents and segregates from the crosses Kanred X Blackhull and Kanred X Kanmarq, respectively, were frozen. The results secured by these investigators are summarized in Table 111. It will be noted that there was a marked and consistent difference in all cases.

Foster Martin *(45)* recorded an average percentage of injury for twelve varieties of spring wheat and one of winter wheat from Moro, Ore., of 50.6 per cent for those lots frozen during the day as compared with 34 per cent for those frozen at night. All varieties were injured the most when frozen during the day.

Suneson *(82)* reported the average injury for ninety pots each of thirteen varieties frozen at night and a similar number frozen during the day. In some pots the greatest injury was sustained when frozen at night, and in others the reverse was true. The average for all pots was 78.2 per cent for the pots frozen at night and 73.2 per cent for those frozen during the day. The results are thus contrary to those

TABLE III.—DAY VERSUS NIGHT FREEZING.

VARIETIES AND STRAINS.	Frozen during night or in the afternoon.		Frozen during day or in the forenoon.		Difference.
	Number of pots.	Estimated percentage of injury.	Number of pots.	Estimated percentage of injury.	
Kanred × Blackhull:					
1927-'28—					
Kanred	23	65.7	54	94.2	28.7
Blackhull	22	92.2	53	98.7	6.5
F_3 hybrids	333	62.4	374	86.6	24.2
1928-'29—					
Kanred	32	59.6	39	67.0	7.4
Blackhull	34	79.5	38	85.5	6.0
F_4 hybrids	371	68.5	446	72.3	4.2
Average	....	....	....	....	28.2±3.17
Kanred × Kanmarq:					
1927-'28—					
Kanred	39	46.5	32	83.9	37.4
Kanmarq	39	59.1	32	91.1	32.0
F_3 hybrids	430	32.9	331	71.2	38.3
1928-'29—					
Kanred	30	65.0	29	94.8	29.8
Kanmarq	35	72.5	30	97.8	35.3
F_4 hybrids	380	56.1	330	77.6	21.5
Average	....	....	....	....	27.7±4.96

secured by Davis and Hubbard. Hubbard also in a later study found no difference. Stevens *(80)* observed no day and night effect, in freezing the parents and segregates of a Kanred X Tenmarq cross.

Davis *(16)* suggested that the difference might be due to the accumulation of carbohydrates during the day in those plants which were frozen at night and the translocation during the night in those plants which were frozen in the morning. Akerman *(3)*, for example, found a marked correlation between the resistance to freezing and certain carbohydrates, principally sugars, and Novikov *(55)* found an increase in carbohydrates of the cell sap in the middle of the day and a decrease in the evening. Tumanov *(85)* has recently shown that plants kept in the dark at temperatures above zero suffer a sharp drop in resistance to cold. The loss in hardiness was especially great with nonhardened plants. Davis suggested, also, that the concentration of the sap might be different for those frozen at night as compared with those frozen in the daytime.

No particular attempt has been made in the experiments reported here to determine whether there is or is not a consistent difference between day and night freezing. The only results are those which have been secured incidental to other experiments, and the only purpose here is to point out that in some cases, at least, differential results have been obtained and this source of error must be considered in making freezing tests. It would appear that until further information is available it will be highly desirable, if not necessary, to conduct all comparable experiments during the day or night; or, better still, as has already been pointed out, to include in one freezing lot all variants which it is desired to compare. In all experiments reported herein this precaution has been observed.

MOISTURE CONTENT OF THE SOIL

Emerson *(19),* as early as 1903, showed that root injury in apple trees is greater in a dry than in a wet soil, a result that seems to agree with general orchard experience. However, there appears to be no unanimity of opinion as to the reason, and the experimental data relating thereto are very meager. Emerson attributed the difference to the more frequent alternate freezing and thawing of a dry soil, although no soil temperatures were recorded. Howard *(36)* found practically no difference in the minimum temperature reached in wet and dry soils during long cold periods in midwinter. Macoun *(43)* assumes that plants in a dry soil will be injured more than in a wet soil if subjected to the same temperature, but Chandler *(10)* interpreted his own experience as indicating exactly the reverse.

Salmon *(68, 69)* , Klages *(38)* Hill and Salmon *(32)* and Tysdal *(87)* have investigated the relation of soil moisture to killing of wheat and alfalfa. As a result, of these investigations it seems to be rather clearly established that under field conditions plants are more often severely injured or killed by the direct effects of low temperature (heaving and similar phenomena excluded) on a dry soil than on a wet soil. Such observations as have been made indicate that the former are actually exposed to a lower temperature, and that the differential effect in the main is attributable to this fact. With artificial freezing experiments, such as reported herein, variations in injury due to differences in moisture content are very marked, and, accordingly, in all experiments reported in this paper, unless otherwise stated, the soil has been thoroughly watered a few hours before freezing. This has been done in order to insure in so far as possible a uniform amount of water in all lots. From time to time, however, special tests have been conducted, as opportunity permitted, similar to those reported by Hill and Salmon *(32).*

In November and December, 1928, Kanred and Blackhull wheat and Dakold rye were frozen at various times, part in dry soil and part in wet soil. A similar experiment was performed on January 29, 1929, with a dry soil, a moderately wet soil and a very wet soil. The results are presented in Table IV. It will be noted that in general the result's agree with those previously reported in that more injury was sustained in the dry soil and least in the wet or very wet soil.

TABLE IV.—EFFECT OF MOISTURE CONTENT OF SOIL ON INJURY TO WINTER WHEAT AND RYE BY FREEZING.

VARIETY.	Estimated percentage of injury.				
	Frozen in Nov. and Dec., 1928.		Frozen in Jan., 1929.		
	Dry soil.	Wet soil.	Dry soil.	Moderately wet soil.	Wet soil.
Kanred wheat	97.4	96.9	95	77	70
Blackhull wheat	98.4	84.9	99	97	91
Dakold rye	59.5	36.0	84	83	80

TABLE V.—EFFECT OF MOISTURE CONTENT OF SOIL ON FREEZING INJURY WHEN FROZEN SLOWLY.

VARIETY.	Average percentage of injury (a) (N=25 for each case).		
	A.	B.	C.
Kanred	91.2	94.4	94.6
Blackhull	57.4	48.5	60.4
Minhardi	75.2	76.3	99.2
Fulcaster	93.2	85.5	89.4
Tenn. Winter barley	93.5	91.6	92.4
Winter Turf oats	90.9	95.0	97.1
Average	76.2	77.6	83.2

(a) The data are not comparable for comparison of varieties since they were frozen at different temperatures.

In January, 1929, an experiment was performed to determine whether in fact the difference in injury is due to lag in temperature. In this experiment seventy-five pots each of Kanred, Blackhull, Minhardi and Fulcaster winter wheat, winter barley and winter oats were divided into three lots. The first lot designated as "A" was not watered for three or four days previous to freezing and, as a consequence, was very dry. Lot "B" was grown with a deficient supply of water for several days before freezing but was watered copiously three or four hours before freezing. Lot "C" was grown continuously in moist soil and was frozen in a moist or wet soil. The moisture content of the soil in which the Minhardi was grown was determined and found to be 10, 28.2, and 23.3 per cent, respectively, for the A, B and C series. The various lots were frozen slowly, the temperatures being maintained only slightly below zero Centigrade until the soil was frozen, after which the temperature was lowered sufficiently to secure differential injury. The average estimated injury for the different series was as presented in Table V. The differences, it will be observed, are within the limits of experimental error, and hence the experiment can be taken to verify the hypothesis that the differences in injury on wet and dry soil are probably due to the specific heat and latent heat of fusion of the water which causes a lag of temperature in the wet soil. Possibly the greater killing of winter wheat on sandy soil can be explained on this basis.

CLUMP SIZE AND RESISTANCE TO FREEZING

In digging plants from the field and transplanting them to flats or pots for freezing, it is practically impossible to insure the transplanting of a single plant or of a uniform number of plants in each lot. Accordingly where plants have been grown in the field, the practice has been to transplant a clump or group of plants as they happen to be distributed in the drill row. These clumps unavoidably vary in size according to the stand, individuality of the plant, rate of growth, etc. It was suspected that there might be a relation between the size of these clumps and the injury from freezing. Accordingly, in December, 1927, 230 lots of Kanred wheat from a field were transplanted into four-inch clay pots and immediately thereafter classified according to clump size into ten different lots, the smallest clump being designated as 1 and the largest as 10. The smallest clump corresponded in size to that of a normal single plant from four to six weeks old with from three to four tillers. In fact, the smaller clumps probably consisted of single plants. The largest clumps filled about half the area of the top of a four-inch pot.

TABLE VI.—RELATION BETWEEN CLUMP SIZE AND PERCENTAGE OF INJURY.

LOT No.	Number of pots.	Average estimated injury.	r.
		Per cent.	
1	64	75.4	—0.438
2	30	80.2	—.737
3	64	69.9	—.905
4	15	88.3	—.678
5	57	80.8	—.409
Average (weighted)			—0.615

These plants were then frozen in five different lots and the correlation between clumps size and the estimated injury was calculated. The number of pots in each lot, the average percentage injury and the correlation coefficients for each lot are given in Table VI.

The coefficients vary from —0.41 to —0.91, having an average value of —0.62. It would thus appear that there is a definite relation between the size of the clump and the injury from freezing, and that in transplanting plants from the field for freezing special efforts should be made to secure clumps of uniform size.

EFFECT OF DISTURBING THE ROOTS ON RESISTANCE TO FREEZING

In transplanting plants from the field, as may be desirable in many cases, the question naturally arises whether the injury to the roots incidental to transplanting may in any way influence the experimental results. An attempt to answer this question was made in December, 1927. Thirty pots were filled with as many clumps of Kanred wheat, in which in each case the soil was thoroughly shaken from the roots before the plants were transplanted. These were then frozen with comparable plants which had been transplanted by distributing the roots as little as possible. The average percentage of injury was 80 per cent for the first lot and 75.4 per cent for the second, with a difference of 4.6 ±1.94. It will thus be seen that although there was a slight tendency for the injury to be increased by shaking the dirt from the roots it was not at all important and of doubtful statistical significance.

Bayles *(7)* in 1928 exposed plants entirely free from the soil for periods of from twelve to twenty hours at temperatures of —10° C. with no apparent injury to the plants. The plants were frozen solid almost immediately when placed in the refrigerator. Similar experiments were performed by the writer on an exteneive scale at other times with similar results. It would appear from these results that whatever effect there may be from disturbing the roots incidental to transplanting it is ordinarily not sufficient to materially influence experimental freezing results.

NATURAL FREEZINGIN POTS

Certain experiments have been conducted in which different varieties were grown in pots in the greenhouse, set outside over winter, and the survival correlated with the injury from artificial freezing. Table VII gives the pertinent data from such an experiment in 1928-1929 and of another reported by Suneson *(82)* in 1929-1930. The agreement with the results of artificial freezing is in both cases very good as shown by the high correlation coefficients. Davis *(16)* also secured good agreement between the survival of Kanred, Kanmarq, and hybrids between them in the field and when grown in pots and exposed outdoors until many of the plants were killed. The results suggest that this method may perhaps be used to supplement field and artificial freezing tests, especially when equipment for artificial freezing is not available.

TABLE VII.—RELATION BETWEEN ESTIMATED PERCENTAGE OF INJURY TO WHEAT VARIETIES FROZEN ARTIFICIALLY AND PERCENTAGE OF SURVIVAL WHEN FROZEN NATURALLY IN POTS OUTDOORS.

DATE OF FREEZING.	Frozen artificially.			Frozen in pots outdoors.			Correlation coefficient.	
	Number of pots frozen.	Mean injury per cent.	E_m	Number of pots frozen.	Mean plants killed per cent.	E_m	N.	r.
December-January, 1928-'29..	31	90 4	1.21	25	84.1	1.4	21	$+.91\pm.027$
December-January, 1929-'30..	31	72.8	0.81	185	82.7	2.7	13	$+.86\pm.050$

RESISTANCE OF VARIETIES OF WINTER WHEAT TO LOW TEMPERATURE

As often happens in investigating a subject concerning which very little is known, many experiments are conducted which furnish very little information. To present all of the details, or even a considerable portion of them, would be to ask the reader to undertake the perusal of a very large amount of data which would add little or nothing to his knowledge and, perhaps, a good deal to his confusion. Consequently it has seemed best to omit all reference to those experiments which supplied no information and to condense much of the remainder in so far as is consistent with furnishing a clear picture of what has been done and the results secured.

Undoubtedly the uniform winter-hardiness nurseries conducted by the Division of Cereal Crops and Diseases, United States Department of Agriculture, in cooperation with the agricultural experiment stations of various states, afford the most extensive and most reliable information extant regarding the relative winter hardiness of a considerable number of varieties of winter wheat. Many of the varieties included in the present study were those which were, or had been, included in these nurseries. However, many of these varieties are of peculiar or special interest near the northern border of the winter-wheat belt and are not of particular interest for the central or southern portion of this belt, because extreme winter hardiness is not needed, and they are unsatisfactory because of their late maturity. Since the purpose of the freezing trials reported here was to secure information as to the ability of many new varieties and strains to survive low temperatures, as well as to determine the usefulness of artificial refrigeration for studying winter killing, it has seemed desirable to include a considerable number that were not included in the winter-hardiness nurseries.

Also, the role of resistance to low temperature in limiting the distribution of varieties seemed worthy of consideration, and for that reason a number of soft wheats from the Eastern United States were included in some of the later tests. These and other varieties not included in the winter-hardiness nurseries are, for convenience, hereafter referred to as regional varieties.

It was pointed out by Hill and Salmon *(32)* that differential response to hardening of varieties of winter wheat may be expected, but, even so, the need of thorough hardening was not fully appreciated in the earlier work presented here, and, moreover, there has not always been the opportunity, because of lack of space and temperature-control equipment, to harden certain varieties sufficiently in all cases. For this reason the results of many of the freezing tests are in some respects not so satisfactory for those groups designated by Quisenberry and Clark *(64)* as very hardy and medium hardy as they are for those classified as slightly hardy or tender. The winter-hardiness nurseries include all the varieties of the first two groups that were included in the artificial freezing trials, and very few of the other varieties. It has, therefore, seemed desirable to present the data secured with varieties from the winter-hardiness nurseries separately from the others.

VARIETIES IN THE WINTER-HARDINESS NURSERIES

In most cases varieties from the winter-hardiness nurseries were grown in the field and allowed to harden naturally before freezing. In a few cases the plants were grown in the greenhouse and frozen without hardening, or were incompletely hardened. Since somewhat different results were secured depending on hardening, the data are considered separately.

Hardened Before Freezing.—The first freezing test, in which there were included a considerable number of varieties from the winter-hardiness nurseries, was conducted in February, 1928, by Mr. B. B. Bayles, of the Division of Cereal Crops and Diseases, United States Department of Agriculture, in cooperation with the author *(7)*. Twenty-three varieties were included, all of which had been in the winter-hardiness nurseries for one or more years. They had been planted in the cereal-crop nursery at Manhattan, Kan., and taken directly from the frozen soil to the refrigerator for freezing.

The mean and mean minimum temperatures outside were 26.9 °F. (-3° C.) and 15.1° F. (-9.5° C.) for December, 33.1° F. (+0.6° C.) and 22.0° (-5.6° C.) for January, and 36.3° (+2.4° C.) and 25.5° (-3.6° C.) for February, respectively. Temperatures as low as -6° (-21.0° C.) were recorded on December 12, -8° (-22.2° C.) on December 31, -9° (-22.7° C.) on January 1, and -8° (-22.2° C.) on January 3. That the plants were reasonably well hardened when frozen is indicated by the fact that it was necessary to subject them to temperatures of -22° to -25° C. to secure differential injury.

Ten determinations were made for each variety. The average estimated injury from artificial freezing for each variety, and the average survival in the winter-hardiness nurseries compared with Kharkof = 100 as reported by Clark, Martin and Parker *(11),* and by Quisenberry and Clark *(64),* are given in Table VIII. The average survival in the winter-hardiness nurseries as given is for all years up to and including 1929, the last year for which data have been published. The probable error of the mean, it will be noted, was 1.5 per cent, and the correlation coefficient for the estimated injury in the freezing trials and the average survival in the winter-hardiness nurseries was -0.65 ±0.085.

TABLE VIII.—RELATIVE INJURY FROM ARTIFICIAL FREEZING OF VARIETIES OF WINTER WHEAT GROWN IN THE FIELD.

Frozen in February, 1928; —22° to —25° C.

VARIETY.	C. I. No.	Average estimated injury from artificial freezing plus (+) or minus (—) Kanred in same tests; (N=10).	Average survival in winter-hardiness nurseries (Kharkof=100).
Kanred × Minhardi	8031	—18.2	116
Minturki × Beloglina-Buffum	8033	—15.3	120
Kanred × Buffum No. 17	8030	—14.5	123
Eureka × Minhardi	8036	—13.8	119
Beloglina	1543	—12.4	115
Minhardi × Minturki	8215	—11.3	127
Turkey × Minhardi	8217	—8.8	126
Minhardi	5149	—8.4	125
Turkey × Minessa	8028	—7.2	128
Minhardi × Minturki	8034	—6.4	116
Karmont	6700	—5.9	100
Odessa	6151	—5.1	117
Turkey	1558	—4.9	....
Buffum No. 17	3330	—4.0	125
Minard	6690	—3.6	117
Minturki	6155	—1.5	119
Regal	7364	—0.6	83
Turkey selection	6152	—0.3	113
Kanred	5146	0.0	103
Newturk	6935	1.1	98
Oro	8220	1.5	100
Blackhull	6251	5.2	78
E_m	....	1.5	....

r= — 0.65±0.085

Bayles *(7)* correlated the results of the artificial freezing with the average survival in 18 winter-hardiness nurseries in 1926 and in 1927, and with 19 nurseries in 1928. He also correlated the average survival in these nurseries with that of the single winter-hardiness nursery at Moccasin, Mont., for comparison. Most winter-hardiness nurseries consist of three distributed rod rows of each variety or strain, but the Moccasin nursery in this case comprised 12 rod rows, *i.e.*, approximately the same number of rod rows as there were pots or their equivalent in the artificial-freezing trials. These coefficients are given in Table IX.

TABLE IX.—CORRELATION BETWEEN AVERAGE SURVIVAL IN THE WINTER-HARDINESS NURSERIES IN 1926, 1927, AND 1928 AND INJURY IN ARTIFICIAL FREEZING TRIALS, ON THE ONE HAND, AND SURVIVAL IN THE MOCCASIN, MONTANA, WINTER-HARDINESS NURSERY ON THE OTHER.

YEAR.	Correlation coefficients.	
	Injury from artificial freezing (a).	Average survival in the Moccasin, Montana, winter-hardiness nursery.
Average survival in all winter-hardiness nurseries:		
1926	—0.66±0.095	0.80±0.062
1927	—.58± .109	.60± .104
1928	—.61± .092	.71± .077

(a) The coefficients here and elsewhere in this paper are negative since the relation is between injury in one case and survival in the other.

TABLE X.—RELATIVE INJURY TO VARIETIES OF WINTER WHEAT GROWN IN THE FIELD AND ARTIFICIALLY FROZEN IN DECEMBER, 1928, (—23° TO 25° C.) COMPARED WITH AVERAGE SURVIVAL IN THE WINTER-HARDINESS NURSERIES.

VARIETY.	C. I. No.	Average estimated percentage of injury from artificial freezing (N=14).	Average survival in winter-hardiness nurseries (Kharkof=100).
Minhardi	5149	65.1	125
Minhardi × Minturki	8034	66.7	116
Kanred × Minhardi	8031	67.4	116
Beloglina	1543	69.0	115
Minard × Minhardi	8218	69.3	114
Turkey × Minhardi	8216	70.5	109
Eureka × Minhardi	8036	71.7	119
Minturki × Beloglina-Buffum	8033	72.6	120
Turkey × Minhardi	8217	72.6	126
Nebraska No. 60	6250	74.6	108
Kanred × Buffum No. 17	8030	74.7	123
Karmont	6700	74.9	100
Buffum No. 17	3300	75.1	125
Minturki	6155	75.3	119
Odessa	6151	75.6	117
Newturk	6935	76.3	98
Oro	8220	76.6	100
Minhardi × Minturki	8215	77.0	127
Kawvale	8180	78.0	93
Turkey	6152	78.5	113
Kanred	5146	79.0	103
Kharkof Selection	6938	79.4	114
Kharkof	1442	81.1	100
Tenmarq	6936	83.9	87
Montana No. 36	5549	85.4	102
Early Blackhull	8856	91.3	75
Blackhull	6251	93.0	78
Superhard	8054	93.1	75
Fulcaster	6471	94.3	72
E_m	....	2.1	....

r = —0 84±0 038

The results show the 12-rod-row nursery to be slightly more accurate as a basis for predicting the relative winter hardiness of varieties than is the average of 10 determinations by artificial freezing. The differences are small, however, and might well have been nil had a larger number of plants been included in the latter tests. This point will be discussed later.

In 1928, 30 varieties, and in 1930, 35 varieties, all of which were in those years, or in previous years had been, included in the winter-hardiness nurseries, were frozen. The freezing took place in December each year, the freezing temperature ranging from —23° to —26° C. The results of these tests together with the average survival in

TABLE XI.—RELATIVE INJURY TO VARIETIES OF WINTER WHEAT ARTIFICIALLY FROZEN IN DECEMBER, 1930, COMPARED WITH AVERAGE SURVIVAL IN THE WINTER-HARDINESS NURSERIES.

Plants grown in greenhouse pots and hardened outside before freezing.

VARIETY.	C. I. No.	Average estimated percentage of injury from artificial freezing (N=18).	Average survival in winter-hardiness nurseries (Kharkof=100).
Minard × Minhardi	8218	49.4	114
Minard × Minhardi	8888	50.8	119
Minhardi × Minturki	8215	54.2	127
Minard × Minhardi	8889	55.5	116
Minhardi	5149	57.2	125
Minhardi × Minturki	8034	60.6	116
Nebraska No. 1062	10015	60.8	104
Oro	8220	61.1	100
Eureka × Minhardi	8036	62.2	119
Newturk	6935	62.8	98
Buffum No. 17	3330	62.8	125
Wheat × Rye	8890	62.8	105
Turkey Selection	6152	63.1	113
Minturki	6155	63.3	119
Turkey × Minessa	8887	63.3	120
Minturki × Beloglina-Buffum	8033	63.9	120
Beloglina	1543	64.4	115
Nebraska No. 60	6250	65.0	108
Kanred	5146	66.3	103
Cheyenne	8885	66.4	101
Kanred × Minhardi	8040	68.3	113
Kharkof	1442	69.7	100
Kawvale	8180	70.0	93
Wisconsin No. 21.25	10018	69.7	100
Kanred × Minhardi	8042	71.4	121
Harvest Queen	6199	72.2	87
Nebraska No. 1069	10016	72.7	102
Beloglina Selection	8884	73.9	103
Karmont	6700	74.2	100
Kanred × Prelude	8886	74.4	77
Early Blackhull	8856	76.7	75
Blackhull	6251	77.2	78
Wisconsin No. 18.4	10019	77.5	81
Tenmarq	6936	78.1	87
Fulcaster	6471	80.3	72
E_m	....	2.7	....

$r = -0.78 \pm 0.046$

the winter-hardiness nurseries[3], as previously noted, are given in Tables X and XI. The appearance of the plants of certain varieties a few days after freezing is indicated in figures 1,3, and 4.

The probable errors of the mean for the artificial-freezing tests in each case were found to be 2.1 and 2.7 per cent, respectively, and the correlation coefficients expressing the relation between injury

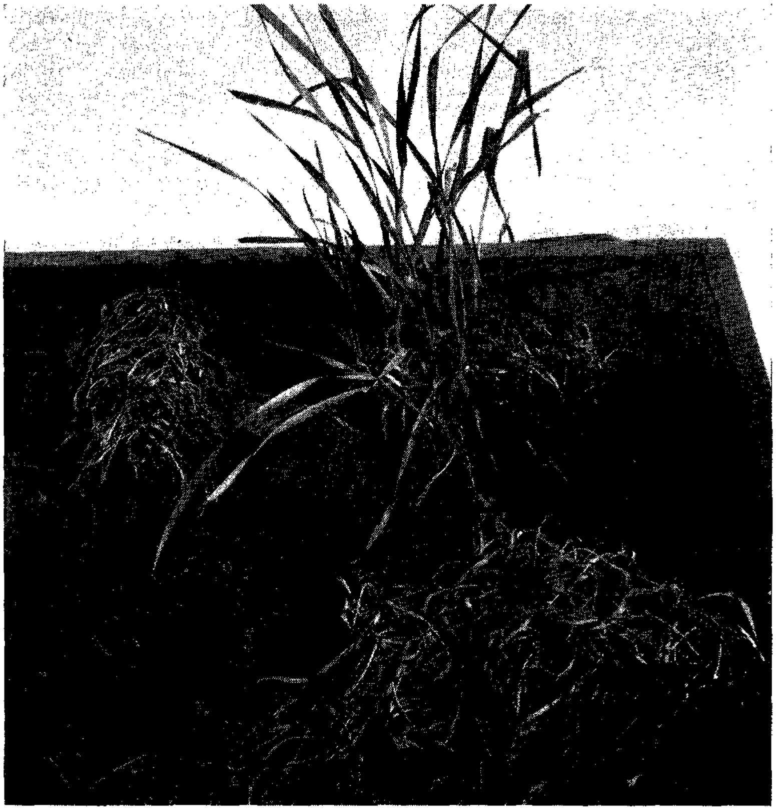

FIG. 3.—Winter wheat frozen artificially. Upper: Left, Kanred; right, Minturki. Lower: Blackhull.

in the artificial-freezing tests and the average survival in the winter-hardiness nurseries were calculated and found to be —0.84 ± 0.038 and —0.78 ± 0.046, respectively.

These may be regarded as fairly high coefficients and indicate beyond doubt that resistance to cold plays an important, if not a major, role in determining winter hardiness in the Great Plains. The significance of these data in relation to predicting relative

3. The writer is under obligation to Messrs. K. S. Quisenberry and J. A. Clark for the data from the winter-hardiness nurseries for those varieties in the freezing tests but not included in winter-hardiness nurseries previous to 1930.

winter hardiness from the results of artificial-freezing experiments will be discussed later.

Not Hardened Before Freezing.—In the fall of 1930 a duplicate set of the varieties from the winter-hardiness nurseries was grown and treated exactly like those previously discussed (Table XI), except they were retained in the greenhouse until they were frozen in December. The growing temperatures were high, mean temperature about, 70° F. (21.1° C,), and the plants made a rank growth.

The average injury in the artificial-freezing tests, the probable error of the mean, the average survival in the winter-hardiness nurseries and the correlation coefficient for the two are shown in Table XII. The correlation coefficient, it will be noted, is low (r=—0.42 ±0.095), though significant, and it is of interest to note that the so-

FIG. 4.—Varieties of wheat after freezing artificially. Left to right: No. 10, Kanred; No. 9, Minard; No. 6, Kanred; No. 5, Minturki × Beloglina-Buffum, C. I. No. 8033.

called very hardy and hardy varieties contribute practically nothing to this coefficient, as shown by the fact that when these varieties are excluded the coefficient is —0.40 ±0.121, and by the fact that the coefficient for these very hardy varieties alone is only —0.178 ±0.188. The probable error of the mean, 7.5 per cent, is unusually high, due perhaps to the high temperature at which the plants were grown.

In January, 1928, nine varieties, including for the most part only varieties of the very hardy or hardy group, were frozen, having been grown in the greenhouse and then exposed before freezing for about three weeks at near freezing temperatures in a specially constructed hardening room. The pertinent data are given in Table XIII. The probable error of the mean, it will be noted, is very low, indicating a very accurate test in so far as the reaction of the plants to low temperature is concerned. The correlation coefficient between estimated injury and survival in the winter-hardiness nurseries, however, is substantially zero. It seems clear that these varieties were not sufficiently hardened before freezing to acquire their full or normal degree of hardiness.

TABLE XII.—RELATIVE INJURY TO VARIETIES OF WINTER WHEAT ARTIFICIALLY FROZEN IN DECEMBER, 1930, COMPARED WITH AVERAGE SURVIVAL IN THE WINTER-HARDINESS NURSERIES.

Plants grown in the greenhouse and frozen without hardening.

VARIETY.	C. I. No.	Average estimated percentage of injury from artificial freezing (N=12).	Average survival in winter-hardiness nurseries (Kharkof=100).
Turkey	6152	53.3	113
Kanred × Minhardi	8040	55.0	113
Wisconsin No. 21.25	10018	75.4	100
Fulcaster	6471	64.2	72
Wisconsin No. 18.4	10019	88.4	81
Cheyenne	8885	65.0	101
Minhardi × Minturki	8034	65.0	116
Minhardi	5149	65.4	125
Kanred × Minhardi	8042	67.1	121
Minard × Minhardi	8889	67.9	116
Kawvale	8180	68.8	93
Minard × Minhardi	8888	69.2	119
Nebraska No. 1062	10015	69.2	104
Beloglina	1543	72.5	115
Minturki × Beloglina-Buffum	8033	74.6	120
Wheat × Rye	8890	75.4	105
Buffum No. 17	3330	78.3	125
Tenmarq	6936	78.8	87
Minhardi × Minturki	8215	79.2	127
Oro	8220	80.4	100
Beloglina Selection	8884	82.1	103
Minturki	6155	82.1	119
Kanred	5146	83.3	103
Nebraska No. 60	6250	84.2	108
Harvest Queen	6199	85.4	87
Karmont	6700	85.5	100
Minard × Minhardi	8218	88.4	114
Eureka × Minhardi	8036	88.8	119
Kharkof	1442	90.4	100
Nebraska No. 1069	10016	94 1	102
Prelude × Kanred	8886	94 2	77
Blackhull	6251	94.2	72
Newturk	6935	96.7	98
Early Blackhull	8856	100.0	75
E_m	....	7.5	....

r = —0 42±0.095

Bayles *(7)*, in work already referred to, froze seventy-seven lots each of Minhardi, Minturki, Kanred and Blackhull at various times during the fall and winter of 1927-'28, beginning on December 3 and continuing until March 3 in order to compare the relative hardiness of these varieties at various times during the fall and winter. The plants were dug from the field immediately before freezing in each case. The results are presented in Table XIV.

It may be observed that in all tests previous to about January 1, Minhardi and Minturki were injured substantially the same or more than Kanred; whereas in those tests after January 1, they survived as well as, or better than, Kanred. On the other hand, Blackhull was injured as much as, or more than, Kanred in all cases.

TABLE XIII.—RELATIVE INJURY TO WINTER WHEAT VARIETIES ARTIFICIALLY FROZEN IN JANUARY, 1928, COMPARED WITH AVERAGE SURVIVAL IN THE WINTER-HARDINESS NURSERIES.

Plants grown in the greenhouse and hardened for about three weeks at, near, or below freezing temperatures before freezing.

VARIETY.	C. I. No.	Average estimated percentage of injury from artificial freezing (N=25).	Average survival in winter-hardiness nurseries (Kharkof=100).
Minhardi	5149	46.9	125
Eureka × Minhardi	8036	52.2	119
Kanred	5146	53.6	103
Kanred × Buffum	8030	59.2	123
Minhardi × Minturki	8034	62.8	116
Minturki × Beloglina-Buffum	8033	63.7	120
Minturki	6155	69.6	119
Turkey (6152) × Minessa	8028	73.8	128
Turkey	6152	86.4	113
E_m	....	0.6	....

$r = -0.01 \pm 0.225$

In a similar study by the writer in 1929-'30 in which Minhardi and other varieties were planted in pots and exposed outside the greenhouse, Minhardi consistently killed more than Kanred when frozen previous to January 1. No freezing tests were made after January 1, but a portion of each lot was left outside until February 8 when it was found that more of the Kanred plants than of the Minhardi had been killed. It is certain from observations on plants brought into the greenhouses that none of the killing outside had taken place previous to January 1. It appears, therefore, that in this case also, Minhardi did not acquire the ability to survive as well as Kanred until some time after the first of the year. Altogether the results would seem to substantiate in a very satisfactory way those reported by Hill and Salmon *(32)*, in which it was shown that Minhardi and similar varieties may not exhibit their complete relative degree of hardiness if frozen without hardening.

Summarizing the results, it would appear that very little dependence can be placed in the results of artificial freezing of unhardened plants of the very hardy varieties as a means of estimating relative winter survival. For fully hardened plants, however, the agreement may be regarded as very good, as shown by the correlation coefficients which ranged from 0.65 to 0.84 when the results of the freezing tests were correlated with the average survival in all of the winter-hardiness nurseries. Quisenberry *(62),* it may be remembered, secured a coefficient of 0.713 ±0.31 for artificial freezing at St. Paul,

TABLE XIV.—RELATIVE INJURY TO MINHARDI, MINTURKI, KANRED, AND BLACKHULL GROWN IN THE FIELD AND ARTIFICIALLY FROZEN AT DIFFERENT TIMES DURING THE WINTER.

DATE FROZEN.	Temperature (C.).	Number of tests.	Average estimated percentage of injury.				E_m
			Minhardi.	Minturki.	Kanred.	Blackhull.	
December 3 to 5	—17 to —21	10	27.5	30.5	33.5	59.5	2.75
December 6 to 10	—16 to —20	12	71.9	65.8	55.8	75.7	1.37
December 10 to 12	—17 to —20	4	80.0	61.3	56.3	87.3	4.37
December 21	—20 to —23	5	67.0	70.0	78.0	85.6	2.02
January 12 to 14	—21 to —24	10	72.5	77.8	82.6	92.5	2.41
February 2 to 5	—22 to —26	20	86.0	92.3	94.6	97.4	0.31
February 18 to 19	—23 to —26	5	60.0	62.0	75.0	82.0	2.65
February 21 to 24	—23 to —26	7	68.6	77.1	77.1	86.4	1.61
March 2 to 3	—22 to —25	4	86.3	92.0	95.0	98.8	1.70

Minn., and the average for two field plantings, one at St. Paul, Minn., and one at Moccasin, Mont., and Foster Martin *(45)* secured a coefficientof 0.762 ±0.085 for artificial freezing of twelve spring wheats at Manhattan, Kan., and winter survival in the field in the Pacific Northwest. Thus it would seem that the relative injury produced by exposure of hardened plants to freezing temperatures agrees reasonably well with winter killing under field conditions.

A better estimation of the reliability of artificial-freezing trials may be made if some consideration be given the accuracy of the results from the winter-hardiness nurseries themselves. Possibly the best measure of this that can be obtained is the interannual correlation coefficients as presented in Table XV, in which the average survival of the varieties in any one year is correlated with the average survival of the same varieties in every other year for the five-year

TABLE XV.—INTERANNUAL CORRELATION COEFFICIENTS OF AVERAGE SURVIVAL IN THE UNIFORM WINTER-HARDINESS NURSERIES OF THE UNITED STATES DEPARTMENT OF AGRICULTURE.

Number of nurseries each year in parentheses.

YEAR.	1926 (18).	1927 (19).	1928 (20).	1929 (21).
(19) 1927	0.95±0.014	....	....	....
(20) 1928	.92± .022	0.94±0.015	....	....
(21) 1929	.91± .025	.95± .014	0.92±0.021	....
(21) 1930	.97± .010	.95± .017	.96± .012	0.94±0.018

period 1926 to 1930, inclusive. It seemed impractical to include in this study data secured previous to 1926, because of the small number of varieties common to two or more seasons before that time. The figures actually used in the calculations are the averages for each year as given in Table 6 of U. S. Dept. Agr. Cir. 141, except for the year 1930, the data of which were kindly supplied by Messrs. Quisenberry and Clark. These averages, it should be noted, do not include seasons and stations in which killing was nil or was complete for all varieties. The correlation coefficients vary from 0.91 ± 0.025 to 0.97 ± 0.010, thus indicating a very high order of agreement from year to year.

In comparing these coefficients with those expressing the relation between injury from artificial freezing and survival in the winter-hardiness nurseries, consideration should be given the fact that the latter are based on from eighteen to twenty-one nurseries, in each of which each variety is represented by from three to nine rod rows comprising several hundred plants; whereas the results from controlled freezing have usually been based on 100 plants or less. Undoubtedly these high correlations reflect to a considerable extent the effect of the fairly large numbers involved in the calculations.

Additional light may be thrown on the accuracy of the winter-hardiness nurseries by calculating the intraclass correlation coefficients for each year. Harris *(27)* has pointed out the usefulness of such coefficients and has developed simple formulae for calculating them. Fisher *(21)* gives the following:

$$k\,S\,(\bar{X}_p - \bar{X})^2 = n^1 s^2 \left[1 + (k-1)\,r\right]$$

which solved for *r*, and using a notation somewhat more familiar to American readers, becomes

$$r = \frac{1}{n-1}\left[\frac{n\,\Sigma\,(\bar{X}_p - \bar{X})^2}{m\,\sigma_x^2} - 1\right] \qquad (1)$$

in which n is the number in each class (in this case stations), m the number of classes (varieties), $\bar{X}_p$ the averages of the individual varieties for all stations, $\bar{X}$ the average survival of all varieties at all stations, and σ_x^2 the variance of all varieties at all stations.

Unfortunately this formula is not applicable when there is differentiation within the classes, which clearly is the case here, since the average survival at various stations is very different. There would appear to be two ways, however, in which this difficulty may be overcome, viz.: (1) express the survival of each variety at each station as a deviation from the mean or average survival of all varieties at the individual stations, as suggested by Harris *(28)*, or (2) modify the formula so as to achieve the same result. The latter procedure involves fewer calculations and is, therefore, followed.

With reference to formula (1) it will be apparent that all values except that for σ_x^2 will be the same, whether survival is expressed in original figures or as deviations from the means of the respective stations; hence a correction needs to be applied only for this expression.

Let $\overline{X}_1, \overline{X}_2, \ldots \overline{X}_n$ be the average or mean survival at stations $X_1, X_2, \ldots X_n$, and $X_{1a}, X_{1b}, \ldots X_{na} \ldots X_{nm}$ be the percentage survival of varieties $a, b, \ldots m$ at these stations. If X_{1a} be the survival of variety a at station X_1, the deviation of this survival from the average survival of all varieties at this station is $(X_{1a}-\overline{X}_1)$. Since the total of the deviations is zero, their mean $\overline{X}$ is zero when all values are expressed as deviations from the mean of their respective stations, and that portion of the sum of the squared deviations, supplied by station X_1 is:

$$(X_{1a}-\overline{X}_1)^2+(X_{1b}-\overline{X}_1)^2+\ldots(X_{1m}-\overline{X}_1)^2=\Sigma X_1^2-m\overline{X}_1^2.$$

Similarly the contributions to ΣX^2 supplied by all stations are:

$$\begin{array}{c} \Sigma X_1^2-m\overline{X}_1^2 \\ + \quad + \\ \Sigma X_2^2-m\overline{X}_2^2 \\ + \quad + \\ \vdots \quad \vdots \\ \vdots \quad \vdots \\ \Sigma X_n^2-m\overline{X}_n^2 \end{array}$$

Summing these we have:

$$\Sigma X^2-m\left[\overline{X}_1^2+\overline{X}_2^2+\ldots\overline{X}_n^2\right]$$

$$\text{and } \sigma^2_x=\frac{\Sigma X^2}{n\,m}-\frac{1}{n}\left[\overline{X}_1^2+\overline{X}_2^2+\ldots\overline{X}_n^2\right]$$

Substituting in formula (1) and simplifying we have:

$$r=\frac{1}{n-1}\left[\frac{n^2\,\Sigma(\overline{X}_p-\overline{X})^2}{\Sigma X^2-m\left[\overline{X}_1^2+\overline{X}_2^2+\ldots\overline{X}_n^2\right]}-1\right]$$

$\overline{X}_p$ is the average survival of any given variety at all stations, and $\overline{X}$ again is the average for all varieties at all stations as in formula (1).

Applying this formula to the data from the 1926 to 1930 winter-hardiness nurseries gives the results presented in the second column of Table XVI.

TABLE XVI.—INTRA-CLASS CORRELATION COEFFICIENTS FOR SURVIVAL IN THE UNIFORM WINTER-HARDINESS NURSERIES, 1926 TO 1930.

YEAR.	Coefficients of correlation.	
	Intra-class, i. e., between individual stations.	Between all stations taken one at a time and the average of all others.
1925-'26	0.23	0.51
1926-'27	.28	.51
1927-'28	.31	.53
1928-'29	.22	.46
1929-'30	.30	.52

It has seemed desirable, also, to calculate the correlation coefficients expressing the relation between survival at individual stations and that at all other stations for the same season. These calculations might be made directly from the tables in the publications referred to above in which the survival percentages for individual stations and the averages for all stations are given, were it not for the fact that the averages as there given include the station being correlated with the average, thus introducing a certain amount of spuriousness. New averages might be calculated, but here again much extra labor is involved. Accordingly a special formula was developed for the purpose.

We make use of the well-known formula:[4]

$$r = \frac{\frac{\Sigma XY}{m} - \frac{\Sigma X}{m}\frac{\Sigma Y}{m}}{\left[\frac{\Sigma X^2}{m} - \left(\frac{\Sigma X}{m}\right)^2\right]^{\frac{1}{2}}\left[\frac{\Sigma Y^2}{m} - \left(\frac{\Sigma Y}{m}\right)^2\right]^{\frac{1}{2}}}$$

in which X is the survival of the variety at any given station and Y the average survival at all stations.

4. It will be noted that the conventional N is here replaced by m in order to avoid confusion with other formulæ in this bulletin.

Let Y_1 equal the average survival of a variety at all stations with station X_1 omitted, Y_2 the average survival with station X_1 omitted, and . , . . Y_n the average survival with station . . . . X_n omitted. The average correlation in terms of the preceding formula then will be:

$$r = \frac{\frac{1}{n}\left[\frac{\Sigma X_1 Y_1}{m} - \frac{\Sigma X_1}{m}\frac{\Sigma Y_1}{m} + \frac{\Sigma X_2 Y_2}{m} - \frac{\Sigma X_2}{m}\frac{\Sigma Y_2}{m} + \cdots \frac{\Sigma X_n Y_n}{m} - \frac{\Sigma X_n}{m}\frac{\Sigma Y_n}{m}\right]}{\left\{\frac{1}{n}\left[\frac{\Sigma X_1^2}{m} - \left(\frac{\Sigma X_1}{m}\right)^2 + \frac{\Sigma X_2^2}{m} - \left(\frac{\Sigma X_2}{m}\right)^2 + \cdots \frac{\Sigma X_n^2}{m} - \left(\frac{\Sigma X_n}{m}\right)^2\right]\right\}^{\frac{1}{2}} \left\{\frac{1}{n}\left[\frac{\Sigma Y_1^2}{m} - \left(\frac{\Sigma Y_1}{m}\right)^2 + \frac{\Sigma Y_2^2}{m} - \left(\frac{\Sigma Y_2}{m}\right)^2 + \cdots \frac{\Sigma Y_n{}^2}{m} - \left(\frac{\Sigma Y_n}{m}\right)^2\right]\right\}^{\frac{1}{2}}}$$

$$\text{But } Y_1 = \frac{n\,Y - X_1}{n - 1}, \qquad Y_2 = \frac{n\,Y - X_2}{n - 1}, \text{ etc.}$$

By substituting for Y_1, Y_2, etc., collecting similar terms, and simplifying, the equation becomes:

$$r = \frac{\frac{1}{m(n-1)}\left\{n\,\Sigma Y^2 - \frac{\Sigma X^2}{n}\right\} - \frac{1}{m^2(n-1)}\left\{n(\Sigma Y)^2 - \frac{1}{n}\left[(\Sigma X_1)^2 + (\Sigma X_2)^2 + \ldots (\Sigma X_n)^2\right]\right\}}{\left\{\frac{\Sigma X^2}{mn} - \frac{1}{nm^2}\left[(\Sigma X_1)^2 + (\Sigma X_2)^2 + \ldots (\Sigma X_n)^2\right]\right\}^{\frac{1}{2}} \Bigg\{\frac{1}{m(n-1)^2}\Big[\Sigma Y^2(n^2 - 2n) + \frac{\Sigma X^2}{n}\Big] - \frac{1}{m^2(n-1)^2}\Big[(\Sigma Y)^2(n^2 - 2n) + \frac{1}{n}\Big\{(\Sigma X_1)^2 + (\Sigma X_2)^2 + \ldots (\Sigma X_n)^2\Big\}\Big]\Big]\Bigg\}^{\frac{1}{2}}}$$

This formula appears complicated, but really is a relatively simple working formula in view of the common terms in the numerator and denominator. Its use avoids the laborious calculation of products. In the present case, with but little more labor than would be required for the calculation of one coefficient, it accomplished the purpose of calculating fifteen to twenty. Applying this formula to the data already referred to gives the coefficients presented in the third column of Table XVI.

These coefficients may be regarded as expressing the relations that may be expected when the results of any single nursery chosen at random are correlated with the results of all others, while the coefficients in the second column of Table XVI may be regarded as expressing the expected relation when the results of a single nursery are correlated with those of any other, both being chosen at random.

In general it would appear not unreasonable to expect correlation coefficients between individual nurseries of the order of 0.25 to 0.30, and between individual nurseries and all others in the same season of the order of 0.50, as compared with from about 0.60 to 0.80 for the artificial-freezing tests and average survival. It will be evident that this cannot be expected to apply to all places in all seasons, and probably should be thought of as a broad generalization useful only for comparison.

These coefficients possibly are lower than would be expected. If so, it should be remembered that the number of rows (or plots) of each variety in any nursery has seldom been more than three, and that the random errors involved in the estimates of survival are no doubt fairly large. There are a few cases where regression is not strictly linear, as when a considerable number of varieties either completely survived or were entirely killed. Also it is probable that conditions are not entirely homogeneous throughout the Great Plains, and, furthermore, differential results have not always been secured, even when killing has been severe but not complete. Thus, at Hays, Kan., in 1928, the killing was decidedly greater for the hardy varieties as a group than for such tender varieties as Blackhull, Superhard, Tenmarq, Harvest Queen, and Fulcaster. In that year the correlation coefficient for survival at Hays and the average of all other nurseries in the same year was —0.72 ± 0.059. At Archer, Wyo., in 1922, and at Morden, Manitoba, and Swift Current, Saskatchewan, in 1929, there was severe winter killing, but there was no consistent difference between members of the various groups as shown by the fact that the correlation coefficients for survival at each of these stations with that at all other stations were only 0.02 ± 0.150, 0.10 ± 0.12 and 0.19 ± 0.119, respectively. Similar relations have been observed in other cases.

The question naturally arises whether the relative survival of varieties might not more satisfactorily be predicted from selected nurseries at which differential killing occurred and which supply homogeneous results than from a single nursery taken at random or from an average of all nurseries. Such undoubtedly is the case, and it may be remembered that the data so far used are from selected nurseries only. It is probable that with information now available it would be possible to make a better selection than has been done. However, it may be doubted whether the omission of additional nurseries can be justified without a critical study from this viewpoint. To make such a study goes beyond the limits set for this bulletin. Moreover, in a preliminary study the omission of certain data which obviously were not homogeneous, and others which were nonlinear, had no material effect on the final result. This, no doubt, is explained by the fact that such data made up a very small proportion of the total. Furthermore it is difficult to reconcile the high coefficients of interannual correlation in Table XV with the supposition that nonhomogeneous data played an important role in determining the value of the intraclass coefficients. It seems more probable that their low values are due mainly to what may be regarded as random errors.

The fact that contradictory results are sometimes secured should not be looked upon as in any sense a criticism of the winter-hardiness nurseries; on the contrary, they are probably due to a differential reaction of varieties to environmental conditions in the field, some of which possibly approximate those encountered in the controlled freezing tests. At Hays, Kan., in 1928, for example, the greater injury to the hardy varieties was attributed to a sudden and severe drop in temperature in early December, following very warm weather during which there was no opportunity for the plants to harden. These seemingly contradictory results may therefore be considered as substantiating, in part, the results of those artificial-freezing tests having to do with the hardening process in which it is shown that the very hardy varieties seem to be no more hardy, or even less hardy, than others if not hardened before freezing.

REGIONAL VARIETIES

As previously noted a number of freezing tests have been conducted in which varieties have been frozen which have not been included in the winter-hardiness nurseries. Each of these tests usually has included a few varieties from the winter-hardiness nurseries, but their inclusion in the experiments now to be reported was incidental rather than otherwise. The distinctive feature of these experiments is that they include relatively few or none of the very hardy group, such as Minhardi and Minturki.

One of the first of these tests included a number of varieties of winter wheat chosen for the range in winter hardiness known to exist and one each of winter rye (Dakold), winter barley, and winter 0ats. The plantings were made in four-inch pots in the greenhouse, November 1, 1927, and were thinned to five plants per pot upon emergence. The plants were kept in the greenhouse (mean temperature about 55° F. or 12.2° C.) until they were frozen in the period from January 4 to January 16, the temperature for the freezing ranging from -9° to -12° C. There were forty-seven pots, or approximately 230 plants, of each variety. The average injury for each variety is indicated in Table XVII. The average survival in the winter-hardiness nurseries compared with Kharkof =100 per cent is included so far as the data were available.

With the exception of Minhardi and Minturki, the agreement with the known survival of the various crops and varieties under field conditions is reasonably good. Thus Dakold rye, which is perhaps the most winter-hardy cereal known in America, was injured the

TABLE XVII.—RELATIVE RESISTANCE OF GREENHOUSE-GROWN CROP PLANTS TO LOW TEMPERATURE. FROZEN JANUARY 4 TO JANUARY 16, 1928 (—9° TO —12° C.), WITHOUT PREVIOUS HARDENING.

VARIETY.	C. I. No.	Average estimated injury (N=47).	Average survival in winter-hardiness nurseries (Kharkof=100).
		Per cent.	
Rye, Dakold	....	8.3	....
Wheat:			
Kanred	5146	39.0	103.0
Kharkof	1442	45.0	100.0
Minhardi	5149	58.1	125.0
Harvest Queen	6199	66.6	87.4
Blackhull	6251	69.2	78.2
Tenmarq	6936	71.2	86.6
Minturki	6155	75.8	119.1
Superhard	8054	76.7	75.0
Fulcaster	6471	79.0	71.8
Nebraska 28	5147	82.7	86.5
Winter barley, Tennessee Winter	....	89.0	....
Winter oats, Winter Turf	....	97.6	....
E_m		1.65	

least, and Kanred and Kharkof, which are known to be among the more winter-hardy wheats, survived better than other varieties of wheat. Harvest Queen, Blackhull, Tenmarq, Nebraska 28, Fulcaster, and Superhard occupy an intermediate position, both with respect to winter hardiness and resistance to low temperature, as shown in these tests. Winter barley and winter oats which were injured more than any of the others by freezing, are well known to be less winter hardy than any of the winter wheats included. The controlled freezing test was not accurate enough to distinguish between Harvest Queen, Blackhull, Tenmarq, Superhard, Fulcaster, and Nebraska 28. The probable reasons for the behavior of Minhardi and Minturki have already been pointed out.

During the winter of 1927-'28, twenty lots each of Harvest Queen, Blackhull, Fulcaster, and Currell were dug from the field plots, transferred immediately to flats and thence to the refrigerator, where they were frozen at temperatures ranging from —22° to —26° C. The average estimated injury together with the average survival in the winter-hardiness nurseries are given in Table XVIII. The probable error is low; viz., 0.43 per cent.

Currell has not been included in the uniform winter-hardiness nurseries, but in several field plots and nursery rows on the Columbus, Kan., experiment field in 1928 and in 1930 it was almost completely killed, whereas Fulcaster survived fairly well and Blackhull and Harvest Queen were scarcely injured. Experience elsewhere with Currell and the distribution of this variety in the United States, as shown by Clark *et al.* *(12),* leave little doubt as to its relative susceptibility to winter injury.

TABLE XVIII.—RELATIVE INJURY OF PLANTS OF FOUR VARIETIES OF WINTER WHEAT GROWN IN THE FIELD AND ARTIFICIALLY FROZEN FEBRUARY 9 AND 10, 1928, AT TEMPERATURES RANGING FROM —22° TO —26° C.

VARIETY.	Average estimated injury from artificial freezing (N=20).	Average percentage survival in winter-hardiness nurseries (Kharkof=100).
	Per cent.	
Harvest Queen	80.8	87.4
Blackhull	84.9	77.9
Fulcaster	84.3	72.6
Currell	94.4	....
E_m	0.43	

It would appear, therefore, that the comparative resistance of these varieties to low temperature under controlled conditions is in agreement with their resistance to winter killing under field conditions. The difference between Blackhull and Fulcaster in the controlled freezing tests is substantially the same as the probable error and hence open to considerable doubt, but it should be observed that the difference between these two in the winter-hardiness nurseries is not clearly established, since the data for the three years for which results have been published indicate only a small difference, in two of them the average difference in each case being less than 0.5 of 1 per cent.

In 1929, twenty-three varieties and strains of winter wheat from various portions of the United States were planted in four-inch pots on October 15, thinned to five plants per pot on emergence, divided into two groups, and frozen during the latter part of November and early December. One of the groups consisting of twenty-five pots of each variety was retained in the greenhouse at a mean temperature of about 55° F. (12.2° C.) and frozen at various temperatures ranging from —10° to —15° C. The other group, consisting of thirty-one pots of each variety, was placed outside the greenhouse and exposed to prevailing conditions and then frozen at various temperatures ranging from —8° to —16° C. Both hard red winter and soft red winter varieties were included in this experiment; also two varieties with erect or spring growth habit, but which often are seeded in the fall, namely, Federation and Sonora. The average estimated percentage of injury and rank for each variety is indicated in Table XIX.

With the exception of Minhardi in the outdoor test the results are not contrary to what would be expected on the basis of the known hardiness of the different varieties, aside from the fact that they fail to differentiate between certain varieties which it is reasonably certain differ in winter hardiness.

Incidentally not a great deal is known regarding the winter hardiness of many of these varieties, except for whatever assumptions may be based on their known adaptation to particular regions. As previously noted most of them have been included in the controlled freezing tests to secure some knowledge of their resistance to low temperatures and not primarily as a measure of agreement between resistance to low temperature and winter hardiness. Nevertheless such deductions as may be made regarding the latter would seem to be of interest.

In general the hard red winter wheats are known to be more winter hardy than the soft red winter varieties as a group, and this relation holds for the resistance to low temperature, as shown in Table XIX. Michikof, Purkof, and Harvest Queen occupy places in the tables just below the hard winter wheats. In the freeze of 1928, when winter injury was very great throughout the soft red winter wheat belt, these three varieties were among those which survived best, whereas Trumbull, Currell, Fulcaster, and others survived very poorly. Purkof survived better than all other varieties in a test at the West Virginia Agricultural Experiment Station, as

TABLE XIX.—RELATIVE INJURY TO VARIETIES OF WHEAT ARTIFICIALLY FROZEN IN NOVEMBER AND DECEMBER, 1929.

VARIETY.	C. I. No.	Average estimated percentage of injury.		Rank.	
		Grown in greenhouse and frozen without hardening (N=25).	Exposed outdoors 8 to 10 weeks before freezing (N=31).	Grown in greenhouse.	Exposed outdoors.
Minhardi	5149	47.8	88.1	1	8
Minturki	6155	56.8	64.4	2	1
Kharkof	1442	60.0	74.7	3	2
Kanred	5146	64.4	76.0	4	3
Michikof	6690	73.8	90.0	5	9
Turkey	1558	76.8	84.8	6	5
Purkof	8381	78.0	82.3	7	4
Blackhull	6251	78.8	92.7	8	10
Denton	8265	80.2	94.7	9	12
Rice	5734	81.4	86.3	10	7
Harvest Queen	6199	85.2	84.8	11	6
Gold Coin	5645	89.8	....	12	....
Fulcaster	6471	90.2	96.1	13	13
Currell	6216	92.4	98.1	14	15
Fulcaster	1945	94.4	98.7	15	17
Poole	3489	94.6	98.9	16	18
Fultz	6215	95.2	99.0	17	19
Purplestraw	1915	95.2	98.4	18	16
Leap	4823	95.6	97.4	19	14
Nittany	6962	97.4	93.9	20	11
Trumbull	5657	97.4	99.4	21	20
Hybrid 128	4326	98.3	99.5	22	21
Fultzo-Mediterranean	1957	98.6	....	23	....
Federation	4734	100.0	....	24	....
Sonora	3036	100.0	....	25	....
E_m	....	2.6	1.2	....	....

reported by Hoover and Garber *(36)*. It is doubtful if either Purkof or Michikof are more hardy than Turkey, as their positions in the table would suggest. Likewise there may be some question as to the hardiness of Denton, although reliable information regarding the winter hardiness of this variety is wanting. These exceptions are easily explained on the basis of chance variations, as shown by the probable errors. There appears to be very little in the published literature regarding the winter hardiness of Purplestraw, although it is common knowledge that it is relatively nonhardy. At Columbus, Kan., in 1930 it was practically all killed, whereas most other varieties survived fairly well or were not severely injured. The fact that Purplestraw wheat is not grown on a commercial scale in the northern states is in accord with the assumption that it is one of the least winter hardy. Nittany is a selection from Fulcaster. In tests reported by Hoover and Garber *(35)* it survived slightly less than Fulcaster, thus agreeing with the controlled freezing results. Federation and Sonora are spring varieties, although often seeded in the fall in mild climates. It will be noted they were more severely injured by freezing than any of the others.

Red Wave, Red Rock, and Lutescens No. 0329 were also included in part of the freezing tests of the nonhardened group, and Red Wave, Sonora, Lutescens, and Fultzo-Mediterranean in a part of the hardened group, but were not included in all because of insufficient seed, and the relative injury is not indicated in the table. In those tests in which they were included Red Rock and Red Wave were injured to about the same extent as Goldcoin. Lutescens No. 0329 was injured slightly more than Kharkof. There seem to be very few dependable observations as to the relative winter hardiness of any of these varieties under natural conditions in the United States. Wilson and Arny *(92)* report the average winterkilling of Red Rock for three years at University Farm, St. Paul, Minn., as 49 per cent compared with 16 per cent for Kanred and 9 per cent for Minturki. The areas of production of Red Wave and Red Rock suggest they are not greatly different from Goldcoin in winter hardiness. Talanov *(83)* classified the strain of Lutescens used here as being among the most winter-hardy varieties in Russia, being as hardy as, or perhaps more hardy than, Minhardi. Fultzo-Mediterranean was injured to about the same degree as Leap, Currell and Purplestraw. It would appear that so far as the information goes, the behavior of these varieties agrees with what would be expected on the basis of their distribution and known behavior under field conditions.

It is of interest to note that Minhardi takes its proper place in the nonhardened group but not in the other. In the latter case the plants were placed outside the greenhouse while they were very young and before they had assumed the procumbent position which is characteristic of most winter-wheat varieties in the fall. Whether this fact, is related to their behavior cannot be determined, but it is perhaps significant that in another study in which the plants were treated exactly like those discussed here, except that they were planted eight days earlier and therefore were more advanced in their growth, the Minhardi survived much better than any others included in that study.

In the fall of 1930 a number of varieties were compared in essentially the same way as in earlier tests except for some reduction in the number of varieties. The plantings were made about the first of October. Soon after emergence the plants were placed outside to harden and were frozen in December. The estimated injury from controlled freezing is indicated in Table XX. The results, it will be noted, agree, at least reasonably well, with what might be expected under field conditions, except that Fulcaster ranks higher than its field hardiness would warrant. The discrepancies, however, are well within the limits of experimental error. Clark No. 40 is another variety concerning which there is very little information as to its winter hardiness, except that in field trials in southeast Kansas in 1930, in which there were severe losses from winter killing, it survived a little better than Harvest Queen and Kawvale.

TABLE XX.—RELATIVE INJURY FROM ARTIFICIAL FREEZING OF REGIONAL VARIETIES OF WINTER WHEAT, FROZEN IN DECEMBER, 1930 (—21° TO —23° C.); HARDENED OUTSIDE BEFORE FREEZING.

VARIETY.	C. I. No.	Estimated percentage of injury (N=5).	Rank.
Kanred	5146	34.6	1
Turkey	1558	44.8	2
Harvest Queen	6199	52.0	3
Purkof	8381	56.8	4
Fulcaster	6471	57.8	5
Clark No. 40	8858	59.3	6
Michikof	6690	60.3	7
Nittany	6962	66.3	8
Denton	8265	67.0	9
Currell	6216	69.8	10
Fultz	6215	71.8	11
Hybrid 128	4326	74.3	12
Trumbull	5657	78.8	13
Purplestraw	1915	82.8	14
Poole	3489	85.3	15
Leap	4823	87.8	16
E_m		2.9	

A duplicate planting of these varieties was grown in the greenhouse and frozen in December without hardening. It was impossible, however, to maintain the greenhouse temperatures as low as desired, the growth was very rank, and all varieties except Harvest Queen and Clark No. 40 were completely killed, and these two were badly injured.

A number of other tests have been made from time to time, the results of which, as a whole, are not of sufficient general interest to justify a complete presentation of all data, and yet they have included certain varieties of interest. Thus Davis *(161)* Hubbard *(37),* Goth *(25),* and Stevens *(80)* , working with the writer and using the equipment described herein, froze the segregates and the parents of crosses between Kanred and Blackhull, Kanred and Tenmarq, Kanred and Kanmarq, and others. The comparative injury of the parents in many cases is of interest in the present study. Also there have been numerous tests of Kanred and Blackhull alone because of the great interest in the latter variety and the urgent need for information regarding its relative winter hardiness as pointed out elsewhere *(72)*. Data pertaining to these varieties are presented in Table XXI. In this table the varieties are listed in pairs, the more winter hardy under field conditions being listed first and the less winter hardy second. In the two columns giving the estimated injury the figures for the varieties are given in the same order. That is to say, the first column under this head gives the percentage of injury for that variety of each pair which is believed to be the more winter hardy under field conditions, and the second column gives the percentages of injury for that variety of the pair that is believed to be the less winter hardy. The differences in the estimated injury of the two and the ratio of the differences to the probable errors are also indicated. The latter have been calculated by the point binominal method suggested by Salmon *(70)*. It will be noted that in all cases, without exception, the variety of the pair known to be the more winter hardy under field conditions proved to be the more resistant to the artificially produced low temperatures. Also the differences in every case are statistically significant.

It is pertinent to note that in this group of varieties as well as those previously discussed the relative injury of all except those of the very hardy or medium-hardy groups, such as Minhardi and Minturki and derivatives of these, appears to be about the same whether hardened before freezing or frozen without hardening. All of them, of course, are much more resistant to low temperature if previously hardened but their relative degree of injury or their rank if ranked according to injury appears to be essentially the same, or at least if different the experiments here have not been such as to demonstrate such a difference.

RESISTANCE OF VARIETIES OF RYE TO LOW TEMPERATURE

Five varieties of winter rye—Dakold, North Dakota No. 9, Swedish (Minn. No. 2), Rosen, and Abruzzi—were included in the freezing tests in 1929-'30 and, also, excepting Swedish, in 1930-'31. The plants were grown in four-inch clay pots in both seasons, five plants per pot. In the first season a part were grown in the greenhouse until frozen, a part were hardened by placing outside, and a part were left outside all winter and frozen naturally. The pro-

TABLE XXI.—RELATIVE RESISTANCE TO CONTROLLED FREEZING OF CERTAIN VARIETIES OF WINTER WHEAT FROZEN IN PAIRS.

Varieties compared.		N (a).	Year.	Approximate date.		Grown in field (F) or green-house (GH) before freezing.	Hardened (H) or not hardened (NH).	Approx. mean temp. (C) previous to freezing (b).	Approximate min. temp. (C) of freezing (c).	Av. estimated percentage of injury.			D-E.
More hardy.	Less hardy.			Planting.	Freezing.					More winter-hardy variety.	Less winter-hardy variety.	Difference.	
Kanred.........	Blackhull.....	67	1926-27	Oct. 8	Dec. and Jan....	GH	NH	37	—10 to —18	51.5	74.6	23.1	10.0
Kanred.........	Blackhull.....	81	1926-27	Oct. 8	Jan. 26.........	GH	NH	37	—10 to —18	64.3	85.1	20.8	7.7
Kanred.........	Blackhull.....	82	1927-28	Oct. 1	Jan. 16-20......	GH	NH	54	—7 to —12	73.6	94.0	20.6	9.0
Kanred.........	Blackhull.....	101	1927-28	Oct. 1	Dec. to Feb.....	F	H	35	—17 to —25	78.6	87.8	9.2	11.4
Kanred.........	Blackhull.....	31	1927-28	Oct. 8	Jan. 29.........	GH	H	....	—18	80.1	96.9	16.8	6.2
Kanred.........	Blackhull.....	61	1928-29	Nov. 5	Jan. and Feb....	GH	NH	55	—10 to —14	59.7	80.0	20.3	7.1
Kanred.........	Blackhull.....	13	1928-29	Nov. 5	Jan. 21.........	GH	H	....	—11.5	77.8	100.0	22.5	5.4
Kanred.........	Tenmarq.....	62	1926-27	Oct. 8	Dec. and Jan....	GH	NH	37	—10 to —18	52.4	73.1	20.7	10.2
Kanred.........	Tenmarq.....	85	1926-27	Oct. 15	Jan. 26.........	GH	NH	38	—10 to —15	66.0	84.0	18.3	9.8
Kanred.........	Tenmarq	19	1927-28	Oct. 29	Jan. 9 and 10...	GH	NH	56	—9 to —12	78.7	90.2	11.5	3.7
Kanred.........	Tenmarq.....	46	1927-28	Nov. 4	Jan. 4 to 16.....	GH	NH	56	—9 to —12	38.5	70.6	32.1	7.3
Kanred.........	Tenmarq.....	46	1928-29	Oct. 15	Jan. 29.........	GH	NH	55	—13	69.8	98.1	28.3	10.1
Kanred.........	Tenmarq.....	175	1928-29	Oct. 15	Jan. 3-18.......	GH	NH	55	—12 to —13	46.8	88.7	41.9	17.3
Kanred.........	Tenmarq.....	90	1929-30	Oct. 18	Jan. 5-25.......	GH	H	....	—12 to —15	67.8	74.8	7.0	8.9
Kanred.........	Nebr. 28.....	110	1926-27	Nov. 3	Jan. 5-15.......	GH	NH	37	—10 to —13	20.5	61.4	40 9	25.0
Kanred.........	Nebr. 28.....	73	1926-27	Oct. 8	Dec. and Jan...	GH	NH	38	—10 to —18	49.0	79.5	30.5	10.7
Kanred.........	Nebr. 28.....	132	1927-28	Oct. 15	Dec. and Jan...	GH	NH	56	—7 to —10	63.3	91.7	28.4	12.2
Kanred.........	Nebr. 28.....	47	1927-28	Nov. 4	Jan. 4-16.......	GH	NH	55	—9 to —12	39.8	82.7	42.9	22.8
Harvest Queen..	Fulcaster.....	62	1926-27	Oct. 8	Dec. and Jan....	GH	NH	38	—10 to —18	62.9	74.7	11.8	6.3
Harvest Queen..	Fulcaster.....	47	1927-28	Nov. 4	Jan. 4-16.......	GH	NH	55	—9 to —12	66.3	79.0	12.5	4.4

(a) N = Number of pots of 5 plants each or equivalent thereof.

(b) The temperatures here given are for the month (or part of a month) immediately preceding the freezing. Since the freezing period extended over several days or even weeks in most cases, and since temperatures for hardening could not be controlled, the temperature previous to freezing was not exactly the same in all cases.

(c) The freezing temperatures given here are those of the air surrounding the plants. The temperature of the soil surrounding the plants and hence the temperature to which the crowns of the plants were exposed was not determined but in general approached that of the freezing chamber.

cedure was the same the second season, except that none was left outside to be frozen naturally. The greenhouse temperatures for the nonhardened lot in 1930-'31 were very high (about 70° F. or 21.1° C.) and the growth was very rank. Also they were not frozen until February, when Dakold was beginning to head and the others were in the boot. The estimated injury for each variety in each test is given in Table XXII. The appearance, a few days after freezing, of the plants frozen in 1929 is indicated in figure 5.

TABLE XXII.—RELATIVE INJURY TO RYE BY CONTROLLED FREEZING IN 1929-'30 AND 1930-'31 AND BY NATURAL FREEZING IN 1929-'30.

VARIETY.	Estimated percentage of injury.				
	Frozen in Nov., 1929, at —7°C., grown in greenhouse, not hardened before freezing (N=12).	Frozen in Dec., 1929, at —13° to —18°C., hardened outside before freezing (N=24).	Frozen naturally outdoors, 1929 and 1930 (N=54).	Frozen Feb. 2, 1931, at —10° C., grown in greenhouse before freezing (N=20).	Frozen Jan. 7, 1931, at —28° C., hardened outside before freezing (N=20).
Dakold	12.5	30.7	10.1	82.0	66.8
N. Dakota No. 9	21.3	44.4	9.9	89.3	62.3
Swedish	65.4	64.0	10.2	....	....
Rosen	82.1	81.4	77.8	100.0	94.5
Abruzzi	92.9	96.3	89.9	100.0	92.1
E_m	2.27	3.02	1.52	....	....

FIG. 5.—Varieties of rye artificially frozen in December, 1929. Left to right: Abruzzi, Rosen, Swedish, North Dakota No. 9, and Dakold.

On the average Dakold was injured the least, but the difference, as compared with North Dakota No. 9 and Swedish, was not great. In general North Dakota No. 9 ranked second, Swedish third, Rosen fourth and Abruzzi was the least resistant of all.

It appears that there is not a great deal of information available regarding the winter hardiness of the three first-mentioned varieties under field conditions, other than the fact that they are relatively hardy. Data secured by Wilson and Arny *(92)* in Minnesota indicate that Dakold is slightly more winter hardy than Swedish. At Crookston, Minn., for example, the average winter killing for a four-year period was 8 per cent for Dakold and 13 per cent for Swedish. Quisenberry and Clark *(63)* report that in a nursery test at Dickinson, N. D., in 1921 and 1922, the survival of Dakold rye grown in a stubble field was 80 per cent as compared with 62 per cent for Swedish. The fact that Abruzzi rye is grown only in the south and has a spring-growth habit supports the common opinion that it is relatively nonhardy. Wilson and Arny *(92)* report a total failure of Rosen rye at University Farm, Minn., in 1928, whereas 42 per cent of Swedish survived. At Grand Rapids, Minn., the average percentage of winterkilling for a four-year period was 33 for Rosen and 3 for Swedish. The results of the freezing tests are therefore in full accord with these observations so far as they go. It is of particular interest to note that the relative position of the varieties is the same regardless of whether they were hardened before freezing or frozen in a nonhardened condition.

RATE OF LOSS OF HARDINESS IN WHEAT

It is of considerable value to know the rate at which the hardiness of varieties is lost after they once become thoroughly hardened. Attempts have been made to secure information on this point in a number of cases. In December, 1927, in February, 1928, and in December, 1930, a number of lots of Minhardi, Minturki, Kanred, and Blackhull, grown in the field, were dug up, transferred to the greenhouse and frozen at various periods thereafter, those not being frozen immediately being kept in the greenhouse (mean temperature 50° to 55° F. or 10° to 12.2° C.). The estimated injury for each separate experiment is given in Table XXIII.

In interpreting these results it should be kept in mind that the comparisons of interest are those between different freezing lots. While all lots in a given experiment were frozen at the same temperature, it is difficult to duplicate exactly the conditions for different freezing lots, and, consequently, the variation between lots supposedly treated alike is, in general, somewhat greater than in previously reported experiments in which all variants are in the same freezing lot. Also the numbers involved in each experiment in the present case are very small.

In spite of these limitations it is clear that hardiness is lost rather rapidly, a perceptible loss usually taking place in the first 12 to 24 hours. In certain cases some of the hardiness was retained for a period of 96 or even 120 hours. It is of particular interest to note that the more winterhardy varieties, namely, Minhardi and Minturki, retain their greater hardiness throughout the duration of the experiments with the exception that, as would be expected, the difference between them and the less hardy varieties becomes less as the injury approaches 100 per cent. In those lots frozen in De-

TABLE XXIII.—RELATIVE INJURY FROM ARTIFICIAL FREEZING OF FIELD-GROWN PLANTS KEPT IN THE GREENHOUSE (MEAN TEMPERATURE, 50° TO 55° F.) FOR VARIOUS PERIODS OF TIME BEFORE FREEZING.

Date of freezing; temperature of freezing; and variety.	Hours in greenhouse before freezing and estimated percentage of injury (N=2).													
	Frozen immediately.	12	24	36	48	60	72	84	96	108	120	132	144	180
December 6 to 13, 1927; —17° to —20° C.:														
Minhardi	67.5	......	......	......	77.5	......	......	......	80.0	......	......	......	95.0	97.0
Minturki	40.0	......	......	......	65.0	......	......	......	75.0	......	......	......	92.5	95.0
Kanred	25.0	......	......	......	65.0	......	......	......	57.5	......	......	......	67.5	92.5
Blackhull	55.5	......	......	......	80.0	......	......	......	70.0	......	......	......	97.0	97.5
December 9 to 12, 1927; —17° to —20° C.:														
Minhardi	......	75.0	......	......	......	85.0	......	......	87.5	......	......	......	......	......
Minturki	......	60.0	......	......	......	62.5	......	......	97.0	......	......	......	......	......
Kanred	......	52.5	......	......	......	60.0	......	......	92.5	......	......	......	......	......
Blackhull	......	80.0	......	......	......	94.5	......	......	92.5	......	......	......	......	......
December 21 to 27, 1927; —20° to —23° C.:														
Minhardi	60.0	......	......	......	......	......	50.0	......	90.0	87.5	95.0	99.0	......	......
Minturki	70.0	......	......	......	......	......	60.0	......	96.5	91.5	100.0	100.0	......	......
Kanred	70.0	......	......	......	......	......	75.0	......	95.0	85.0	100.0	100.0	......	......
Blackhull	75.0	......	......	......	......	......	90.0	......	87.5	95.0	100.0	100.0	......	......
February 18 to 22, 1928; —23° to —26° C.:														
Minhardi	58.3	62.5	75.0	75.0	75.0	......	72.5	......	85.0	......	......	......	......	......
Minturki	60.0	65.0	77.5	80.0	75.0	......	77.5	......	90.0	......	......	......	......	......
Kanred	70.0	82.5	82.5	85.0	85.0	......	90.0	......	87.5	......	......	......	......	......
Blackhull	80.0	87.5	90.0	90.0	87.5	......	95.0	......	95.0	......	......	......	......	......
February 21 to 25, 1928; —23° to —26° C.:														
Minhardi	60.0	70.0	72.5	80.0	72.5	90.0	85.0	82.5	......	......	......	......	......	......
Minturki	70.0	72.5	82.5	80.0	82.5	94.0	92.5	92.0	......	......	......	......	......	......
Kanred	72.5	72.5	85.0	85.0	87.5	96.5	87.5	92.5	......	......	......	......	......	......
Blackhull	75.0	87.5	90.0	85.0	92.5	94.5	97.0	97.5	......	......	......	......	......	......
December 5 and 27, 1930; —12° to —14°C.:														
Minhardi	58.9	......	86.7	......	70.0	......	73.9	......	83.7	......	......	......	......	......
Kanred	72.8	......	97.7	......	86.9	......	96.4	......	95.2	......	......	......	......	......
Kawvale	78.4	......	99.0	......	91.5	......	98.3	......	99.5	......	......	......	......	......
Blackhull	93.9	......	99.3	......	97.4	......	98.0	......	98.2	......	......	......	......	......
Harvest Queen	89.5	......	98.3	......	95.7	......	98.7	......	98.9	......	......	......	......	......

cember, 1927, Minhardi and Minturki were in general injured as much or more than Kanred, probably because they were insufficiently hardened.

The above results were supplemented in the spring of 1928 by digging lots of the same varieties from the field after growth had started and freezing immediately at a temperature of -25° C. The average percentage of injury for 16 lots of each variety is given in Table XXIV. This experiment is of interest in showing that Minhardi and Minturki retained their relative hardiness as compared with Kanred and Blackhull, although the difference between Minturki and Kanred is no greater than might be attributed to random errors.

In the spring of 1929 several varieties known to differ in hardiness, including Kanred, Oro, Currell, Fulcaster, and Harvest Queen, were transferred from the field to the greenhouse and frozen at temperatures of — 10° to — 16°C. These experiments were repeated at various times between April 1 and April 17 after growth had become quite marked. Currell killed more than the other varieties up till the last date of freezing, but the differences between the others were well within the limits of experimental error. All varieties were almost completely killed, but such differences as were apparent were in agreement with the results just presented.

TABLE XXIV.—RELATIVE RESISTANCE TO LOW TEMPERATURE OF VARIETIES OF WINTER WHEAT DUG FROM THE FIELD AND FROZEN IN THE SPRING OF 1928.

VARIETY.	Average percentage of injury (N=20).
Minhardi	85.9
Minturki	92.7
Kanred	95.4
Blackhull	97.6
E_m	1.23

These results would seem to agree with those secured by Harvey *(30),* who noted that plants acquire and lose hardiness rather rapidly, and, likewise, with Tumanov's *(85)* data which indicate a perceptible loss in hardiness in a single day with plants kept at greenhouse temperatures, and that the rate of loss of hardiness is much greater than the rate of acquisition of hardiness. They are also in agreement with the general opinion that a warm period of only a few days greatly decreases the ability of wheat to survive low temperatures. It may be observed, however, that the rate of change under field conditions would probably be less than in the experiments reported here, because of the insulating effect of the soil which in the field must be considerable.

Based on these results it may be expected that under field conditions Minhardi, Minturki, and similar varieties will prove as hardy or more hardy than others following a short period of unusually warm weather in the winter or early spring in contrast with their relatively low survival when frozen suddenly in the fall following warm weather before they have had an opportunity to harden fully, as at Hays, Kan., in 1928.

USE OF ARTIFICIAL FREEZING IN BREEDING HARDY VARIETIES

It has been pointed out that one of the serious difficulties in breeding winter wheat is inability to determine the relative winter hardiness of new varieties and selections. The need of some such measure will be apparent from the fact that at Manhattan, Kan., no winter since 1917 has been severe enough to differentiate between such varieties as Kanred, Turkey, and Kharkof on the one hand, and such relatively tender varieties as Blackhull, Superhard, Harvest Queen, and Fulcaster on the other. Even Currell, which is known to be the least hardy of any variety grown in Kansas, has not winter killed during the six-year period it has been grown. One of the purposes of the present study was to determine whether controlled freezing might be used for this purpose and, accordingly, Dr. John H. Parker of the Kansas station and several of his students have cooperated with the writer in testing this method with several hybrid populations.

Davis *(16)* made an extensive study of a Kanred X Kanmarq (Kanred X Marquis) cross. He froze 72 pots of each parent and 761 F_3 hybrids. They were grown in the greenhouse and frozen without hardening. The average percentage of injury for Kanred was 63.1, for Kanmarq, 72.2, and for the hybrids, 49.9. In a space-planted test in the cereal crop nursery at Manhattan in the same year, the percentage of plants killed during the winter was 6.9 per cent for Kanred, 27.8 per cent for Kanmarq, and 16.2 per cent for 201 F_3 hybrid lines. Those hybrids which survived the best in the nursery test were in general injured the least in the controlled freezing test. In a similar test the following year involving 710 F_4 hybrids and approximately 65 plants each of the parents, the average percentage of injury was 77.2 per cent for Kanred, 84.2 per cent for Kanmarq, and 66.2 per cent for the hybrids. In a space-planted test in the nursery in the same year the percentage of winter killing was 11.5 per cent for Kanred, 15.4 per cent for Kanmarq, and 0.5 per cent for the hybrids. There was reasonably good agreement between the percentage of injury in the controlled freezing tests of the F_3 and F_3 lines and the same was true in the nursery test.

Hubbard *(37)* made a study of the inheritance of cold resistance in the cross, Kanred X Blackhull, similar to that made by Davis. The average injury was 78.5 per cent for Kanred and 96.2 per cent for Blackhull. The average injury for the F_3 hybrids was 76.6 per cent. In a similar study in the following year the average percentage of injury was 54.3 per cent for Kanred, 77.9 per cent for Blackhull and 65.4 per cent for the F, hybrids. Transgressive segregation was observed, some of the lines being more resistant than Kanred, the more hardy parent.

Stevens *(80)* reported the results of a similar study of a Kanred X Tenmarq (P1066 X Marquis) cross and of a Kanred X Kansas No. 443 (Kanred X Marquis) cross. For the first cross the average injury was 78.7 per cent for Kanred, 90.2 per cent for Tenmarq and 86.7 per cent for 264 hybrids representing 39 F_3 lines. In the following year the average estimated injury was 72.2 per cent for Kanred, 98.3 for Tenmarq and 53.2 per cent for 346 hybrids from 36 F_3 lines. Some of the F_4 hybrids were observed to be as hardy as Kanred. In a continuation of this study the third year, in which a part of the plants were grown in the greenhouse and frozen without hardening, the injury to Kanred was 84.5 per cent, to Tenmarq, 97.8, and to the hybrids, 86.6 per cent.

In the study of the Kanred X Kansas No. 443 cross, the average percentage of injury was 71 per cent for Kanred, 81 per cent for Tenmarq, which is known to he very similar to the other parent of the cross and grown in place of it, and 64.9 per cent for the 34 F_3 hybrid families.

A fairly extensive study was made by Parker and the writer of the cold resistance of F_4 and F_5 segregates of a Kanred X Nebraska 28 cross, in which the segregates were classified according to time of maturity into very early, early, intermediate, late, and very late groups. Parker *(56)* has reported in part on this cross. Plants of these various groups were grown in the greenhouse in 1926-'27 and in 1927-'28 and frozen without previous hardening at temperatures of —10° to —13° C. The' average estimated injury the first season was 34 per cent for Kanred, 67 per cent for Nebraska 28, and 40.5 per cent for the hybrids. In the second season the percentages of injury were 63.9 per cent for Kanred, 88.7 per cent for Nebraska 28, and 77 per cent of the hybrids.

In both seasons the intermediate and late classes were injured to about the same degree as the Kanred parent, being distinctly more resistant than the Nebraska 28 parent and more resistant than the early-maturing hybrids.

Parker in April, 1929, using the equipment described herein, froze 20 plants each of the parents and a number of F, hybrids of the cross Chinese wheat X Dakold rye, which previously had been made by Mr. W. J. Sando, of the Division of Cereal Crops and Diseases, United States Department of Agriculture. The estimated injury was 42.8 per cent for the rye, and 99.1 per cent for the wheat. The injury to the F_1 hybrid plants was intermediate. The injury to the parents and a few of the hybrids is illustrated in figure 1,B.

Goth *(25)* studied the relative cold resistance of certain selections from the cross Kanred X Hard Federation. He found evidence of marked differences in resistance to low temperature of these various strains, some of them apparently being nearly as hardy as the Kanred parent.

It will be observed that in all of the crosses herein mentioned the relative injury of the parents to low temperature is in accord with what would be expected under field conditions, and the same also is true of the various hybrid lines in so far as information on winter hardiness in the field is available. Because of the mildness of the winters, however, there has been very little opportunity to verify the relative hardiness of the hybrid lines under field conditions.

Further evidence of the potential usefulness of the artificial freezing method has been supplied in relation to other varieties and strains. Thus information as to the probable winter hardiness of Kawvale, a new variety recently released for distribution in eastern Kansas, in relation to Fulcaster was secured during the period of field testing by this method. The fact that Early Blackhull is probably less hardy than Blackhull was first demonstrated in the same way and the probable relative hardiness of Kanred X Prelude (C. I. 8886), a new early variety of promise for western Kansas, as compared with Kanred, was first made known in the same way. The first information as to the resistance of Provence alfalfa (S. P. I 34486), as reported by Salmon *(71)* and verified by Peltier and Tysdal *(58)*, was secured by freezing artificially. Peltier and Tysdal *(58)* and Timmons *(84)* using this method have shown that Hardistan alfalfa is more resistant than Grimm to low temperature.

Additional evidence for the belief that nonhardy segregates may be eliminated from a cross by controlled freezing was afforded by a simple experiment in 1929-'30 in which a fifty-fifty mixture each of Fulcaster and Currell and a similar one of Kanred and Currell were grown in the greenhouse and subjected to controlled freezing. The surviving plants were grown until they headed, when they could be identified by the fact that the Currell is awnless and the others are bearded. In a single test conducted in this way, in which there were 125 plants of each variety in each set, it was found that 16 per cent of the Fulcaster survived and 6.4 per cent of the Currell. In the Kanred-Currell mixture 61.6 per cent of the Kanred survived and 4 per cent of the Currell. Currell is one of the least hardy of the winter wheats under field conditions, being distinctly less winter hardy than either Kanred or Fulcaster.

Based on these results it would appear that controlled freezing merits consideration by the plant breeder. Breeders in the northern Great Plains, where winterkilling occurs nearly every year, may not find it necessary or profitable to resort to such devices; but for those in the central and southern portion of this area artificial freezing should make it possible to eliminate effectively cold-susceptible segregates from hybrid populations with certainty and rapidity, which often cannot be done under natural conditions, thus greatly reducing the number that must be grown in field tests. It seems, also, that reasonably reliable information as to the winter hardiness of new varieties may be secured in this way.

COLD RESISTANCE AND THE ADAPTATION OF CROP PLANTS

It has been pointed out that one of the objectives of the artificial freezing tests was to determine whether resistance to cold might be a factor in limiting the distribution of crop plants. The subject would appear to be of interest from two more or less distinct viewpoints: (1) That of the plant ecologist and others whose interests are more or less academic and theoretical, and (2) that of the agronomist and plant breeder, whose interests are for the most part immediately practical. Merriam *(50)*, Livingston *(41)*, Livingston and Livingston *(42)*, Hopkins *(34)*, Finch and Baker *(20)*, Smith *(77)*, White *(89)*, Shreve *(75,76)*, Uphof *(88)*, and others have made noteworthy contributions to the general subject of crop distribution and temperature, but it is only in recent years that a distinction has been made between those plants which survive the winter season and those which do not. The distinction would seem to be of basic importance.

The writer has pointed out elsewhere *(66)* the close agreement between the minimum winter temperature in the United States and the northern limits of winter wheat, winter barley, and winter oats. Tumanov and Borodin *(86)* determined the relative resistance to low temperature of nine Afghan winter wheats by the direct freezing method, and found that resistance increased regularly with the altitude of their habitat. Klages *(39)* has pointed out that inability to survive cold winters constitutes a limiting factor in the distribution of winter vetches. Thus, he says, "some species of vetches such as varieties of common vetch *(Vicia sativa)* and monantha vetch *(V. monantha)* have some very decided points of advantage over hairy vetch, yet the inability of these types to survive winter conditions constitutes, in many localities, the limiting factor in production." It is common knowledge that yield of varieties of alfalfa is to some extent a function of winter survival and for twenty years or more the superior winter hardiness of Grimm alfalfa has been the chief or only reason for growing it in the northern United States as compared with strains of Common. Timmons *(84)*, as previously noted, has shown a definite relation between the place of origin of alfalfa and its resistance to low temperature. Contributions' by Arny *(5, 6)*, Wiggans *(90, 91)*, Megee *(49)*, Cox and Megee *(14)*, Pieters *(59)*, McRostie *(44)*, and Delwiche *(17)* show beyond doubt that ability to survive severe winters plays a predominant role in the adaptation of foreign and domestic varieties and strains of red clover in the United States. Steinbauer *(78)* also found marked differences in the resistance of varieties of red clover to artificially produced low temperatures and that, "in general, European or southern varieties proved less resistant than those grown in northern areas of the United States."

It is likewise common knowledge that soft red winter and soft white winter wheats cannot be grown successfully in the Great Plains because of winterkilling, and Wilson and Arny *(92)* have pointed out that the supriority of Minturki winter wheat in southern and central Minnesota is in the main due to its winter hardiness. Likewise they have shown that Rosen rye is a productive variety worthy of recommendation for southern Minnesota, whereas it is too easily winterkilled to be considered satisfactory for the central and northern parts of the state. On the other hand, Dakold rye, which is very winter hardy, has given the best yields at Crookston, which is in the northern part of the state, and this variety is grown extensively in Canada.

Quisenberry and Clark *(64)* have emphasized the importance of winter hardiness by pointing out that the losses in the wheat crop from low temperatures are nearly as great as from all diseases combined, and they have shown a definite relation between yield and winter hardiness in the winter-hardiness nurseries. There would, therefore, seem to be ample reason for the belief that ability to survive severe winters is one of the important factors, indeed the most important factor, determining the northern limit of winter annual, biennial, and perennial crops in the northern hemisphere. There remains only to be discussed the possible bearing of winter hardiness on the distribution of particular varieties and strains.

In Kansas the role of cold resistance in limiting distribution may be very clearly seen. The isotherms for the minimum winter temperatures extend diagonally across the state from the southwest to the northeast *(73)*. Currell, the least resistant to cold of any variety of wheat grown on a commercial scale in the state, is limited almost to a single county in the southeastern corner of the state where the average minimum winter temperature is 25° F. (—3.9° C.) or higher. Fulcaster has been shown to be somewhat more resistant to cold and it is grown commercially somewhat farther north. Harvest Queen, which is distinctly more resistant to cold than either Currell or Fulcaster, is grown to the exclusion of these varieties in northeastern Kansas. Both Fulcaster and Harvest Queen are rather definitely limited to the eastern part of the state, probably because they are not resistant to drought. This limitation, however, does not apply to Blackhull, a variety usually classed as a hard wheat, but which nevertheless is in some respects similar to the soft wheats. The northern limit of Blackhull in Kansas in 1924, as shown by Clark *et al (12),* coincides very well with the winter isotherm of 20° F. (—6.7° C.); whereas, Kanred and Turkey, typical hard wheats and distinctly more resistant to cold, are grown to the practical exclusion of Blackhull in northwestern Kansas.

The distribution of Harvest Queen as compared with Fulcaster and of Fulcaster as compared with Currell and Purplestraw in the United States is also in agreement with the supposition that resistance to low temperature plays a leading role. Fulcaster, it has been pointed out, possesses a moderate degree of cold resistance and is widely distributed throughout the eastern United States. However, it is not grown extensively north of Kentucky, Tennessee, and West Virginia, except in southeastern Pennsylvania, where it occupies a considerable acreage. It is scarcely grown in New York and only very sparingly in Michigan, Ohio, Indiana, or Illinois. Harvest Queen distinctly tends to predominate over Fulcaster in northern Missouri and Illinois and in northwestern Indiana. It is a relatively new variety, and its distribution in certain areas has been restricted because of its susceptibility to flag smut and mosaic disease. Possibly for these reasons, among others, it is not so widely grown as its winter hardiness would suggest.

Purplestraw is among the least cold resistant of any of the varieties that were tested, excluding only Sonora and Federation. It is limited to the southern border of the soft winter-wheat belt. Currell, which is intermediate in winter hardiness between Purplestraw and Fulcaster, occupies in general an intermediate position with respect to distribution.

In the extreme northern part of the soft winter-wheat belt, white wheats, principally Dawson and Goldcoin or Fortyfold, predominate, Very little experimental data are available regarding the cold resistance or the winter hardiness of these varieties as compared with the soft red winters. Dawson is often referred to in the literature as one of the most winter-hardy varieties, and in at least several experimental trials in which winter killing was severe, it survived as well or better than others. In a very limited number of freezing trials with controlled temperatures, Goldcoin appeared to be about as resistant as Harvest Queen. It would thus appear that these varieties are at least as cold resistant. and as winter hardy as Fulcaster, and probably more so. It should be pointed out, however, that the mere fact that Fulcaster is not grown extensively in New York and parts of Michigan, where white wheats predominate, proves nothing as to its winter hardiness, since there is in this region a special demand for white wheats of low protein content, such as Dawson and Goldcoin, which is sufficient alone to account for the predominance of these varieties. Probably with respect to these varieties little can be claimed other than that their distribution is not contrary to the assumption that it is determined to some extent by ability to survive low temperatures and heaving.

Red Rock and Berkeley Rock, which are among the most important red wheats in Michigan, are referred to in the literature of the Michigan Agricultural Experiment Station as being very winter hardy but at the Minnesota station *(92)*, with more severe and quite different conditions, Red Rock is nonhardy. These varieties appear to be only moderately resistant to low temperatures, although the information on this point is very meager.

In the Pacific Northwest, the dependence of varietal adaptation on winter hardiness appears in one or two cases to be clear cut. Winterkilling does not frequently occur, due not only to the fact that winters are relatively mild, but also, perhaps, to the fact that there is less fluctuation from season to season and as a consequence, farmers soon learn what varieties will not survive and do not plant them. In other words, they are not so likely to be misled by a few unusually mild seasons. Turkey wheat and allies of Turkey are the principal winter varieties in the Big Bend area of central Washington. Here winterkilling sometimes occurs, but it usually is so closely related to unfavorable conditions for seeding in the fall and drought injury, that the two factors would seem to be inseparable with present information.

In the more humid sections of eastern Washington, northeastern Oregon, and northern Idaho, Turkey and similar varieties are on the average so much less productive than others they are seldom grown. In this area, Hybrid 128, Albit, Ridit, Triplet, Goldcoin or Fortyfold, and Federation are the principal fall-sown varieties. Federation, though a spring wheat, is able to survive in the southern part of the area because of the snow cover that is ordinarily present. The northern limits of this variety are rather clearly recognized, and clearly dependent on its ability to survive the winters. Federation, as shown by the controlled freezing tests, is among the least resistant to cold, and it is highly probable that its failure north of its present limits is due to this defect.

As would be expected a relation between resistance to cold and adaptation cannot always be clearly established. The hard winter wheats, for example, are less winter hardy as a class than the soft winter wheats in the eastern United States. Trumbull, which is one of the leading varieties in Ohio, is relatively nonhardy, as judged by artificial freezing tests. Rice, a little-known variety, appears to be about as resistant to low temperature as Harvest Queen, but is grown no farther north than Tennessee and North Carolina.

Such exceptions are easily explained by the well-known fact that cold resistance is not the only factor determining winter hardiness, and that winter hardiness is only one of many factors determining adaptation and distribution. Heaving, for example, is well known to be an important cause of winterkilling in the eastern United States and it is equally well known that the hard winter wheats are relatively susceptible to heaving. Govorov *(26)* states that in the Moscow district of Russia plants perish under the snow chiefly for lack of oxygen and Talanov found that those varieties which are very hardy at Saratov, Russia, including Minhardi, Minturki, and Kanred, are relatively nonhardy in the north with its abundant snow.

Trumbull no doubt possesses desirable characteristics sufficient to offset whatever deficiency it may possess with respect to winter hardiness, and Rice, on the other hand, may be deficient in other respects though relatively cold resistant. Fulcaster is grown in central Kansas and southeastern Nebraska, possibly because no severe winter killing has occurred in those areas in recent years, and because Fulcaster possesses desirable characteristics, such as stiff straw, which are not characteristic of the hard red winter wheats commonly grown. Hence these exceptions would seem not to invalidate the general rule that cold resistance plays a leading role in determining the adaptation of crop varieties.

SUMMARY AND CONCLUSIONS

The injury, and in some cases the survival, has been recorded for more than 125,000 plants of winter wheat, winter rye, winter oats, and winter barley artificially frozen in about 30,000 four-inch greenhouse pots, or flats during a period of five years. All varieties of wheat recently grown in the uniform winter-hardiness nurseries of the United States Department of Agriculture were included, and in addition a number of varieties of commercial importance, principally from the eastern United States. The data from the artificial freezing experiments were correlated with the average survival from the winterhardiness nurseries so far as possible, and the relations between resistance to cold and varietal adaptation are discussed. A considerable amount of data has been accumulated relating to the technic of artificial freezing and certain precautions which should be observed are indicated.

Because of a secondary effect, probably physiological, which appeared in many of the experiments soon after freezing, it was not possible to use the survival of the plants as a criterion of injury. Consequently the relative injury was estimated and recorded in percentages, based roughly on the proportion of the visible plant tissue that appeared to be killed. A comparison of the percentage of plants killed for those experiments in which this secondary effect did not appear and of estimated injury based on the appearance of the plants a few days after freezing, indicated that the latter may be safely used in most cases. The coefficients of correlation for the two measures varied from 0.76 $\pm$0.048 to 0.95 $\pm$0.017.

It was observed that plants frozen at night were frequently injured less than similar plants frozen during the day. This was not universally true, but it is considered essential in making artificial freezing tests that all plants to be compared be frozen at approximately the same time of day.

The moisture content of the soil was found to affect materially the degree of injury. Invariably, with the procedure regularly followed, plants in dry soil were injured more than those in wet soil. If, however, the soil mass was frozen before the plants were subjected to temperatures sufficiently low to cause injury, no marked difference in the injury to plants on a wet or a dry soil was observed. The lesser injury which commonly occurs on a wet soil is therefore, in the main, attributed to a lag in temperature.

In taking plants from the field for freezing, it was observed that the size of the clump materially influenced the results. In a study of five different lots totaling 230 pots of Kanred wheat, in which the clumps were classified according to size, the correlation coefficient between size of clump and injury varied from —0.41 to —0.91.

Disturbing the roots, which must of necessity occur in transplanting plants from the field to pots, was found to have very little effect on the injury or survival of the plants.

The possibility of determining relative resistance to cold by growing plants in pots (or flats) and exposing them to natural freezing out-of-doors during the winter was investigated. The results were found to correlate very well with artificial freezing, and it is suggested that this method may be useful when refrigeration equipment is not available.

High correlations between the results of artificial freezing of thoroughly hardened varieties of wheat and survival under field conditions were obtained. In three separate trials involving varieties from the winter-hardiness nurseries the correlation coefficients for estimated injury and winter survival were —0.65 ± 0.085, —0.84 ± 0.038, and — 0.78 = 0.046. Attention is called to the fact that Quisenberry *(62)* secured a coefficient of —0.713± 0.031 in similar trials in Minnesota, and that Foster Martin *(45),* working with the writer with spring wheat, secured a coefficient of —0.762 ± .085, the survival in the latter case being from fall seeding in the Pacific Northwest. In each of these cases the number of plants of each variety in the artificial freezing trials and in some of the field trials was small, and it appears reasonable to assume that somewhat higher coefficients would have been obtained with larger numbers.

The accuracy of the artificial freezing trials was compared with that of single winter-hardiness nurseries under field conditions by determining the intra-class correlation coefficients for the latter each year, and also by determining the correlation between the survival at all stations taken individually and the average at all other stations in the same year. The coefficients in all cases were materially lower than those secured for the artificial freezing trials. It appears that a single artificial-freezing test under the conditions specified may be expected to furnish a more reliable prediction of relative winter hardiness in the Great Plains than would the survival of a single winter-hardiness nursery selected at random, but less reliable than the average of all winter-hardiness nurseries for a single season. The results of the single winter-hardiness nursery at Moccasin, Mont., in 1926, 1927 and 1928, in which the number of rows was greater than usual, when correlated with the average of all other nurseries in the same seasons, gave coefficients comparable with or slightly larger than those secured by artificial freezing.

Without exception the injury by artificial freezing of regional varieties of winter wheat, other than those included in the winter-hardiness nurseries, was in agreement with their relative hardiness under field conditions in the Great Plains, so far as information regarding the latter is available. It is also in agreement with the supposition that the distribution of varieties in other portions of the United States is often limited by their inability to survive low temperatures.

The resistance to low temperature of winter barley, winter oats, and varieties of winter rye is also in accordance with the supposition that resistance to cold is a predominating factor in determining adaptation and distribution. Timmons *(84)* working with the writer found a similar relation in alfalfa.

It was found that the group of varieties classified by Quisenberry and Clark *(64)* as very hardy and medium hardy were frequently no more hardy than the normally tender varieties if frozen without previous exposure to low temperatures, thus verifying the observations by Hill and Salmon *(32)*. For these varieties it appears that exposure to low temperatures for a considerable period before freezing is essential if a true expression of their relative hardiness is to be obtained. These varieties apparently do not in some cases acquire their true relative hardiness under field conditions at Manhattan, Kan., before about January 1.

Growing the plants at high temperatures (22° to 25° C.) reduces the difference between varieties with respect to cold resistance, but, excepting the varieties of the very hardy and medium-hardy groups, their relative resistance tends to remain the same so far as it can be determined.

No evidence of a differential response to hardening was found in winter rye, the relative resistance to low temperature appearing to be the same, except for the degree of differences, regardless of the temperature at which the plants were grown.

Hardened plants of several varieties of wheat exposed to greenhouse temperatures suffered a perceptible loss in hardiness in from 12 to 24 hours, and most of the differences in hardiness disappeared in about 120 hours. The rate of loss appeared to be substantially the same for all varieties, or slightly greater for the more hardy varieties.

Artificial refrigeration was used apparently with success to eliminate nonhardy varieties in mixtures and nonhardy segregates in hybrid populations of a number of crosses.

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