THE RESISTANCE OF CERTAIN VARIETIES AND REGIONAL STRAINS OF ALFALFA TO CONTROLLED LOW TEMPERATURES

by

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INTRODUCTION

During the last thirty years, extensive experience and a considerable number of experiments have shown marked differences in the adaptation of different varieties and strains of alfalfa. In general, Grimm and other variegated varieties and strains have proved best in northern regions while various strains of common alfalfa or of the non-hardy group (Peruvian) are better adapted to the central and southern areas. It is reasonable to suppose that differences in adaptation are correlated with resistance to low temperature: indeed it is well known that adaptation in many cases is limited primarily by inability to survive severe winters and hence presumably by susceptibility to freezing temperatures. In a few cases differences in resistance to low temperatures have been experimentally demonstrated. In connection with other work of a similar nature at the Kansas station, it seemed desirable to make a more extensive study of this relation in order to determine (1) whether or to what extent resistance to low temperatures is a factor of major importance in determining the adaptation of alfalfa varieties and (2) whether artificial freezing may be usefully employed in determining the relative resistance of varieties and strains to winter killing and (3) to work out a satisfactory

technic for conducting controlled freezing trials with alfalfa and interpreting the results.

REVIEW OF LITERATURE

The literature on the general subject of cold resistance in plants has been comprehensively reviewed by Chandler (5), Newton (21) and Martin (15) et al. In this paper mention will be made only of those contributions to cold resistance in alfalfa.

Lyon and Hitchcock (14) in a field test conducted at the Nebraska Agricultural Experiment Station from 1898 to 1903 observed that strains of common alfalfa from Arizona and California winterkilled completely and that common alfalfas from Utah, Colorado and Kansas were injured more severely than those from Nebraska.

Brand (1) reported the results of winter hardiness studies carried on in Minnesota and North Dakota. At the Minnesota Agricultural Experiment Station 1901 to 1908, strains of northern grown common alfalfa winter killed much more severely than Turkestan and Grimm while Grimm proved to be hardier than Turkestan. At Tappen, North Dakota the percentages of survival in 1909 were as follows: Grimm and Montana Common 90, Kansas and Nebraska Common 85, Colorado Common and Russian 60, New York Common and Utah dry land

alfalfa 30, Utah irrigated 10 and common alfalfa from Texas, Germany, France and Southern Europe 5. Brand and Waldron (2) reported that at Dickinson, North Dakota in 1906 to 1909, Arabian, African and South American strains killed out almost completely, European strains from 83.9 to 100 per cent, Utah Common 90.4 per cent, Colorado Common 86.1 per cent, Kansas Common 84.8 per cent, Nebraska Common 76.4 per cent, Montana Common 65.4 per cent, Turkestan strains 56 to 91 per cent, Mongolian 39 per cent, Turkestan selections 9.2 to 34.6 per cent and Grimm from 2 to 7 per cent.

Piper (23) called attention to the results from experiments that were conducted at Havre, Montana 1916-17 and at Redfield, South Dakota in 1916 to 1920. At Havre the following percentages of winterkilling were recorded: Medicago falcata 0.0, Baltic 4.3, Grimm 4.6, Montana Common 7.8, Canadian variegated 13.8, Turkestan 17.8, Liscomb 27.8, Kansas Common 32.0, Utah non-irrigated 42.9, Utah irrigated 47.9 and India alfalfa 100. At Redfield, South Dakota the percentage reduction in stands was 21.1 in Grimm, 34.9 in Baltic, 52.6 in Canadian variegated, 49.3 to 66.9 in Dakota Common, 83.6 in Kansas Common and 95.3 in Utah Common.

Miller (16, 17) noted differential winterkilling at Morris, Minnesota, 1914 to 1918. Grimm winterkilled 10 to 36 per cent, northern grown common strains 25 to 40 per cent and southern grown common strains almost 100 per cent.

Moore and Graber (18), Graber (8) and Nelson and Graber (20) reported the results from field trials with alfalfa at the Wisconsin Agricultural Experiment Station. Common alfalfa from New Mexico, Arizona and California proved decidedly less hardy than common strains from Kansas and states farther north, and in an extensive comparison of common alfalfas, those from Kansas and Nebraska were equal in winter hardiness to those from Dakota and Montana. Grimm and Turkestan were superior in hardiness to both southern and northern grown strains of common.

Experiments conducted at the Iowa Agricultural Experiment Station (10) showed Grimm and Turkestan to be the most winter hardy, Nebraska and Kansas Commons intermediate in hardiness and strains from Utah, Oklahoma and New Mexico the least hardy of those included in the comparison.

Singleton (27) found that common alfalfas from Kansas, Dakota and Montana were as hardy as variegated alfalfas under irrigated conditions in Washington, whereas Hairy Peruvian winterkilled severely.

In Connecticut, Brown and Slate (3) observed that Grimm, Turkestan, Canadian variegated, Baltic, Northern grown common, Peruvian, Kansas Common, Provence and Arabian alfalfa maintained their stands in the order named during the period 1910 to 1915.

In an experiment started at the Illinois Agricultural Experiment Station in 1923, Burlinson and Hackleman (4) noted that Grimm and Cossack showed practically no winter killing, while South Dakota No. 12, Kansas Common, Colorado Common and Idaho Common were intermediate and Argentine winterkilled severely.

Cox and Megee (6) and Rather and Wenner (24) concluded, on the basis of extensive field trials in Michigan, that Hardigan, Grimm, Cossack, and Ontario variegated comprised the most hardy group. Common alfalfas from northern and western states were intermediate in this respect and those from Utah and Kansas were somewhat less hardy than strains from Dakota, Montana and Michigan. The non-hardy group included Hairy Peruvian, Arizona Common and strains from South America and Africa.

Kiesselbach and Anderson (12) reported the stand survivals in two rather extensive field trials with alfalfa strains carried on at the Nebraska Agricultural Experiment Station from 1922 to 1927. In one test the "stand survival" after six years expressed as a percentage, were Cossack 89, Baltic 85, Grimm 83, Turkestan 81, Nebraska Common 72, Canadian variegated 69, Sand Lucern 13 and Peruvian 0. Common alfalfas from twenty different sources were compared in another field test. A strain from Dawson County Nebraska,

later named Hardistan (13) maintained the best stand. Provence (F. C. I. 34886) and other strains of common alfalfa from Nebraska, Kansas and South Dakota, North Dakota, Montana, Wyoming and Colorado showed a somewhat greater loss of plants while those from Oklahoma, New Mexico and Texas killed out very s everely. The stands of Argentine, Spanish and Italian alfalfas were practically destroyed. The loss of plants in these experiments appears to have been due chiefly to winter injury.

In an experiment conducted by Nelson (19) at the Arkansas Agricultural Experiment Station from 1925 to 1929 the varieties ranked according to stand at the end of the period were: Grimm, Baltic, Dakota Common, Utah Common, Argentine, Kansas Common, Oklahoma Common, Texas Common and Peruvian. Winter injury was considered responsible for most of the loss of plants.

Nelson and Graber (20 p. 37) exposed plants of Grimm, Turkestan and common to artificially produced low temperatures (-4°F). Eighty-four per cent of the common was killed as compared with 18 per cent for the Grimm. Turkestan was also killed less than the common.

Steinmetz (28) dug up Grimm and Kansas Common plants from the field at different times during the winter and froze them under controlled conditions. Potted greenhouse

plants of both varieties were also frozen. Grimm was distinctly more resistant than Kansas Common. Resistance to cold increased in both varieties as winter approached and disappeared with the approach of spring, the critical period for both being at the time of the spring thaw.

Steinmetz also compared Grimm and Kansas Common with respect to the freezing point of the root tissue, the freezing point depression of the sap expressed from the roots, the viscosity of the cell sap from the roots, and the sugar, pentosan and amino acid content of the roots. He found no definite relation between any of these factors and the relative cold resistance of the two varieties and concluded that "as positive measures of the differences between the varieties under study, the freezing of potted plants or of roots removed from the soil has been found to be the most practicable and reliable method."

Martin (15) reached a similar conclusion with regard to wheat.

Salmon (25) compared certain alfalfa varieties in two different controlled freezing tests. Plants of Provence (F. C. I. 34886), Ladak, Grimm, Cossack and Kansas Common were grown in twelve-inch clay pots in the greenhouse and frozen for twelve hours at -11°C. The estimated percentages of injury were 44.3, 70, 74.4, 78.2 and 94.7 respectively. In another test the estimated percentages of injury for one

year old plants transplanted from the field and frozen were Provence 35.2, Grimm 37.4, Ladak 46.2, one year old Kansas Common 50.2 and two year old Kansas Common 45.1

Peltier and Tysdal (22) froze six weeks old greenhouse grown plants in controlled freezing chambers. The ratios of survival compared with 100 for a standard strain of Turkestan were; Provence (F. C. I. 34886) 115, Hardistan 115, Ladak 105, Grimm 87, Nebraska Common 72 and Arizona Common 26. Several strains of Turkestan showed varying degrees of survival.

MATERIALS AND METHODS

The experimental work reported here was conducted in two different seasons with different sets of plant material and with slightly different methods. The first experiments were made in the fall and early winter of 1929 with five varieties which had been seeded on August 23, 1929 in square rod plots on the Agronomy farm of the Kansas Agricultural Experiment Station, Manhattan. These varieties were Provence (F. C. I. 34886)[1], Ladak (from Redfield, South Dakota), certified Grimm (from Idaho), Kansas Common and Utah Common.

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1. This strain has recently been officially named "Kaw". See "Kaw - A New Alfalfa", by S. C. Salmon. Submitted for publication in Jour. Am. Soc. Agron., Sept., 1931.

Clumps of from six to ten plants were transplanted from the field into four-inch clay pots with a small garden trowel, one clump being placed in each pot without seriously disturbing the soil about the roots. The pots were then transferred directly to the refrigerator or held outside of the greenhouse until frozen. They were frozen in the carbon dioxide automatic temperature controlled refrigerator described by Sellschop and Salmon (26). Each freezing lot consisted of fifteen pots of each variety distributed so that three pots of each variety were in each of three flats 16" x 16" x 5" and two pots of each variety were in each of three flats 12" x 24" x 4". Transplanted but unfrozen plants of each variety of each freezing lot were retained in the greenhouse as checks. In most cases one large flat and one small flat (five pots of each variety) were removed from the refrigerator at the end of twelve, fifteen and eighteen hours. In two cases the period of freezing was 48 hours. Since the relative degree of injury for each variety seemed to be approximately the same regardless of the period of freezing, no distinction is made with respect to this factor in the presentation of the data. The different lots were subjected to various freezing temperatures ranging from about -11°C to -28°C depending upon the degree of hardening of the plants. Those temperatures were chosen which

previous experience had indicated would produce a moderate injury in the more resistant varieties. After each lot had been frozen it was placed in the greenhouse to thaw and was then maintained under optimum conditions of moisture and temperature for several weeks for observations on recovery.

The injury was estimated for each pot of each variety at various intervals, ranging from four to fifteen days after freezing for the first estimates and from two to eleven weeks after freezing for the final estimates. The estimates were based on the appearance of the above ground parts as compared with the unfrozen checks of the same lot and variety.

Immediately after making the final estimate of injury the plants were removed from the soil and the number of dead and surviving plants recorded. The surviving plants were then examined for root injury and the number of those with injured and with uninjured roots were recorded. Those with roots frozen off so that the plants were subsisting entirely by new rootlets from the crown were recorded as severely injured.

The second set of experiments was conducted in the fall and winter of 1930-31 and comprised eleven regional strains of alfalfa, secured from Mr. H. L. Westover of the United States Department of Agriculture, Kansas Common from

northwestern Kansas and Hardistan, Ladak and Grimm. The seed was planted in a field nursery and the plants were transplanted to four-inch clay pots for freezing as in the previous year except that the tap roots were cut off at a uniform length of four inches from the crown and three plants were placed in each pot. Care was taken to place each plant about one inch from the side of the pot and so place the plants that the soil in the pot came to the same point on the crown as it had in the field. As in the previous season, all pots were heavily watered a short time before freezing. Each freezing lot consisted of five pots of each variety, one pot of each variety being placed in each of five flats 16" x 16" x 5". One additional pot of each variety of each lot was retained unfrozen as a check. The plants were subjected to temperatures varying from -10.5° to -13°C and the time of exposure varied from eleven to thirteen hours. Estimates of injury and determinations of living, injured and dead plants were made as in the preceding season. In addition, an attempt was made to measure the size of the roots of each variety to determine whether differences in varieties might be influenced by differences in size such as might result from unequal stands.

EXPERIMENTAL RESULTS

Experiments in 1929

The broadcast seeding made August 23, 1929 produced good, uniform stands of all varieties except Provence (F. C. I. 34886), which had a stand of only fifty per cent, due to seed of low germination. The plants in all varieties were from four to six inches tall and the roots from one to five millimeters in diameter when transplanted for freezing.

Survival from Freezing Injury. Seven different lots comprising a total of 105 pots of each variety were frozen in the fall of 1929 beginning November 2 and continuing until December 19. The date of freezing, the temperature of freezing, the percentage of plants killed in each lot and the average per cent killed for each variety for all lots are given in Table I. The probable error of the mean was determined from the probable error of the experiment, calculated by the method suggested by Student and Fisher (29) and discussed by Goulden (7).

It is apparent that Kansas Common and Utah Common were killed to practically the same extent, Grimm considerably less and Provence (F. C. I. 34886) least of all. Ladak was intermediate between Grimm and Provence. Peltier and Tysdal (22) have also found this strain of Provence to be

Table I. The Relative Resistance of Alfalfa Varieties to Low Temperature

Variety	Plants Killed - Per cent							
	Date of Freezing and Temperature of Freezing							
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6[2]	Lot 7	
	Nov. 2	Nov. 10	Nov. 16	Dec. 7	Dec. 8	Dec. 19	Dec. 19	Mean
	-11°C	-11°C	-11°C	-21°C	-21°C	-28°C	-28°C	
Provence F. C. I. 34886	47.1	30.6	24.6	38.2	9.6	75.9	33.3	37.0
Ladak	81.4	55.0	66.6	68.3	16.4	46.7	25.8	51.5
Grimm	80.1	97.1	82.0	83.2	29.7	78.3	61.8	73.2
Kansas Common	97.9	94.3	96.5	99.0	87.1	100.0	87.1	94.5
Utah Common	97.3	99.2	98.1	98.8	86.6	95.1	92.8	95.4

P.E. of a variety in a single lot of the experiment = 9.14 per cent
P.E. of the mean of a variety = 3.46 per cent

2. The plants in Lot 6 were kept outside the greenhouse after they were transplanted December 6 and may have been injured by freezing previous to December 18 and 19. The plants in Lot 7 were transplanted December 5 and 6 but were held in the greenhouse until December 15 after which they were put outside with those in Lot 6.

more resistant to low temperature than Grimm and Ladak. It is of interest to note that with the possible exception of Ladak and Provence the varieties retained approximately the same position relative to each other whether frozen early in the fall before they had had an opportunity to harden as was the case with the first three lots or later in the fall after having been subjected to freezing temperatures as were the last four lots. The minimum daily temperatures for the last week in October and first two weeks in November ranged from slightly below to above 32°F, while the maximum daily temperatures were below 50°F on only four days. On the other hand the four lots frozen last were subjected to rather severe temperatures reaching -1°F on November 22.

Comparison of Different Methods of Measuring Cold Resistance. A study was made of the agreement between three different measures of freezing injury. The average percentages of dead plants, of dead and injured plants and of estimated injury are given in Table II. The same data, together with the first estimate of injury, are shown graphically in Figure I. When the plants which survived but suffered root injury in varying degrees were considered with those that failed to survive, the differences between the varieties were reduced somewhat, especially between Provence and Ladak. This was due to the tendency for the proportion

Table II. Comparing Different Methods for Determining Relative Injury From Low Temperatures

Variety	Total No. of Plants Frozen	Average Per cent Killed	Average Per cent Killed and Injured	Average Per cent of Estimated Injury
Provence F. C. I. 34886	340	37.0	57.6	51.9
Ladak	799	51.5	63.5	51.0
Grimm	757	73.2	79.8	70.2
Kansas Common	718	94.5	96.7	95.0
Utah Common	897	95.4	96.7	91.8
Probable Error of the Mean		3.46	3.50	4.34

of plants sustaining root injury in the different varieties to decrease as the per cent of survival decreased. The average per cents of plants showing root injury were 20.5, 12.0, 6.5, 2.2 and 1.3 respectively for Provence, Ladak, Grimm, Kansas Common and Utah Common. The freezing exposure was apparently too severe for any of the varieties with the possible exception of Provence, to exhibit their complete range of hardiness in the form of plants sustaining only a moderate degree of injury.

On the whole the estimated injury agreed rather closely with the other two measures of cold resistance. The variation, however, was greater as shown by the higher probable error. Giving due weight to chance fluctuation it will be seen that the varieties would be ranked in the same order regardless of which measure is used with the exception of Provence and Ladak. As measured by the estimated injury, Provence was no more hardy than Ladak, whereas by either of the other two measures it would be considered slightly superior to Ladak.

In Figure I it will be noted that an initial estimate of injury made from four to fifteen days after freezing showed smaller differences between varieties than either of the other three measures. Provence, Ladak and Grimm made a much greater recovery than Kansas Common and Utah Common as

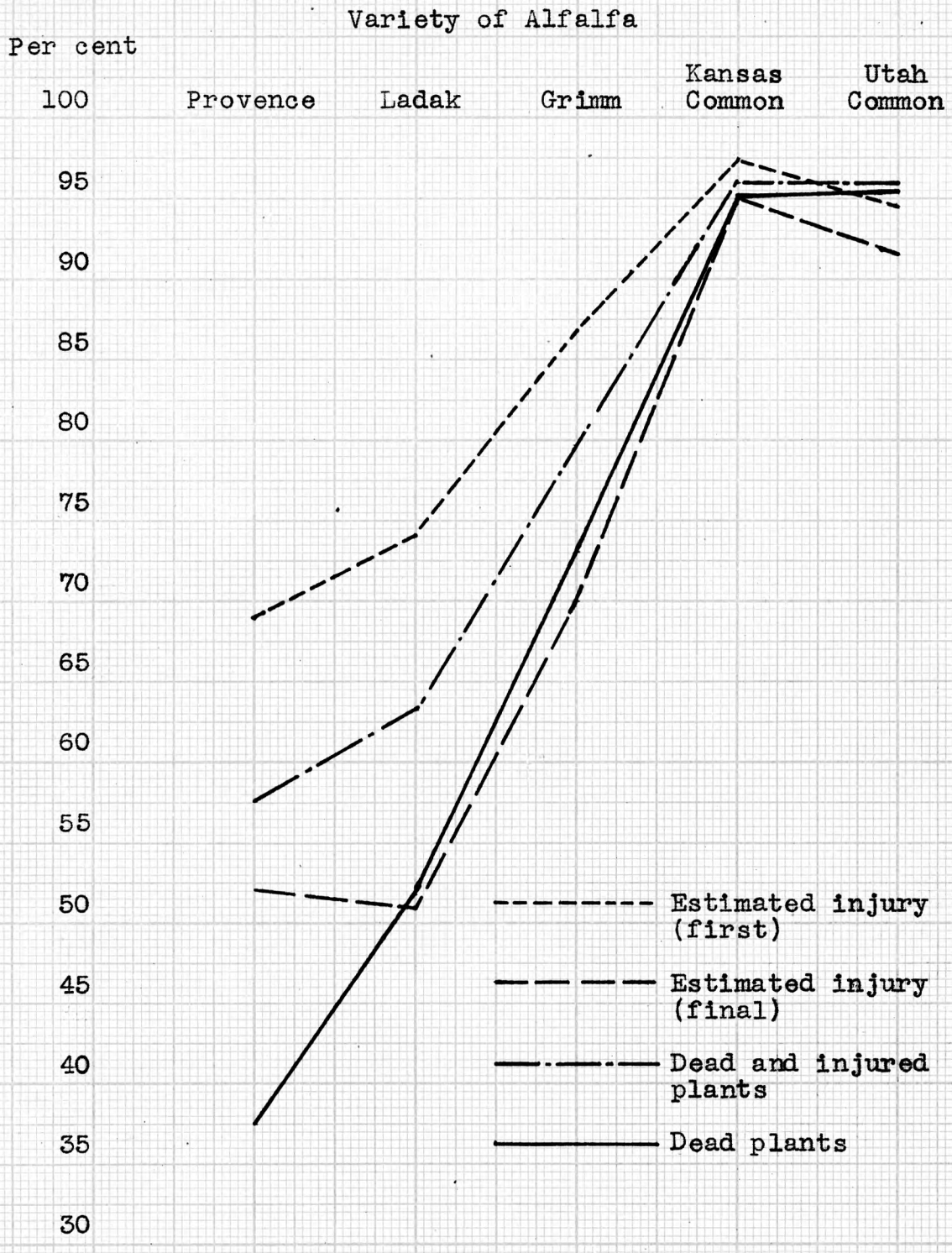

Fig. 1. A graphic comparison of four different measurements of the relative cold resistance of the alfalfa varieties under study.

shown by the final estimate of injury taken two or more weeks after the first estimate. Thus, it is probable that an estimate of the injury from freezing made too soon after the plants are frozen does not show the true differences between the varieties. However, if the treatments had been less severe it is possible that the same differences between initial and later readings would have been noted in Kansas Common and Utah Common as are shown in the other varieties.

Influence of Time of Observation on Injury Recorded. Figure II shows graphically, the effect which a difference in the length of the interval between the time of freezing and the time of final observation had upon the type and degree of injury observed. The average percentage of plants showing root injury was nearly fifteen per cent in each of the first three lots where final observations were made from two to four weeks after freezing while it was about five per cent in each of the last four lots, in which case the interval was from nine to eleven weeks. The most plausible explanation for this is that a large per cent of the plants which were not killed outright but sustained root or crown injury, actually died during the nine to eleven weeks before the final observations were made on the last four lots, whereas they were still living at the end of two to four weeks in the case of the first three lots. Plants were

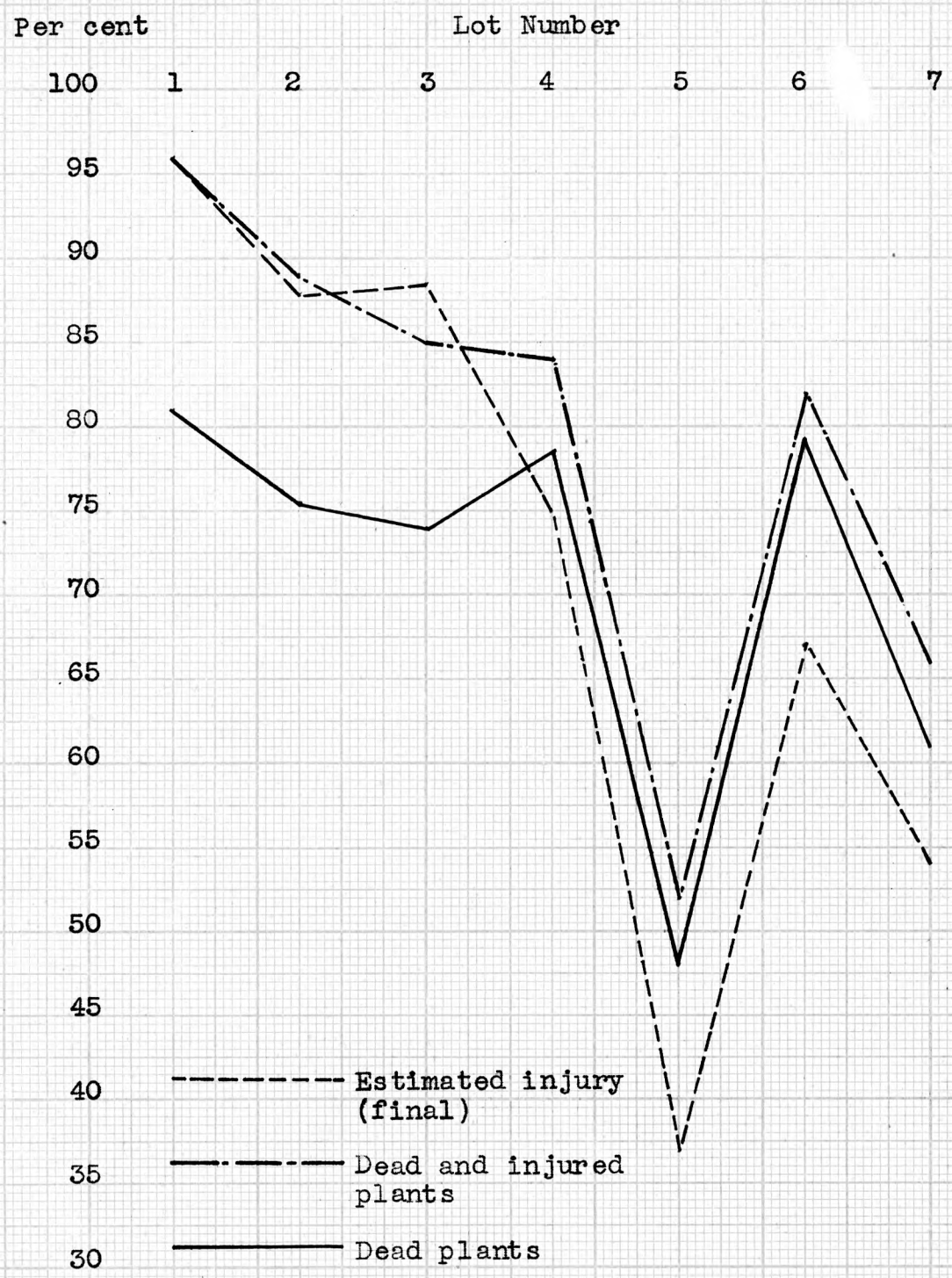

Fig. 2. A graphic comparison of three different measurements of the average freezing injury to all varieties in each experimental lot.

observed to die several weeks after they had been frozen. Further support is lent to this explanation by the closer correlation of the final estimate of injury with the per cent of both dead and injured plants than with per cent of dead plants in Lots 1, 2 and 3. The new growth made by the surviving plants during the longer interval in the case of the last four plots, probably accounts for the observer's tendency to estimate the injury to be less than that shown by an actual count of dead and injured plants. Provence suffered the greatest proportionate loss of plants with injured roots when the final observations were taken at a longer interval after freezing. In the first three lots the percentages of plants showing root injury were 35.4, 16.0, 9.3, 3.9 and 1.5 for Provence, Ladak, Grimm, Kansas Common and Utah Common, while in the last four lots they were 9.3, 9.1, 4.3, 1.7 and 1.1, respectively.

Experiments Conducted in 1930-31

Survival from Freezing Injury. Uniformly good stands were obtained for the fifteen varieties and regional strains planted in April 1930 and a vigorous growth was secured during the summer. The plants attained a height of ten to sixteen inches and bloomed profusely in the early fall. The top growth was left on until after the first heavy frost.

Nine lots of each variety were transplanted and frozen in November and three lots in January and February, making a total of 12 lots. Each lot consisted of five pots of each variety, making a total of 60 pots or 180 plants of each variety frozen.

Unusually mild temperatures prevailed throughout the winter and it is probable that in none of the lots were the plants as thoroughly hardened as is usually the case. The minimum daily temperatures during the last week in October and first three weeks in November, when the first nine lots were transplanted and frozen, were materially below freezing only three times; viz 17°F, 19°F and 23°F on October 31, November 6 and 7 respectively. During this time the maximum daily temperature was below 50°F only once and exceeded 65°F on eighteen different days. Later in the year the minimum daily temperatures ranged mostly below 32°F and were as low as 3°F. The plants in Lot 10 which were frozen on February 7 had been transplanted January 3 and had remained outside of the greenhouse in four-inch pots from January 3 to February 7 except on January 14 when they were put in the greenhouse for one day to protect them from a minimum temperature of 3°F. Lots 11 and 12 were transplanted February 7 and 28 respectively and were probably only slightly less hardened to cold than Lot 10.

Table III. Relative Injury of Varieties and Regional Strains of Alfalfa Artificially Frozen

Variety or Strain	Plants Killed: Per cent in Each Experimental Lot					
	1	2	3	4	5	6
	Date and Temperature of Freezing					
	Nov. 13 -13°C	Nov. 2 -11°C	Nov. 3 -11°C	Nov. 20 -12°C	Nov. 11 -12°C	Nov. 11 -13°C
Hardistan (Nebr.)	53	19	7	27	13	25
Ladak (S. Dak.)	82	59	25	44	7	35
Grimm (Utah)	72	21	47	58	43	69
Nebr. Com. (F.C.I.15897)	92	56	53	67	36	64
Colo. Com. (F.C.I.14482)	75	53	46	71	50	65
Dakota Com.(F.C.I.16081)	88	75	60	87	53	52
Utah Com. (F.C.I.15986) Uinta Basin, Utah	100	43	100	87	29	50
Kansas Common	93	67	50	80	27	69
Oklahoma Com. (F.C.I. 15902)	90	73	80	80	67	80
Idaho Com. (F.C.I.17397)	85	87	87	93	46	44
Utah Com. (F.C.I.15995) Millard Co., Utah	90	87	94	86	43	62
New Mexico Com. (F.C.I. 14470) Roswell, N. M.	100	80	80	93	53	67
New Mexico Com. (F.C.I. 15877) Loving, N. M.	100	80	87	100	80	86
Arizona Com. (F.C.I. 15837) Yuma, Arizona	100	100	93	100	77	86
California Com. (F.C.I. 15889) Palo Valley, Cal.	100	100	100	100	92	100

P.E. of a variety in a single lot of the experiment = 9.11 per cent

P.E. of the mean of a variety = 2.63 per cent

Table III. (Continued Right Hand Marginal)

Variety or Strain	Plants Killed: Per cent in Each Experimental Lot						
	7	8	9	10	11	12	
	Date and Temperature of Freezing						
	Nov. 12 -13°C	Nov. 13 -13°C	Nov. 22 -12°C	Feb. 7 -13°C	Feb. 7 -13°C	Mar. 1 -10.5°C	Mean
Hardistan	6	45	27	55	23	25	27.1
Ladak	47	64	50	40	29	41	43.6
Grimm	20	75	38	22	40	29	44.5
Nebr. Com.	47	94	66	61	87	37	63.3
Colo. Com.	74	100	80	42	66	41	63.6
Dakota Com.	29	100	93	41	75	61	68.3
Utah Com.	88	87	73	47	56	83	70.3
Kansas Com.	73	100	93	65	82	100	74.9
Oklahoma Com.	56	93	73	75	71	83	76.8
Idaho Com.	65	100	100	78	74	64	76.9
Utah Com.	100	100	69	40	88	67	77.2
New Mexico Com.	100	100	87	88	95	94	86.4
New Mexico Com.	100	100	93	94	100	83	91.9
Arizona Com.	100	100	100	88	95	100	94.9
California Com.	100	100	100	94	100	100	98.8

The date and temperature of freezing, the percentage of plants killed for each lot and the averages for all lots are given in Table III. Lots 1 and 4 were refrozen since the first freezing exposure was not severe enough to give satisfactory results. Hardistan, a new strain discovered by the Nebraska Experiment Station (13), was distinctly the most cold resistant strain in the test. Ladak and Grimm survived to about the same extent, being considerably more resistant to cold than any of the common alfalfas. It is of interest to note that this agrees with Peltier and Tysdal (22) who found Hardistan more resistant to low temperature than any other variety tested, including Ladak and Grimm, excepting only Provence (F. C. I. 34886) which it equaled. The strains of common alfalfa grouped themselves in two broad classes with respect to survival. The non-hardy group consisting of strains from California, Arizona and New Mexico, survived considerably less than the intermediate group which included strains of common from Nebraska, Colorado, Dakota, Utah, Kansas, Idaho and Oklahoma. The differences within this latter group are rather small; however, Nebraska Common and Colorado Common appeared to be significantly more hardy than the strains from Utah, Kansas, Idaho and Oklahoma. Dakota Common also exhibited somewhat more resistance to cold than the latter strains. The strains from Utah, Kansas, Idaho and Oklahoma were killed to about the same

extent.

A study of the percentages of plants killed in the different lots reveals that in Lots 10, 11 and 12, Hardistan survived no better than Ladak and Grimm, whereas it maintained a distinct superiority in the first nine lots. Colorado Common had a higher per cent of survival in relation to other varieties in the last three lots than in the first nine lots. It is possible that the greater degree of hardening to low temperature received by the last three lots may have changed to some extent, the relative cold resistance of some of the strains in the test.

Root Injury From Freezing. At the time that the counts were made of the dead and surviving plants in each lot, the surviving plants were divided into three groups on the basis of root injury. Plate I shows the representative roots from each group. Those plants in which the root injury extended entirely across the root or crown were classified as severely injured (A). These plants possessed living crown buds and shoots but no new branch rootlets had been formed to indicate that enough uninjured root tissue remained to support the top growth and continue the life of the plant. Such plants with severely injured crowns and roots due to freezing were observed by Nelson and Graber (20) to die in the field during the late spring and summer. It is quite

Plate I. Photograph Showing Representative Plants With (A) Severely Injured Roots, (B) Slightly Injured Roots and (C) Uninjured Roots

A B C

probable that practically all of the plants with severely injured roots in this experiment would have died in a few more weeks under optimum growth conditions.

A second group of plants (B) in which the root injury was more localized and in which the uninjured tissue produced new branch rootlets, was considered to be slightly injured. Jones (11) working with winter injured plants obtained from the field, found that when the plants could isolate the injured areas, new tissue was formed abundantly and the plants continued to grow with unabated vigor. It seems likely that most of the plants with slightly injured roots in this experiment would have recovered at least temporarily under optimum conditions.

The third group consisted of those plants which survived without root injury (C). The roots of all unfrozen check plants in each lot were also examined at the same time as the frozen plants and in no case was there any root injury evident beyond the scar at the tip of the root where it had been severed in transplanting.

Comparison of Different Measures of Relative Cold Resistance. A study was made to determine what was the most reliable index to the relative cold resistance of the alfalfa varieties and strains under study. In Table IV the average percentages of dead plants in each variety are compared with the average percentages of plants dead and

severely injured and with the average estimated percentages of estimated injury taken just before the plants were examined for survival and root injury. When the plants with severely injured roots were considered with the dead plants the relative ranking of the varieties was changed slightly from that based on dead plants only. By the former, Ladak was significantly superior to Grimm, whereas from the standpoint of dead plants alone the difference was very small. When severely injured plants were considered, Nebraska Common was about equal to Dakota Common and common from Millard County Utah, while on the basis of dead plants alone, Nebraska Common was as hardy as Colorado Common and the Millard County Utah strain no more hardy than Idaho and Oklahoma Common. Since most of the severely injured plants would undoubtedly have died, the combined percentages of dead and severely injured plants are probably the most reliable measure to the relative freezing injury sustained by the varieties.

The rank of the varieties with respect to cold resistance as based on the average percentages of estimated injury taken at the final observation, was practically the same as that based on the percentages of dead and severely injured plants. The average percentages of estimated

Table IV. Comparing Plants Killed, Plants Killed and Severely Injured and Estimated Injury as a Measure of Resistance of Alfalfa Varieties to Low Temperature

Variety	Dead Plants Per cent	Plants Dead and Severely Injured Per cent	Estimated Injury Per cent
Hardistan (Nebraska)	27.1	44.7	49.1
Ladak (S. Dakota)	43.6	63.8	50.3
Grimm (Utah)	44.5	74.9	62.5
Nebraska Com. (F.C.I.15897)	63.3	86.9	78.8
Colorado Com. (F.C.I.14482) Great Divide, Colorado	63.6	80.4	74.0
Dakota Com. (F.C.I.16081)	68.3	87.6	81.5
Utah Common (F.C.I.15986) Uinta Basin, Utah	70.3	92.7	88.1
Kansas Common	74.9	92.2	88.1
Oklahoma Com. (F.C.I.15902)	76.8	94.1	85.9
Idaho Common (F.C.I.17397)	76.9	92.9	86.3
Utah Common (F.C.I.15995) Millard Co. Utah	77.2	88.8	84.7
New Mexico Com. (F.C.I.14470) Roswell, New Mexico	86.4	98.3	94.6
New Mexico Com. (F.C.I.15877) Loving, New Mexico	91.9	97.9	97.5
Arizona Com. (F.C.I.15827) Yuma, Arizona	94.9	99.4	98.6
California Com. (F.C.I.15889)	98.8	100.0	99.1
Probable error of the mean of a variety	2.63	1.91	2.17

injury ranged midway between those of dead plants and of dead and severely injured plants except in the case of Hardistan. The average per cent of estimated injury was practically as great for Hardistan as for Ladak, whereas, Hardistan was significantly superior from the other two standpoints. Observation throughout the experiment indicated that Hardistan produced new top growth more slowly after freezing than any of the other varieties. In some cases well developed and uninjured crown buds on uninjured or slightly injured roots of Hardistan plants were just beginning to produce new shoots at the time of the final observation from three to four weeks after freezing. This condition was undoubtedly reflected in the higher per cent of estimated injury which was based entirely on the vigor of the top growth at the final observation. The same condition was observed in relation to Provence F. C. I. 34886, in the work of the previous year. It probably accounts for the failure of the observer to estimate accurately, the degree of injury sustained by these two varieties. With all other varieties it was possible to estimate the degree of injury with a reasonable degree of accuracy.

The data in Table IV are shown graphically in Figure 3. The estimated per cent of injury showed a correlation of

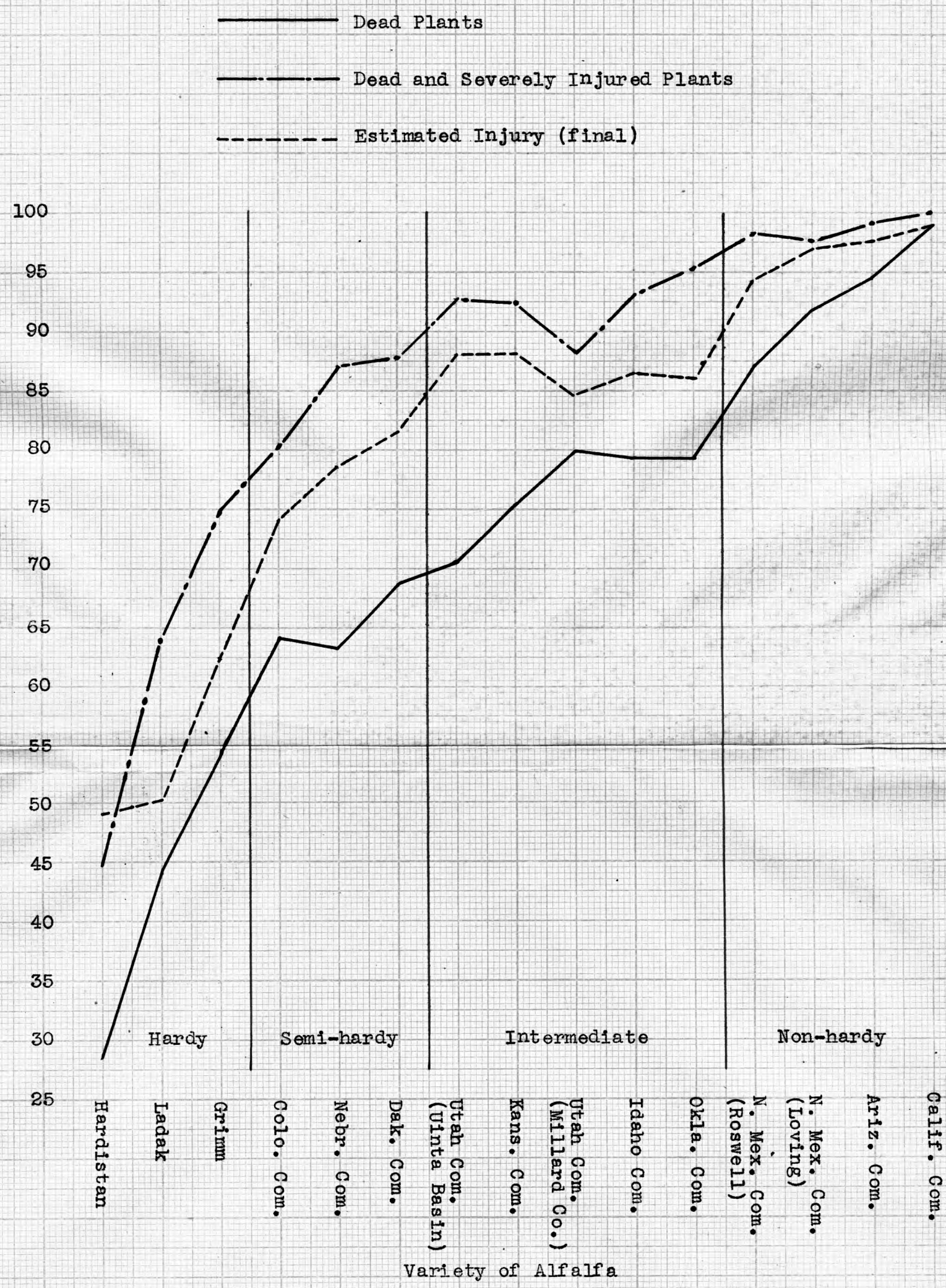

Fig. 3. A graph comparing three different indices to the cold resistance of alfalfa varieties.

.970 ± .010 with the per cent of dead plants and .956 ± .015 with the per cent of dead and severely injured plants. Thus it is evident that on the whole the relative cold resistance of the varieties of alfalfa under study are very much the same as measured by any of the three indices. All three showed a distinct separation between the hardy group, consisting of Hardistan, Ladak and Grimm; the intermediate group including common strains from Colorado, Nebraska, Dakota, Utah, Kansas, Idaho and Oklahoma; and the non-hardy group which included common alfalfas from New Mexico, Arizona and California. The three methods of measurement differed slightly in the ranking of the strains within the first two groups. The estimated injury did not include a significant superiority for Hardistan over Ladak while for all other strains the agreement of the per cent of dead and severely injured plants was very close. The average per cent of dead plants did not show a significant difference between Ladak and Grimm and also indicated a lower relative cold resistance in the strains of common alfalfa from Colorado and Millard County Utah than was evident when the severely injured plants were considered together with those killed outright by freezing.

Influence of Time of Observation on Estimate of Injury. Since estimates of injury from freezing were taken on each freezing lot at three different intervals after freezing,

it was possible to study the progressive recovery of the different varieties from the time of freezing to the time when the plants were examined for survival and root injury. Table V gives the length of the intervals for each lot, between the time of freezing and the time the different estimates of injury were made. The interval for the third estimate of injury in each case is also the interval that elapsed between the time of freezing and the time of examination for survival and root injury.

The estimates of injury made at three different intervals are compared with the average percentages of dead and severely injured plants in Figure 4. The final estimates of injury taken from two to four weeks after freezing showed a correlation of .989 ± .004 with the first estimate of injury made from three to thirteen days after freezing. The average percentages of dead and severely injured plants showed a correlation of .956 ± .015 with the final estimate of injury but only .854 ± .047 with the first estimate. The greatest deviation occurred in the case of Oklahoma Common in which the first estimate of injury from freezing was over twenty-five per cent less than the final estimate. The first estimate of injury was over fifteen per cent less than the final estimate in Nebraska Common and Idaho Common. On the other hand, in Hardistan and Ladak, the first estimate

Table V. Intervals Between Time of Freezing and That of the Different Estimates of Injury

Lot Number	Date Frozen	Days Between Date of Freezing and Estimate of Injury		
		First Estimate of Injury	Second Estimate of Injury	Third Estimate of Injury
1	Nov. 13[3]	4	8	18
2	Nov. 2	5	12	15
3	Nov. 3	7	11	14
4	Nov. 20[4]	4	11	16
5	Nov. 11	3	10	30
6	Nov. 11	3	10	31
7	Nov. 12	3	10	29
8	Nov. 13	4	10	28
9	Nov. 22	13	20	24
10	Feb. 7	10	16	28
11	Feb. 7	10	16	23
12	Mar. 1	6	11	15

(3) Refrozen. Frozen first on November 1
(4) Refrozen. Frozen first on November 4

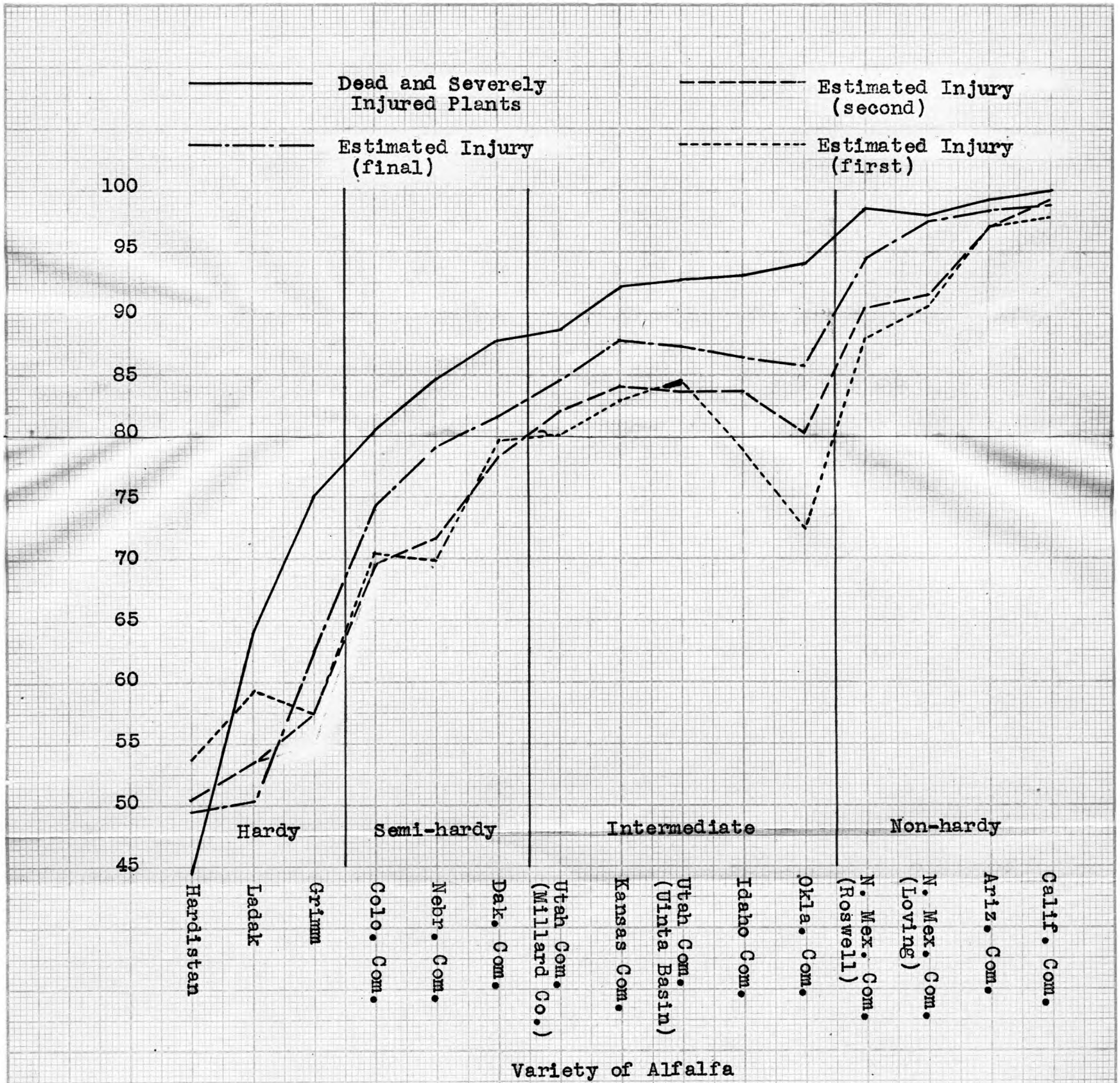

Fig. 4. Estimates of freezing injury taken at three different intervals compared with the average percentages of plants killed and severely injured.

of injury was about fifteen per cent more than the final estimate. Thus it appears that the first estimate of injury taken soon after freezing was probably a less reliable measure of the resistance of the different varieties to cold than the second and the third estimates taken somewhat later.

The Range of Injury and Survival Within Varieties. The range in injury and survival from freezing within each variety is shown in Figure 5. All varieties and strains except the non-hardy ones from New Mexico, Arizona and California exhibited every degree of injury and survival from dead to uninjured plants. With the exception of Grimm and Utah Common from Millard County, those in the hardy and intermediate groups had about the same proportion of plants with severely injured roots. Grimm contained a considerably larger per cent than other strains in these groups, while Utah Common from Millard County had a somewhat smaller proportion of severely injured plants. Within the non-hardy group the proportion of severely injured plants decreased as the per cent of dead plants increased. The percentages of plants showing slight root or crown injury tended to decrease as the percentages of dead and severely injured plants increased. This tendency was very marked in all of the varieties in the experiment with the exception of the Millard

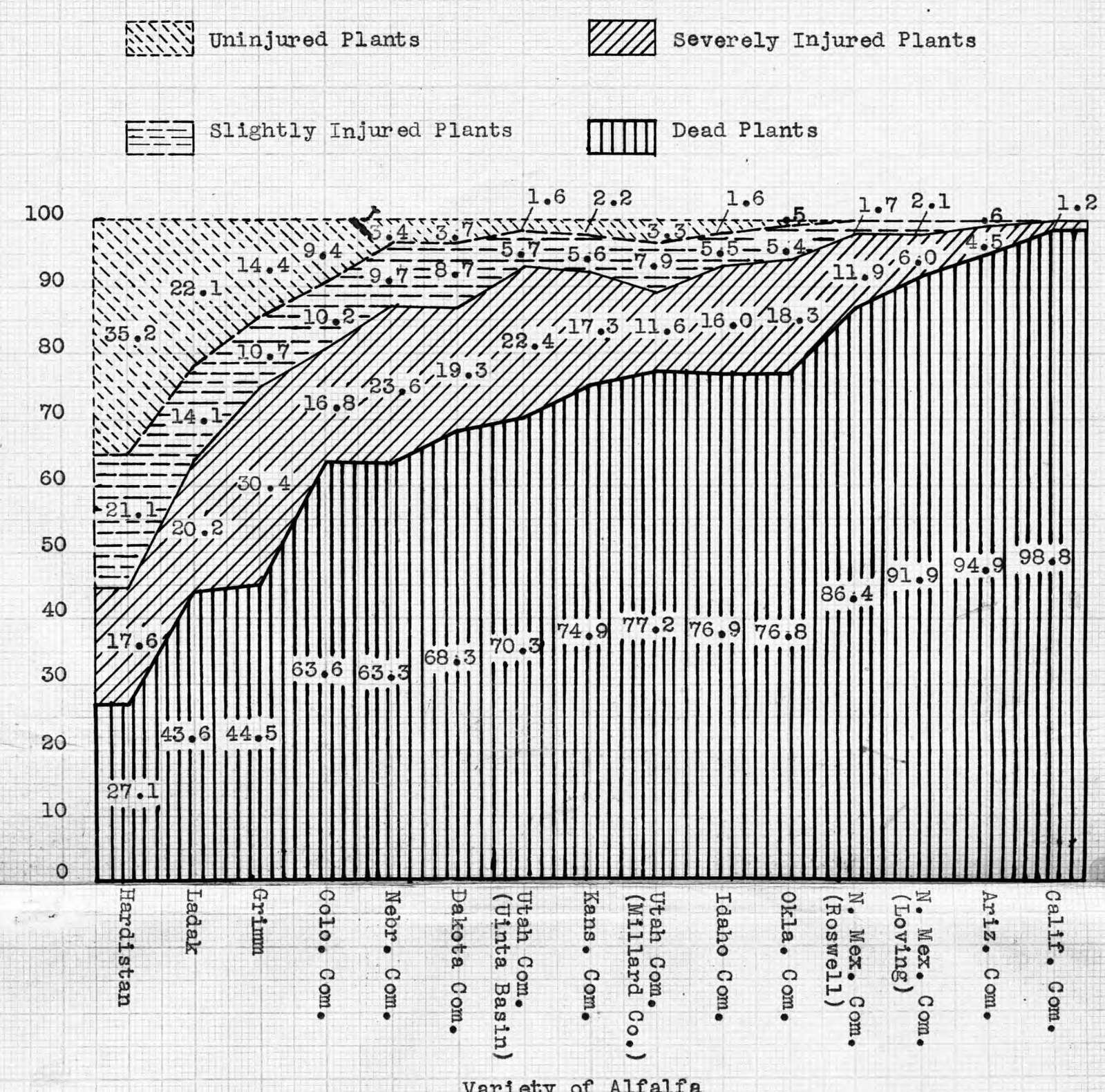

Fig. 5. Diagram showing the range in injury and survival within each variety.

County Utah strain and was no doubt due to the fact that the freezing exposure was so severe that most of the varieties were prevented from showing their complete range in survival.

A further study of the relation of the severity of the freezing exposure to the proportion of injured plants, is shown in Figure 6. It will be noted that the proportion of severely injured plants was practically the same in all lots in which the per cent of dead plants was below seventy. The proportion of severely injured plants varied inversely with the per cent of dead plants, especially when the latter was over seventy. This tendency was much more evident within the different experimental lots than within the various varieties as can be seen by comparing Figures 5 and 6. This indicates that the exceptions in the case of Grimm, common from Millard County Utah, and Nebraska Common were due to varietal differences rather than to chance variation. The proportion of slightly injured plants in the different lots was almost perfectly inversely proportional to the per cent of dead and severely injured plants as was the case within the varieties. This further indicates that the freezing exposure used was too severe for many of the varieties to exhibit their full range in cold resistance. It seems

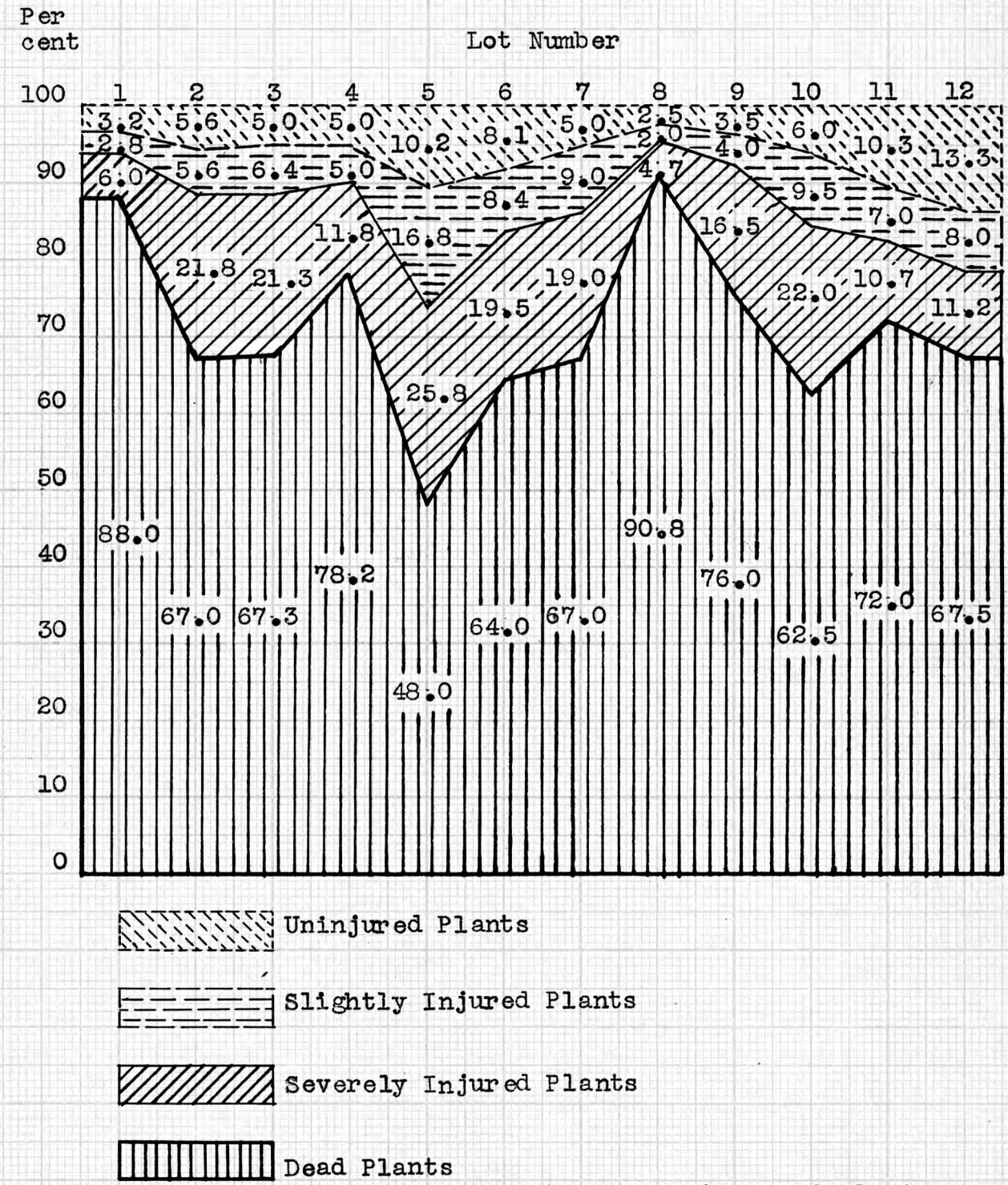

Fig. 6. Diagram showing the proportions of plants exhibiting the different degrees of injury and survival in each lot.

possible that a less severe freezing exposure would have brought out more clearly, the differences in cold resistance between the varieties in the intermediate group which were so closely grouped in the results obtained in these experiments.

Cold Resistance and Distribution of Varieties and Strains. A close relation between the cold resistance of the commercially important varieties and strains and the severity of the winters in the regions where they have become adapted is apparent. Very little is known about the history or the adaptation of Provence F. C. I. 34886 and Hardistan, the most resistant varieties in these experiments, hence, they cannot be considered in this connection. Ladak and Grimm, which are known to be especially adapted to northern states characterized by severe winters, were much more resistant to controlled freezing exposure than any of the strains of common tested. The Dakota Common which came from the Black Hill section, was perhaps less resistant to low temperatures in comparison with Nebraska Common and Colorado Common than its origin would seem to suggest. However, the winters in and near the Black Hills are less severe than other portions of the state. Also the Great Divide region of Colorado from which the Colorado Common here used was obtained, is an area of more than 6,000 feet elevation

and is characterized by very severe winters. The experiments did not show any consistent or marked difference between the various strains from Idaho, Kansas, Utah and Oklahoma. Thus the difference between the two Utah strains was greater than that between any other two of the group. This is not entirely in accord with the survival of these strains under field conditions nor with what would be expected. However, large differences would probably not be expected and no doubt the differences between lots secured within the boundaries of each state would vary as much as those reported here. It is probable that establishment of a relation between resistance to low temperature and distribution of these strains would require more extensive experiments and especially more exact knowledge as to the source of seed and its previous history than was available for the experiments reported here. The small proportion of survival from freezing of the strains from California, Arizona and New Mexico is in agreement with the mildness of the winter seasons in those regions.

Influence of Size of Roots on Cold Resistance. The root diameters of all plants frozen were measured at the time the plants were examined for root injury. The size of roots in all varieties ranged from four to eleven millimeters in diameter and the average root diameters for the

different varieties or strains ranged from slightly below to slightly above six millimeters. No consistent or definite influence of the size of roots on cold resistance was noted in any variety. Plants with root diameters ranging from the smallest to the largest were found in the dead, injured and uninjured groups within nearly every variety. In some of the varieties the roots of dead and injured plants averaged slightly smaller than those of slightly injured or uninjured plants, however, in other varieties the reverse was true. It is apparent then, that the size of the roots was not an important factor in the relative cold resistance of the different alfalfas in this experiment.

SUMMARY

Several hundred plants of five varieties of alfalfa were transplanted from fall sown field plots and frozen at controlled temperatures in the fall and early winter of 1929. About two hundred plants of each of fifteen varieties and regional strains were transplanted from spring seeded field plots and frozen at controlled temperatures during the fall and winter of 1930-31.

Provence F. C. I. 34886 and Hardistan, proved to be more resistant than any other variety or strain tested. Grimm and Ladak were second. A third group consisted of

Dakota Common, Nebraska Common and Colorado Common. Kansas Common, Utah Common, Idaho Common and Oklahoma Common made up the fourth group and a fifth group consisting of strains from Arizona, New Mexico and California was the least resistant of any.

In general, the resistance of the various strains to low temperatures was found to correlate well with the severity of the winters of the regions to which these strains had become adapted.

On the whole, there was a close agreement between the different indices to the relative cold resistance of the varieties under study. The correlation between estimated injury and dead plants was found to be .970 ± .010 and between estimated injury and dead and severely injured plants .956 ± .015. Hardistan and Provence F. C. I. 34886 survived somewhat better than would have been expected on the basis of estimated injury.

The first estimate of injury made soon after freezing showed a correlation of .854 ± .047 with dead and severely injured plants but appeared to be a less reliable index to cold resistance than the estimate made several weeks after freezing in which the correlation with dead and severely injured plants was .956 ± .015.

The per cent of plants with root injury was much larger

when the final observations were made from two to four weeks after freezing than when they were delayed until nine to eleven weeks after freezing, the principal reason being the death of injured plants during the longer period of observation.

The proportion of plants with root injury varied inversely with the per cent of dead plants in all experimental lots and in most of the varieties. Some varietal differences were apparent.

The size of roots was apparently not an important factor in the varietal differences obtained.

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