FREEZING INJURY TO GERMINATION OF SORGHUM SEED WHEN FROZEN AT DIFFERENT STAGES OF MATURITY, AT DIFFERENT TEMPERATURES, AND FOR DIFFERENT LENGTHS OF TIME

by

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INTRODUCTION

Grain sorghum has firmly established itself as a major crop in the Southern Great Plains and is continuing to spread into other areas. In the plains area of Kansas, Oklahoma, and Texas, it is the second most important grain crop, being second in value only to wheat. With grain sorghum production that important, along with a large acreage of forage sorghums, production of sorghum seed has become an extensive business. Since most grain sorghums grown are hybrids, seed production is handled primarily by commercial companies or special farmers who produce hybrid seed. Many forage types are not hybrids, and seed could easily be grown by anyone, however, this is not the usual practice.

Assuming that seed of the proper varieties or hybrids is being produced, it becomes the duty of seed producers to place on the market high quality seed with good germination. A number of factors influence the germinability of seeds. One of these is freezing damage to immature seed. Although the majority of sorghum seed is produced in areas where freezing damage is either non-existent or of very minor importance, considerable seed is produced in areas where freezing injury can be an important problem. In a normal year probably little attention is given to freezing damage, but when an early freeze occurs, the question of how much the germination of immature sorghum seed was lowered becomes important. Thus, the purpose of this study was to determine the extent of reduction in germination that occurs when sorghum grain, at different stages of maturity, is frozen at different temperatures for different lengths of time.

REVIEW OF LITERATURE

The effect of freezing temperatures on the germination of sorghum has not been studied widely. Robbins and Porter (7) reported the only published information regarding this subject. Results of freezing temperatures on the germination of some other crops also will be reviewed. Interesting observations pertaining to germination studies also will be reviewed briefly.

Robbins and Porter (7), in their study with sorghum, used as cold treatments, 33°, 20°, and -20° F. for 8 and 12 hours. No seed exposed to 33° F. was injured. In 1939, at the 20° F. level, no sorghum seed with 40 percent moisture or less was injured when exposed for 12 hours. Feterita at 42 percent moisture was not injured by 20° F., but Hegari of the same moisture had a germination decrease of from 94 to 62 percent by an exposure of 12 hours to 20° F. This indicated a varietal difference existed. In 1940, when the main study was made, the varieties used were Hegari, Early Sumac, Early Kalo, Leoti, Waconia Orange, Honey Drip, Black Amber, Grohoma, and Sooner Milo. About the same results were obtained as seed of 30 to 35 percent moisture or less was not reduced in viability by 20° F. An exception was Early Kalo, which at 25.9 percent moisture was injured by 20° F. Also of interest was that two samples, one of Leoti and one of Grohoma, were over 40 percent in moisture, but no damage occurred. At the -20° F. level, seed with a moisture of 19 percent or less showed little damage, but when the moisture was above 22 percent and especially above 30 percent, the germination was seriously reduced, being zero for the higher moistures.

A study of germination of artificially frozen sorghum grain was conducted at Kansas State University in 1939 and 1940 as reported by the Annual Report of 1941.[1] The results burned in a fire in 1957 and were never published. Blackhull, Atlas, and Club were frozen from one to eight hours at temperatures of 20° to 30° F. Mature sorghum of 15 to 20 percent moisture withstood considerable freezing without injury. The germination of Blackhull was reduced only slightly by 20° F. for eight hours.

Kiesselbach and Ratcliff (4) made a detailed study of the effect of freezing temperatures on open-pollinated corn. Often a great difference was found in the response of two ears with the same moisture to the same temperature treatment. A transition moisture was set up for the different temperatures used. This transition moisture indicated the moisture above which little if any damage occurred, and below which damage was quite frequent and severe. All ears were exposed for 24 hours without the husks. The temperatures at which the corn was frozen

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[1] Annual Report of Small Grain and Sorghum Breeding, 1941. On file in Agronomy Department, Kansas State University, Manhattan, Kansas.

and the transition moistures for each temperature are given as follows:

a. 32° to 28° F. - Transition between 40 and 50 percent.

b. 24° to 20° F. - Transition between 28 and 35 percent. All with 50 percent or above were killed.

c. 16° to 12° F. - Transition between 25 and 30 percent. All with 33 percent or above were killed.

d. 8° to 4° F. - Transition between 19 and 25 percent.

e. 0° to -5° F. - Transition between 18 and 20 percent.

As moisture decreased, injury from exposure to a given temperature became less. With immature corn, freezing injury at a given moisture content increased as the temperature lowered. Dry, mature corn showed no reduction in vitality. The vitality of corn exposed to freezing temperatures in the field was more variable, but in general, substantiated the principles established under controlled conditions.

Rossman (9) studied the freezing injury of two hybrids at 30, 40, and 50 percent moisture levels. Temperatures used were 26°, 20°, and 14° F. for 2, 4, 8, and 16 hours. Results of that study:

a. 26° F. - No injury at any moisture or time.

b. 20° F. - No injury at 2 and 4 hours, but 8 and 16 hours reduced viability in the 40 and 50 percent moisture levels.

c. 14° F. - 4, 8, and 16 hours reduced viability of the 40 and 50 percent moisture levels.

d. 30 percent moisture was uninjured in all except 8 and 16 hours at 14° F.

Temperature, time, and moisture, along with the first and second-order interactions, were all significant. Also the two hybrids differed significantly in their damage. The injury was progressive; first a reduction in seedling vigor, then abnormal seedlings, and finally complete death of the seeds. This was in contrast to the study in which mature, dry seed was soaked to various moisture levels and then frozen. In the latter case, the seed was either killed or remained completely viable. Porter (6) reported on hybrid seed corn collected from the field after a frost when the temperatures ranged from 18 to 22° F., and only two lots, one with 40 percent and the other with 52 percent moisture, were injured.

Kern (3) reported results of freezing seed corn on the ear, with and without husks, of two hybrids at 30°, 18°, 10°, and 0° F. for 12 hours. No injury occurred in any material frozen at 30° F. At 18° F., with no husks, samples of 26 percent moisture or below were not injured, but those above 26 percent were damaged in direct proportion to moisture content. At 10° F., ears without husks with 20 percent moisture or less were not injured. Samples without husks frozen at 0° F. were not injured when the moisture content was below 17 percent. Samples of over 19 percent moisture dropped abruptly to 0 germination at 30 percent moisture. Samples with husks, at 18°, 10°, and 0° F., showed slightly better germination than those of similar moisture without husks.

Reaction of oats to freezing temperatures was given by Fryer (2) who germinated samples before and after frost.

No reduction in germination occurred with 2° to 3° F. of frost. Two plots germinated 10 percent better after the frost, suggesting that the frost may have made the grain more physiologically mature. Also, no damage occurred in any of the maturity stages with 4° to 6° F. of frost. Two consecutive nights of hard freeze, one of 5° F. and one of 8° F., lowered the germination of the samples that were high in moisture. The dough to dry-mealy were not injured but the less mature were all injured with the severity increasing for the more immature, reducing the germination of grain in the milk stage from 66 to 13 percent. Another study with oats by Booth (1) revealed that controlled temperatures ranging from 31° to 27° F. for periods of one to three hours had no apparent effect on germination of immature kernels.

A study by Porter (6) showed that soybean seed with a moisture of 50 to 63 percent withstood a temperature of 20° F. but was seriously damaged when the temperature reached -20° F. When the moisture was near 30 percent, no appreciable injury occurred from a temperature of -20° F.

Various factors may influence freezing injury to grain. Rossman (9) listed the following as influencing the reduction in viability due to freezing maturing corn:

1. Temperature
2. Duration of exposure
3. Moisture
4. Variety
5. Husk protection
6. Physiological maturity
7. Rate of drying after freezing

All but husk protection would apply to sorghums as well as to corn. Physiological maturity was measured by Rossman (10) as the percentage of total dry matter accumulated. Corn with 30 percent moisture was considered to be physiologically mature. Grain grown in hot, dry weather was less physiologically mature at a given moisture level than grain grown under moderate temperatures and ample moisture. Rapid drying after freezing was shown by Rossman (9) to have a highly significant injurious effect as the slowly dried seed germinated 20.3 percent higher. Rate of thawing had no significant effect. Repeated freezing and thawing was less injurious than continuous freezing when total exposure time was the same. Rossman (10) stated that freezing tolerance of seed corn was not related to hybrid vigor, and the relationship of seed size to tolerance also was not significant. Tolerance was significantly related to seedling vigor.

Koehler, Dungan, and Burlison (5) showed evidence that weathering apparently lowered germination since corn when first mature gave the highest germination with a slow decline thereafter.

The method of curing and storing may influence germination as Robbins and Porter (7) found that sorghum seed cured by hanging up while still in the head, germinated better than that threshed and cured in cloth sacks. They also found that threshed seed kept in the laboratory four months declined materially in viability. The decline was not associated with the moisture at which it was harvested. Porter (6), however, reported that sorghum seed showed little loss in germination after storage in a dry, heated room for eight years. Robertson and Lute (8) reported that Black Amber sorghum seed stored in sacks in an unheated room decreased in germination only 2 percent in six years.

MATERIALS AND METHODS

General

Equipment and facilities used in this study were property of the Department of Agronomy, Kansas State University, and all material was grown on the Agronomy Farm located two miles northwest of Manhattan.

The variety, Plainsman, a grain sorghum widely grown in this area before the advent of hybrid grain sorghums, was used in this study. The Plainsman samples were collected in the fall of 1958 except for a few samples collected in the fall of 1959. In both 1958 and 1959, a few samples of Atlas, an important forage sorghum, were collected and in 1959, several samples from the grain sorghum hybrid RS610[1] (Combine kafir-60 x 7078) were collected to determine its tolerance to freezing.

Three variables, temperature at which grain was frozen, time of exposure to that temperature, and moisture content of the grain were used in the study. Four levels of each variable

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[1] The seed was open-pollinated F_2 seed taken from F_1 plants of RS610.

were used and were equally spaced to aid in analysis. The temperatures used were 31°, 28°, 25°, and 22° F. The time of exposure was 2, 4, 6, and 8 hours. The moisture levels used were approximately 45, 34, 23, and 12 percent.

All possible combinations of the variables were used. Six heads were harvested for each combination, e.g., at one moisture level and one temperature, 24 heads were collected, six for each time of exposure. Four heads that were closest to the desired moisture percentage were selected for germination tests. Each head was split in half; one-half serving as the control while the other half was frozen. The reduction in germination was obtained for each possible combination of temperature, time of exposure, and moisture.

Sampling and Freezing

Plainsman[1] was collected from two fields that differed in maturity by about 10 days and were located one-fourth mile apart. The field designated as A was the earliest with field C being the late field.

Only one temperature was used at a time and the maturity range of the sorghum was such that at most only two moisture levels, but generally only one, could be collected the same day. Collection of heads began on September 11 and was done intermittently as heads reached the desired moisture level, through

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[1] Unless otherwise designated, "Plainsman" refers to that collected in the fall of 1958.

October 16. The heads were selected at random except for selection of at least average-sized, healthy heads that appeared to be of the same maturity. The samples generally were taken in the morning after the dew had evaporated, but the actual time varied from day to day.

The harvested heads were placed in a large paper sack and taken immediately to the Crop Physiology Laboratory where the freezer and the oven were located. The heads were taken at random from the sack, split in half, and given an identification number. The first six heads chosen were frozen for two hours, the next six for four hours, etc. The appropriate half-heads[1] then were put into the freezing chamber which previously had been adjusted to the desired temperature; thus, no attempt was made to freeze the heads gradually. The freezer consisted of a home-made, wooden, insulated chamber containing a Larkin refrigerator unit inside with an automatic thermostatic control that held the temperature $\pm$ 0.5° F. of that desired. The chamber was about three feet wide, four feet high, and five feet long. The half-heads were laid on the floor of the freezer, and circulating air assured even cooling. Moisture percentage of the seed on each head was determined by taking 40 seeds from each control half-head. Ten seeds each from four locations ranging from tip to base made up the 40-seed sample for each head. Rossman (9) and Kiesselbach and Ratcliff (4) reported on the

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[1] Half-heads refer to half a head, either the frozen half or the control half.

difference in moisture in corn kernels from tip to base. The seeds were weighed to the nearest one-thousandth of a gram, then placed in a Precision Thelco Model 18 oven at 75° C. for 24 hours. They were then weighed again and the percentage moisture calculated on a green weight basis.

The appropriate heads were removed from the freezer after two, four, six, and eight hours. When removed, they were placed loosely in a box at room temperature until all heads had been removed, then taken to the greenhouse where they were strung on twine and hung up to dry. The control half-heads were held at room temperature and then taken to the greenhouse the same time as the corresponding treated half-heads. The temperature in the greenhouse varied considerably from near 100° F. on sunny days to 55° F. some nights. An effort was made to keep the temperature at approximately 70° F. All heads were hung there until the middle of December when they were threshed; each head individually in a small Vogel thresher designed to thresh individual heads. The threshed seed was placed in envelopes and stored at room temperatures in metal boxes.

Germination Procedure

Two hundred seeds were taken from each half-head of the four heads nearest the selected moisture percentage for germination studies. Thus, each original head had four 100-seed samples taken from it; two from the control half and two from the frozen half. In selecting seeds for germination, an effort was made to use the normal, non-cracked, disease-free seeds. Germination tests were started March 10, 1959 and continued through the middle of May. Germinations were run in a germinator similar to those used by the Kansas State Seed Laboratory. The temperature control mechanism was a refrigerator unit modified to meet the requirements for germination. There was a fan at the top of the chamber, a water spray mechanism at the bottom to keep humidity high, an automatic temperature control, and sliding screens on which to lay the blotters. Little differences in temperature occurred at different locations in the germinator. Some areas were prone to dry out sooner than others, but the blotters were checked every day and sprinkled with water when necessary to prevent drying. Alternating temperatures of 20° and 30° C. for 16 and 8 hours, respectively, were used. The seed to be germinated was divided into four groups; each group consisting of one head from each temperature, time, and moisture combination, making 64 heads per group. As each head was split, 128 100-seed samples were in each group. All in one group were germinated at one time. Each group was germinated twice because of the two samples from each half-head.

The position of the blotters for each sample was randomized, except that the samples of frozen and control for each head were side by side. Gray blotting paper cut 9 x 6 inches was used. The sheets were folded over with the 100-seed samples put between the folds.

To help prevent molds from developing, a very small amount of Spergon was mixed with each sample before germination. As Spergon does not injure germination, the amount applied was not accurately measured as only minute quantities were needed. A small amount of Spergon on the end of a flat toothpick was mixed with each sample.

Germination counts were made following the regulations in the U.S.D.A. Handbook (12). It was recommended that counts for sorghum be made on the fourth and tenth days. As some samples were rather slow to germinate, and also because it was sometimes difficult to determine whether the coleoptile contained a normal plumule on the fourth day, preliminary counts were made on the fifth day. On both the preliminary and the final count, the number of normal and abnormal seedlings and dead seeds was recorded. They were added to give the percentage normal, abnormal, and dead in each sample. During the preliminary count, the frozen and the control samples were compared by sight, and the frozen given an arbitrary value indicating the degree of reduction in germination due to freezing. A rating of 0 meant no apparent injury, while a 1 rating meant slight damage, and a 2 rating indicated considerable damage. Some 2++ ratings were given and they represented very severe injury to the frozen seed when compared to the non-frozen seed from the same head.

Analysis

An analysis of variance was run on the results to test for significant differences. In analyzing the material, the germinations were transformed to angles, according to Bliss' table as given by Snedecor (11). The two angle values for each half-head were averaged and the difference between the control and the frozen half was used in the analysis. In determining differences, the value always represented control minus frozen. Since only a few samples were harvested, no analysis was made on the Plainsman collected in 1959 or on the Atlas or RS610, other than comparing germination differences with the main study, that of Plainsman collected in 1958.

Additional Material Collected

The Atlas in 1958 and 1959, and the Plainsman samples in 1959 were handled the same as the Plainsman in 1958, except that samples of only a few combinations of temperature, time of exposure, and moisture level were collected. Only two heads were selected to run germination tests at a particular temperature, time and moisture level. The samples of Atlas in 1958 and 1959 were taken from border rows next to experimental sorghum plots. The Plainsman collected in 1959 was taken from a small area in the sorghum experimental field that was planted to Plainsman. The RS610 used in 1959 was taken from a similar small area that was planted with that hybrid. Samples were first taken in 1959 on August 26. That was earlier than in 1958, as the weather conditions were rather dry during the summer of 1959, making the grain mature earlier.

Soil and Weather

The sorghums used were grown on Geary silty clay which was reasonably fertile and deep. The topography was gently rolling.

The weather in 1958 was cool and wet. June was wet and the last half very cool while July was cool and very wet with 12.39 inches of rain. August was cool with moisture slightly above average. September was characterized by mild temperatures and wet weather with one inch or more falling on each of five days with 7.53 inches for the month. A frost occurred the night of September 30 with a low temperature of 29° F. Only five samples were collected after this, one with 23 percent moisture and all four samples with 12 percent moisture. October was mild and dry. The weather in 1959 was normal in temperature but rather dry, especially in August; however, no drought symptoms appeared on the sorghum, as apparently there was ample subsoil moisture.

RESULTS

Plainsman - 1958

Germination was severely reduced in some of the moisture-temperature combinations, especially in the high-moisture samples exposed to the lower temperatures. Time of exposure to freezing generally had no important effect.

Means of all germination percentages were calculated, and the mean reduction in germination percentage for each treatment calculated (Table 1). All heads frozen at 31° F. showed no

Table 1. The average germination of control and frozen samples, and the difference (control minus frozen) of heads with different moisture, frozen at different temperatures for different lengths of time.

		Moisture percentage												
	Time	12			23			34			45			Average
Temp. ° F.	frozen hrs.	Control	Frozen	Difference	Control	Frozen	Difference	Control	Frozen	Difference	Control	Frozen	Difference	age difference
22	2	63.5	64.1	-0.6	57.1	37.2	+19.9	80.1	9.6	+70.5	72.8	1.4	+71.4	+40.3
	4	49.6	53.6	-4.0	63.5	43.9	+19.6	81.5	6.4	+75.1	81.2	0.6	+80.6	+42.8
	6	63.8	66.0	-2.2	61.9	46.1	+15.8	75.8	16.0	+59.8	75.9	0.2	+75.7	+37.3
	8	51.1	61.5	-10.4	62.4	53.9	+8.5	78.0	22.4	+55.6	79.4	15.0	+64.4	+29.5
	Avg.	57.0	61.3	-4.3	61.2	45.3	+15.9	78.8	13.6	+65.2	77.3	4.3	+73.0	+37.45
25	2	62.8	71.1	-8.3	55.9	59.8	-3.9	83.1	40.5	+42.6	81.1	39.8	+42.0	+18.1
	4	62.6	66.2	-3.6	55.5	51.1	+4.4	79.4	23.8	+55.6	79.8	12.9	+66.9	+30.8
	6	69.6	72.1	-2.5	69.8	63.2	+6.6	78.6	44.6	+34.0	86.1	17.9	+68.2	+26.6
	8	73.8	68.1	+5.7	67.2	65.2	+2.0	83.4	32.9	+50.5	80.5	21.0	+59.5	+29.4
	Avg.	67.2	69.4	-2.2	62.1	59.8	+2.3	81.1	35.4	+45.7	82.1	22.9	+59.2	+26.25
28	2	59.4	61.8	-2.4	62.0	63.4	-1.4	88.6	89.0	-0.4	85.4	86.6	-1.2	-1.4
	4	58.4	66.0	-7.6	61.9	63.9	-2.0	87.4	82.0	+5.3	81.8	74.9	+6.9	+0.6
	6	63.2	58.6	+4.6	70.5	66.5	+4.0	87.6	63.9	+23.7	86.1	70.2	+15.9	+12.0
	8	63.9	61.0	+2.9	62.0	64.1	-2.1	86.8	67.1	+19.7	82.9	83.1	-0.2	+5.1
	Avg.	61.2	61.8	-0.6	64.1	64.5	-0.4	87.6	75.5	+12.1	84.1	78.7	+5.4	+4.12
31	2	65.0	62.0	+3.0	57.5	63.1	-5.6	79.1	80.2	-1.1	80.5	85.1	-4.6	-2.1
	4	63.5	67.2	-3.7	61.2	60.2	+1.0	83.5	84.2	-0.7	77.4	82.0	-4.6	-2.0
	6	65.6	68.2	-2.6	60.9	60.8	+0.1	84.2	82.4	+1.8	80.2	83.4	-3.2	-1.0
	8	67.4	69.2	-1.8	63.9	60.8	+3.1	86.4	84.8	+1.6	82.5	81.1	+1.4	+1.3
	Avg.	65.4	66.7	-1.3	60.9	61.2	-0.3	83.3	82.9	+0.4	80.1	82.9	-2.8	-1.0
Grand Avg.		62.7	64.8	-2.1	62.1	57.7	+4.4	82.7	51.9	+30.8	80.9	47.2	+33.7	

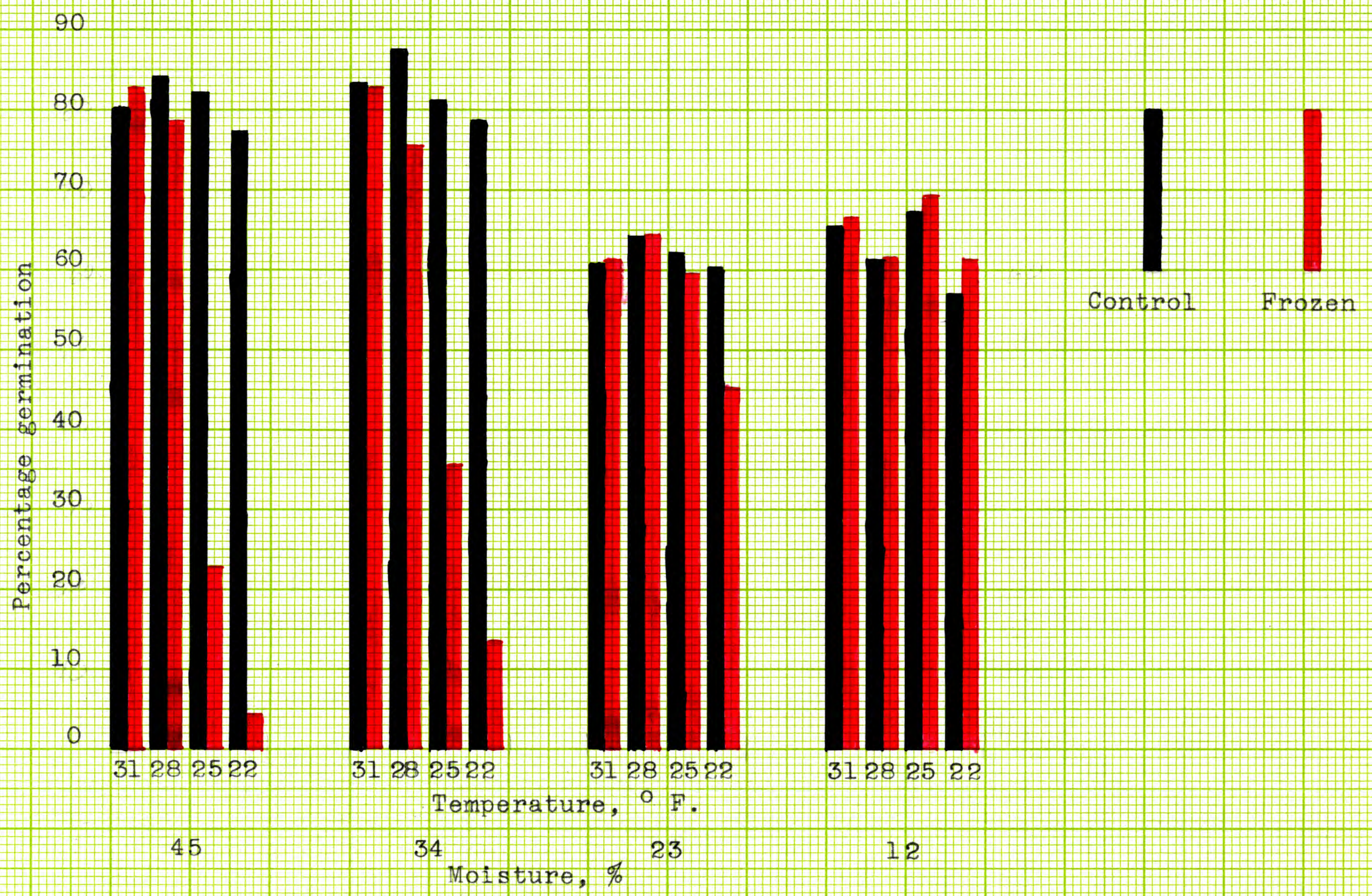

Fig. 1. A comparison of the control and frozen samples of sorghum seed frozen at different moisture levels and at different temperatures.

reduction in germination due to freezing, regardless of the moisture content of the seed. At 28° F., no injury occurred at any moisture level except the 45 percent moisture level where six hours of exposure lowered the germination 15.9 percent, and at the 34 percent moisture level where exposures of six and eight hours lowered the germination 23.7 and 19.7 percent, respectively. No reduction occurred, however, in the 45 percent moisture level frozen for eight hours. Seed with 45 and 34 percent moisture was severely injured by exposure to 25° F. with the average reduction in the 45 percent group being 59.2 percent and in the 34 percent moisture group, being 45.7 percent. Heads containing 23 percent moisture, exposed to 25° F., showed no appreciable reduction in germination. A temperature of 22° F. severely lowered germination in the 45 and 34 percent moisture groups and lowered it some in the 23 percent moisture group. The 45 and 34 percent moisture groups were lowered by 73.0 and 65.2 percent, respectively, while the germination was lowered 15.9 percent in the 23 percent moisture group. No seed with 12 percent moisture was injured at any temperature.

For all moistures, samples frozen at 31° F. showed a germination increase of 1 percent, while those frozen at 28° F. were reduced in germination by 4.1 percent. Freezing at 25° F. lowered germination 26.2 percent, and 22° F. lowered it an average of 37.4 percent. Thus, the lower the temperature, the more injury that occurred.

Grouping all temperatures together, the 45 percent moisture samples showed the greatest injury, with a gradual decrease as moisture was lowered. Germination in all the 45 percent moisture samples was decreased an average of 33.7 percent. The 34 percent moisture samples were decreased in germination by 30.8 percent; the 23 percent moisture samples by 4.4 percent; and the 12 percent moisture group showed a slight increase of 2.1 percent in germination in the frozen samples.

The effect of time of exposure, in general, was not very important. However, in the moisture-temperature combinations where the most injury occurred, that is, in the 45 and 34 percent moisture groups exposed to 28°, 25°, and 22° F. and in the 23 percent moisture group exposed to 22° F., six out of the seven groups showed less injury in the samples frozen for eight hours than in those frozen for six hours.

Thus, injury increased with increasing moisture and decreasing temperature, being severe in the 45 and 34 percent samples exposed to 22° and 25° F. Seed with 23 percent moisture, frozen at 22° F., showed considerable but not severe injury. Seed with 34 percent moisture, frozen at 28° F., showed slight injury, while that with 45 percent moisture, frozen at 28° F., and the 23 percent moisture group at 25° F., showed a very slight reduction in germination.

Plates I, II, and III illustrate how increasing moisture of seed at the time they were subjected to freezing, increases injury. Plates II, IV, and V illustrate increasing injury as

EXPLANATION OF PLATE I

Seed with 45 percent moisture, frozen for two hours at 22° F. Figure 1 is the control and Fig. 2 is the frozen seed.

Germination count of seed in Plate I

	Percent			
	Normal	Abnormal	Dead	Rating
Fig. 1 (control)	95	4	1	
Fig. 2 (frozen)	3	29	68	2^{+++}

PLATE I

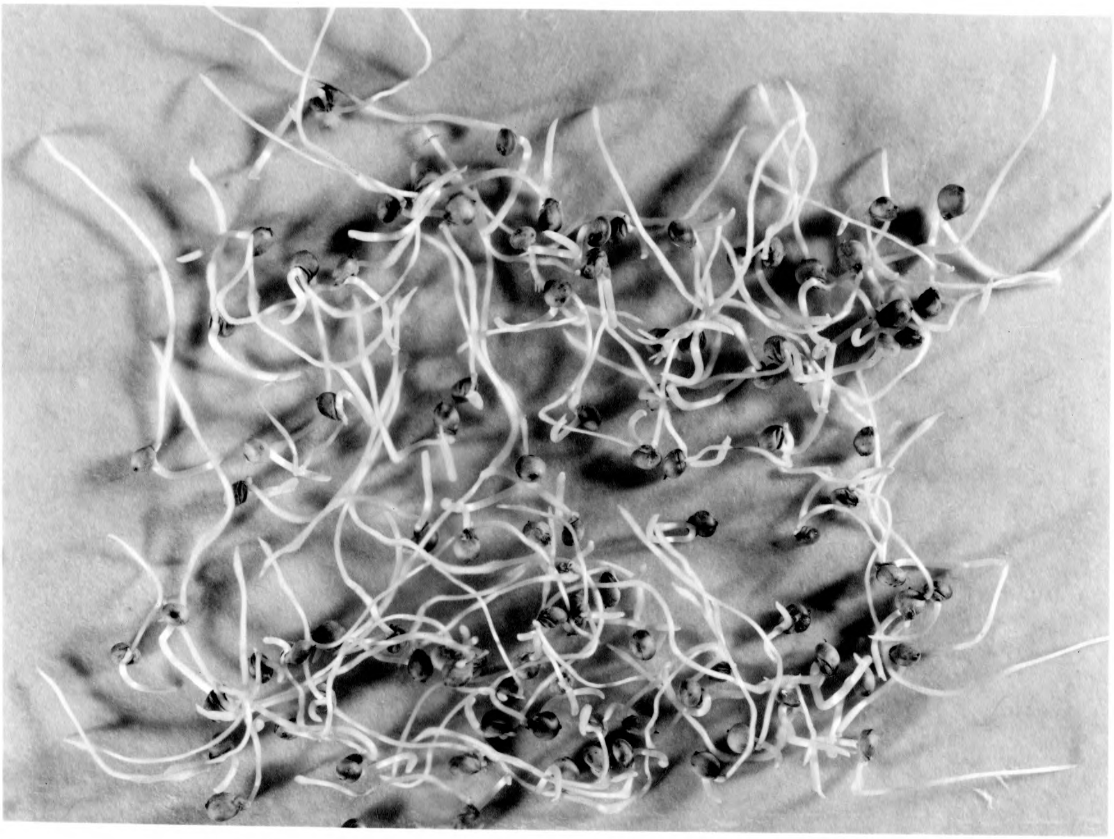

Fig. 1

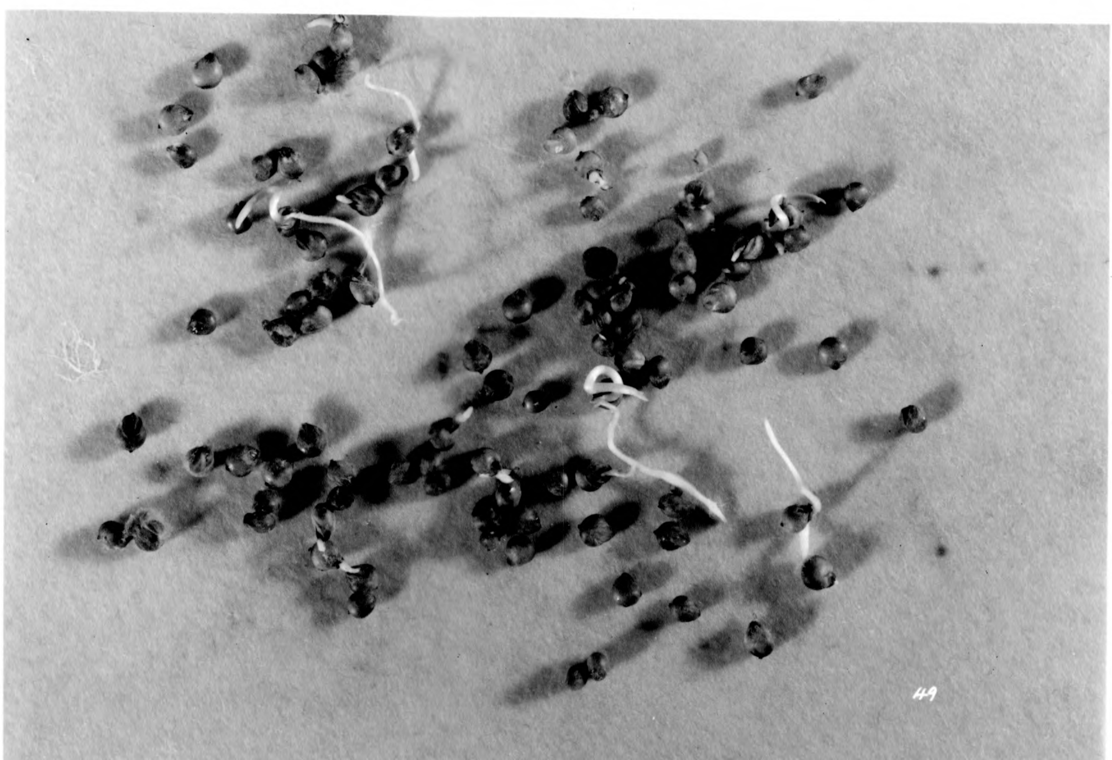

Fig. 2

EXPLANATION OF PLATE II

Seed with 34 percent moisture, frozen for two hours at 22° F. Figure 1 is the control and Fig. 2 is the frozen seed.

Germination count of seed in Plate II

	Percent			
	Normal	Abnormal	Dead	Rating
Fig. 1 (control)	86	9	5	
Fig. 2 (frozen)	7	45	48	2^{++}

PLATE II

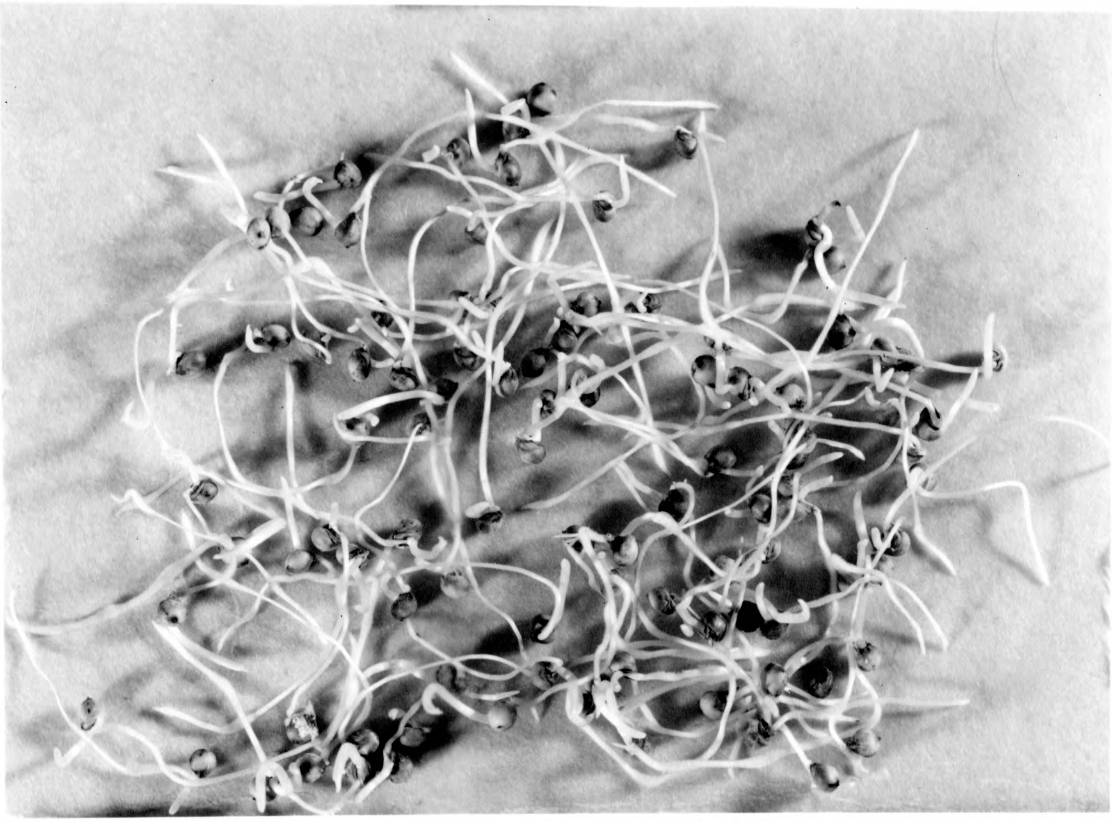

Fig. 1

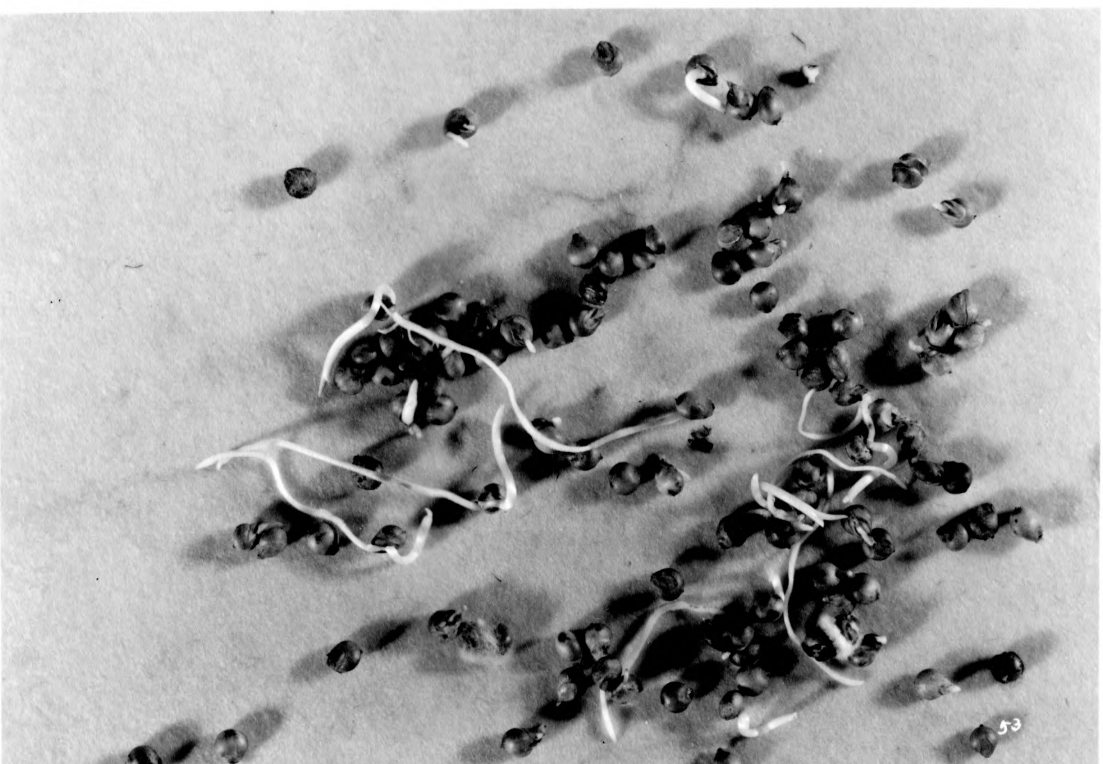

Fig. 2

EXPLANATION OF PLATE III

Seed of 23 percent moisture, frozen two hours at 22° F. Figure 1 is the control and Fig. 2 is the frozen seed.

Germination count of seed in Plate III

	Percent			
	Normal	Abnormal	Dead	Rating
Fig. 1 (control)	62	24	14	
Fig. 2 (frozen)	27	35	38	2

PLATE III

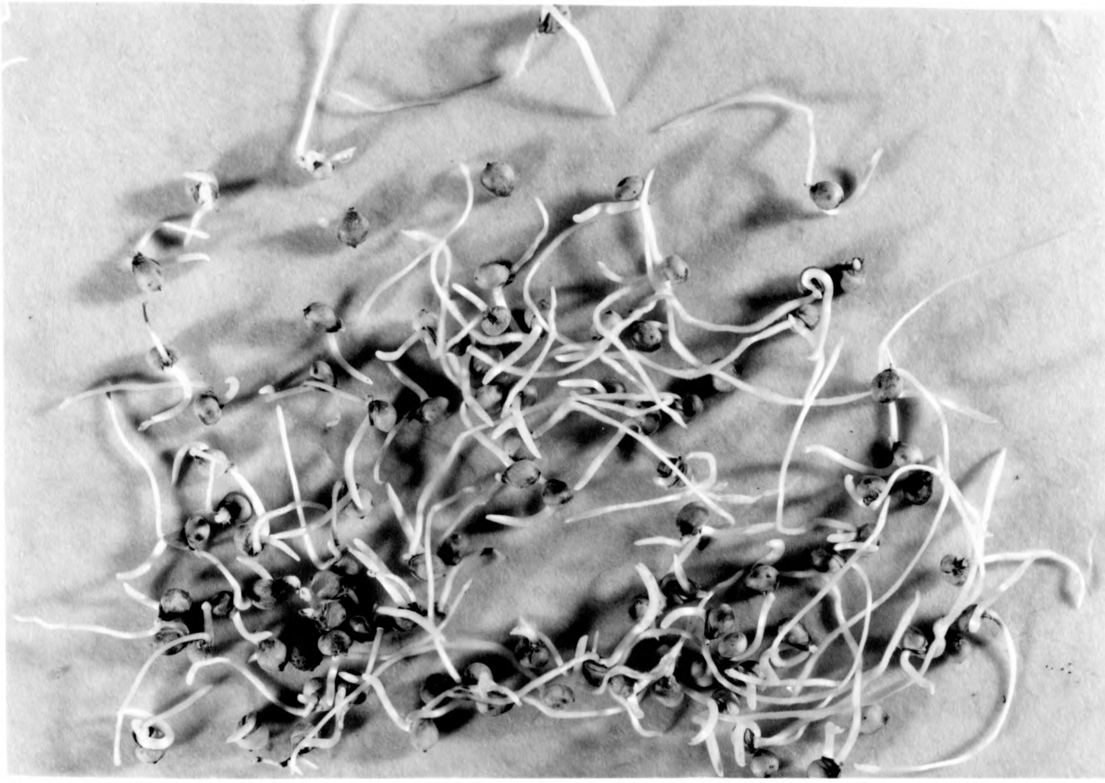

Fig. 1

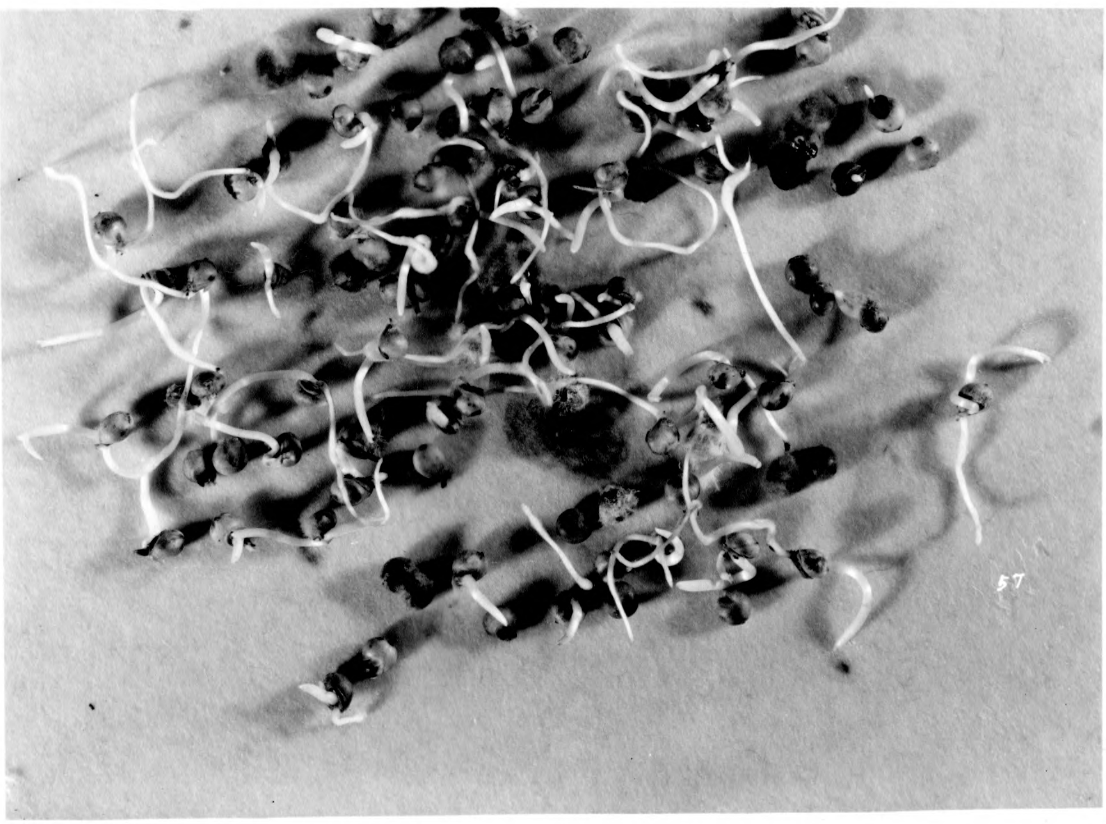

Fig. 2

EXPLANATION OF PLATE IV

Seed of 34 percent moisture, frozen two hours at 25° F. Figure 1 is the control and Fig. 2 is the frozen seed.

Germination count of seed in Plate IV

	Percent			
	Normal	Abnormal	Dead	Rating
Fig. 1 (control)	88	11	1	
Fig. 2 (frozen)	46	35	18	1-2

PLATE IV

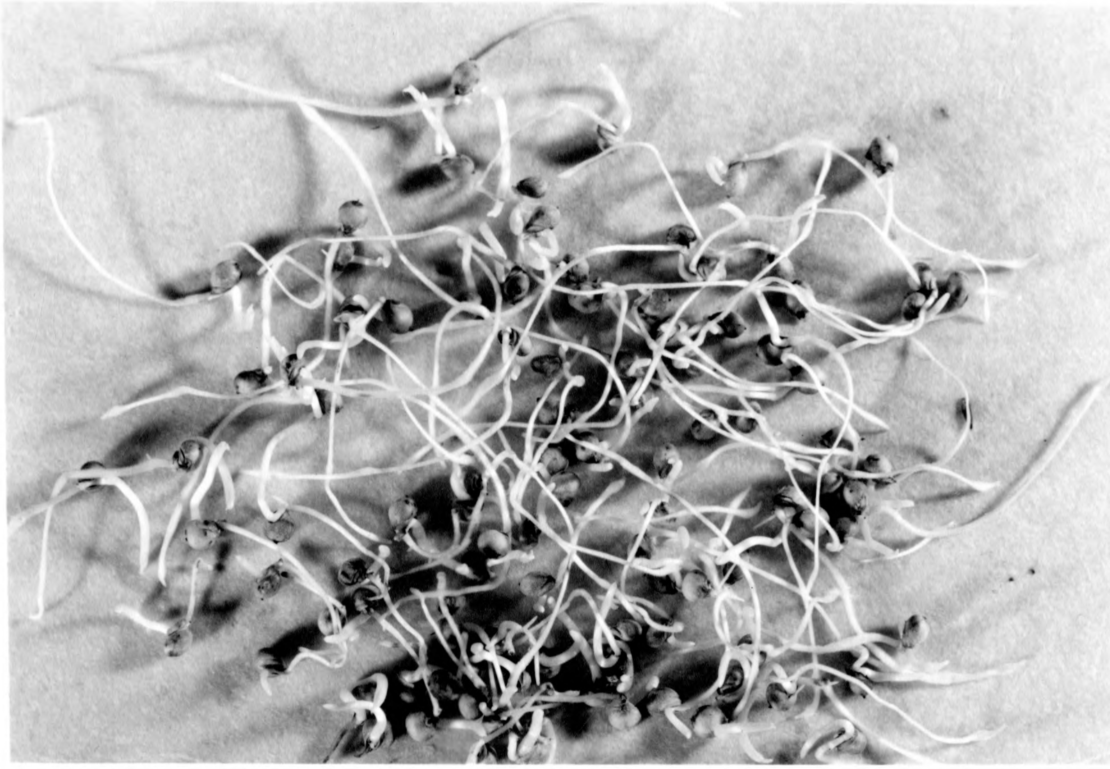

Fig. 1

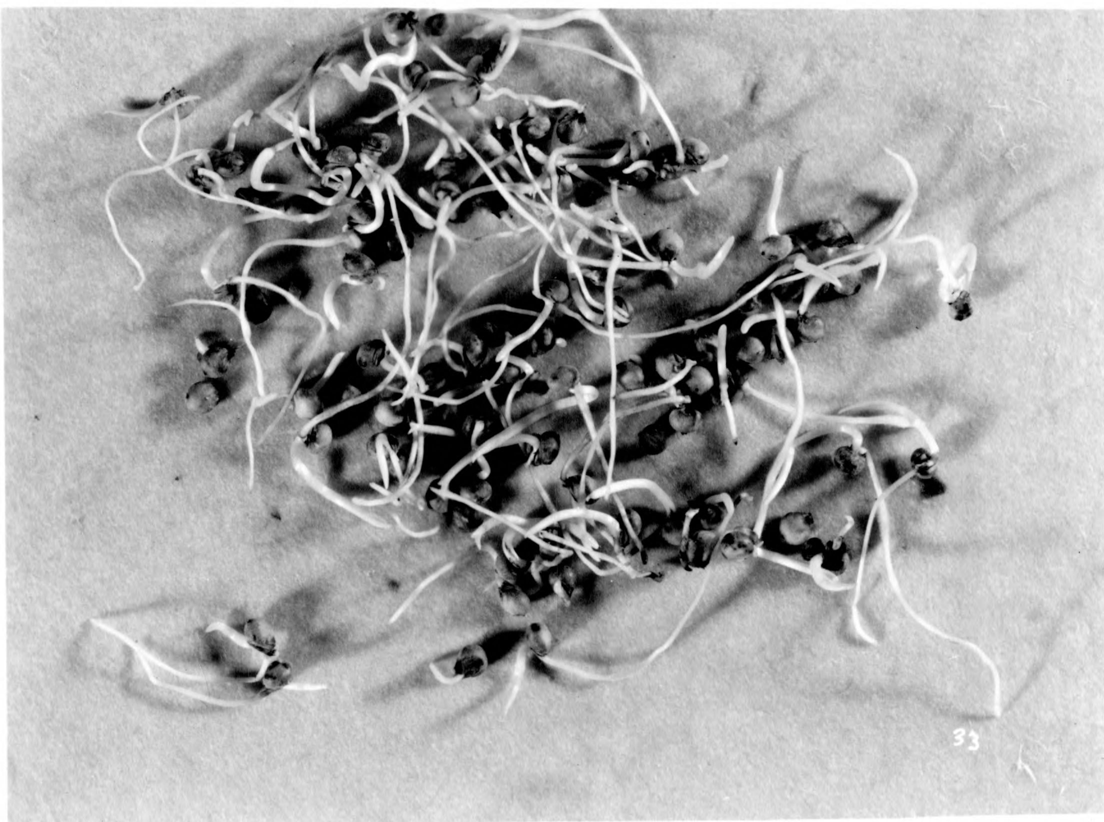

Fig. 2

EXPLANATION OF PLATE V

Seed of 34 percent moisture, frozen two hours at 28° F. Figure 1 is the control and Fig. 2 is the frozen seed.

Germination count of seed in Plate V

	Percent			
	Normal	Abnormal	Dead	Rating
Fig. 1 (control)	98	2	0	
Fig. 2 (frozen)	94	5	1	0

PLATE V

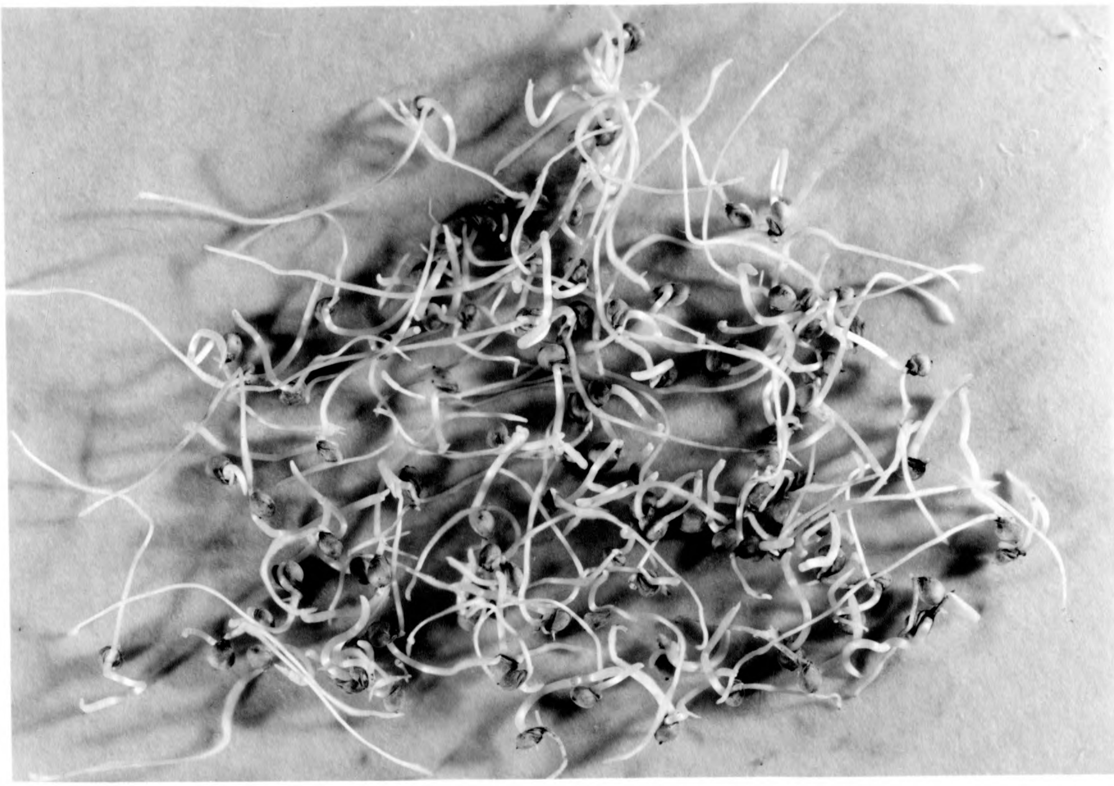

Fig. 1

Fig. 2

the temperature of freezing became lower. All pictures were taken on the sixth day. The upper picture in each plate is from the control half-head, while the lower represents seed from the frozen half of the same head.

In the statistical analysis, all germination percentages were transformed to angles, and the angle values for each half-head were averaged and the difference, control minus frozen, obtained. The differences of each group of four heads were added and presented in Table 2 and used in the analysis presented in Table 3. The linear components of moisture and temperature explained most of the variation; however, quadratic temperature effect also was significant but was much less important than the linear component. Time of exposure accounted for only a small portion of the variation, and the only significant component of time was the quadratic. The cubic component was significant in both moisture and temperature but was not considered important since only four points were used. In the first order interactions, moisture x temperature was the most important source of variation. However, moisture x time was close to significance, and time x temperature was significant but much less so than moisture x temperature. The second order interaction, moisture x time x temperature, was not significant.

Separate analyses were made on each two-factor combination, e.g., the linear, quadratic, and cubic components were computed for each time of exposure at each temperature and at each moisture, etc. (Tables 4 through 9). Figures 2 through 7 were taken from the means (Tables 10a, 11a, 12a) of the sums used in the

Table 2. The effect of freezing at different temperatures for different lengths of time upon germination of seed at various moisture levels as shown by the sum of differences (control minus frozen) of angle values at each moisture, temperature, and time combination.

Temp. frozen in ° F.	Moisture percentage 12					23					34					45					Grand total
	2 hrs.	4 hrs.	6 hrs.	8 hrs.	Total	2 hrs.	4 hrs.	6 hrs.	8 hrs.	Total	2 hrs.	4 hrs.	6 hrs.	8 hrs.	Total	2 hrs.	4 hrs.	6 hrs.	8 hrs.	Total	
22	-1.3	-4.4	-5.3	-24.5	-35.5	+31.9	+36.4	+36.8	+19.8	+124.9	+185.0	+206.5	+150.8	+134.9	+677.2	+216.5	+248.3	+237.8	+168.0	+870.6	+1637.2
25	-20.6	-9.5	-6.9	+15.8	-21.2	-9.7	+10.1	+15.5	+4.6	+20.5	+106.7	+140.8	+84.5	+124.4	+456.4	+106.3	+172.1	+176.2	+152.6	+607.2	+1062.9
28	-3.7	-18.4	+10.0	+6.3	-5.8	-2.5	-4.6	+8.2	-9.9	-8.8	-0.4	+17.8	+67.4	+53.7	+138.5	-4.3	+20.3	+41.7	-2.8	+54.9	+178.8
31	+2.2	-9.5	-5.7	-4.0	-17.0	-13.9	+3.7	+0.7	+2.4	+2.4	+1.1	-2.8	+6.4	+5.8	+10.5	-16.8	-13.8	-9.1	+4.1	-29.6	-43.2
Total	-23.4	-41.8	-7.9	-6.4	-79.5	+5.8	+45.6	+61.2	+16.9	+129.5	+292.4	+362.3	+309.1	+318.8	+1282.6	+307.7	+426.9	+446.6	+321.9	+1503.1	+2835.7

Table 3. Statistical analysis of variance. All are tested with error mean square.

Source of variation		Degrees of freedom	Sum of squares	Mean square	F	Significance
Moisture:	linear	1	27203.6100	27203.6100	639.18	***
	quadratic	1	.5166	.5166	.01	ns
	cubic	1	2751.5648	2751.5648	64.45	***
Time:	linear	1	38.5378	38.5378	.90	ns
	quadratic	1	529.8629	529.8629	12.41	***
	cubic	1	.3348	.3348	.01	ns
Temperature:	linear	1	27429.0469	27429.0469	642.46	***
	quadratic	1	484.8254	484.8254	11.86	***
	cubic	1	737.9606	737.9606	17.28	***
Moisture x time		9	719.1315	79.9035	1.87	near *
Moisture x temperature		9	24662.8166	2740.3130	64.18	***
Time x temperature		9	1968.5150	218.7239	5.12	***
Moisture x time x temperature		27	1472.4536	54.5353	1.28	ns
Error		192	8177.2575	42.6940		
Total		255	96196.4340			

ns = non-significant.
* = significant at the 5% level.
*** = significant at the 0.1% level.

Table 4. Significance of the trends of germination with different freezing temperatures of seed at various moisture levels. (See Fig. 2.)

	Sum of squares
12% moisture	
linear	15.71 ns
quadratic	10.16 ns
cubic	2.40 ns
23% moisture	
linear	565.25 ***
quadratic	175.89 *
cubic	6.08 ns
34% moisture	
linear	16791.01 ***
quadratic	134.56 ns
cubic	257.40 *
45% moisture	
linear	33066.75 ***
quadratic	500.08 ***
cubic	1789.36 ***

ns = non-significant.
* = significant at the 5% level.
*** = significant at the 0.1% level.

Table 5. Significance of the trends of germination with different lengths of time frozen of seed of various moisture levels. (See Fig. 3.)

	Sum of squares
12% moisture	
linear	22.51 ns
quadratic	6.19 ns
cubic	22.42 ns
23% moisture	
linear	7.47 ns
quadratic	110.51 ns
cubic	3.98 ns
34% moisture	
linear	2.11 ns
quadratic	56.63 ns
cubic	108.11 ns
45% moisture	
linear	12.13 ns
quadratic	929.49 ***
cubic	6.30 ns

ns = non-significant.
*** = significant at the 0.1% level.

Table 6. Significance of the trends of germination with different moisture percentages of grain at the different freezing temperatures. (See Fig. 4.)

	Sum of squares
22° F.	
linear	33427.58 ***
quadratic	17.02 ns
cubic	1761.56 ***
25° F.	
linear	16835.95 ***
quadratic	185.89 *
cubic	1442.03 ***
28° F.	
linear	339.08 **
quadratic	101.51 ns
cubic	454.10 **
31° F.	
linear	1.28 ns
quadratic	39.06 ns
cubic	13.37 ns

ns = non-significant.
* = significant at the 5% level.
** = significant at the 1% level.
*** = significant at the 0.1% level.

Table 7. Significance of the trends of germination with different lengths of time frozen on grain frozen at the different freezing temperatures. (See Fig. 5.)

	Sum of squares
22° F.	
linear	685.62 ***
quadratic	487.31 **
cubic	13.70 ns
25° F.	
linear	281.06 *
quadratic	164.80 ns
cubic	191.12 *
28° F.	
linear	257.04 *
quadratic	175.56 *
cubic	242.21 *
31° F.	
linear	33.67 ns
quadratic	4.52 ns
cubic	0.65 ns

ns = non-significant.
* = significant at the 5% level.
** = significant at the 1% level.
*** = significant at the 0.1% level.

Table 8. Significance of the trends of germination with different moisture levels of seed frozen for different lengths of time. (See Fig. 6.)

	Sum of squares
2 hours	
linear	5119.20 ***
quadratic	3.02 ns
cubic	873.51 ***
4 hours	
linear	9275.12 ***
quadratic	8.12 ns
cubic	724.21 ***
6 hours	
linear	8114.41 ***
quadratic	73.10 ns
cubic	261.36 *
8 hours	
linear	5174.54 ***
quadratic	6.38 ns
cubic	1041.85 ***

ns = non-significant.
* = significant at the 5% level.
*** = significant at the 0.1% level.

Table 9. Significance of the trends of germination with different freezing temperatures of seed frozen for different lengths of time. (See Fig. 7.)

	Sum of squares
2 hours	
linear	7547.58 ***
quadratic	891.77 ***
cubic	50.64 ns
4 hours	
linear	10419.61 ***
quadratic	288.15 *
cubic	465.61 **
6 hours	
linear	6349.27 ***
quadratic	3.90 ns
cubic	.01 ns
8 hours	
linear	3918.60 ***
quadratic	22.80 ns
cubic	662.40 ***

ns = non-significant.
* = significant at the 5% level.
** = significant at the 1% level.
*** = significant at the 0.1% level.

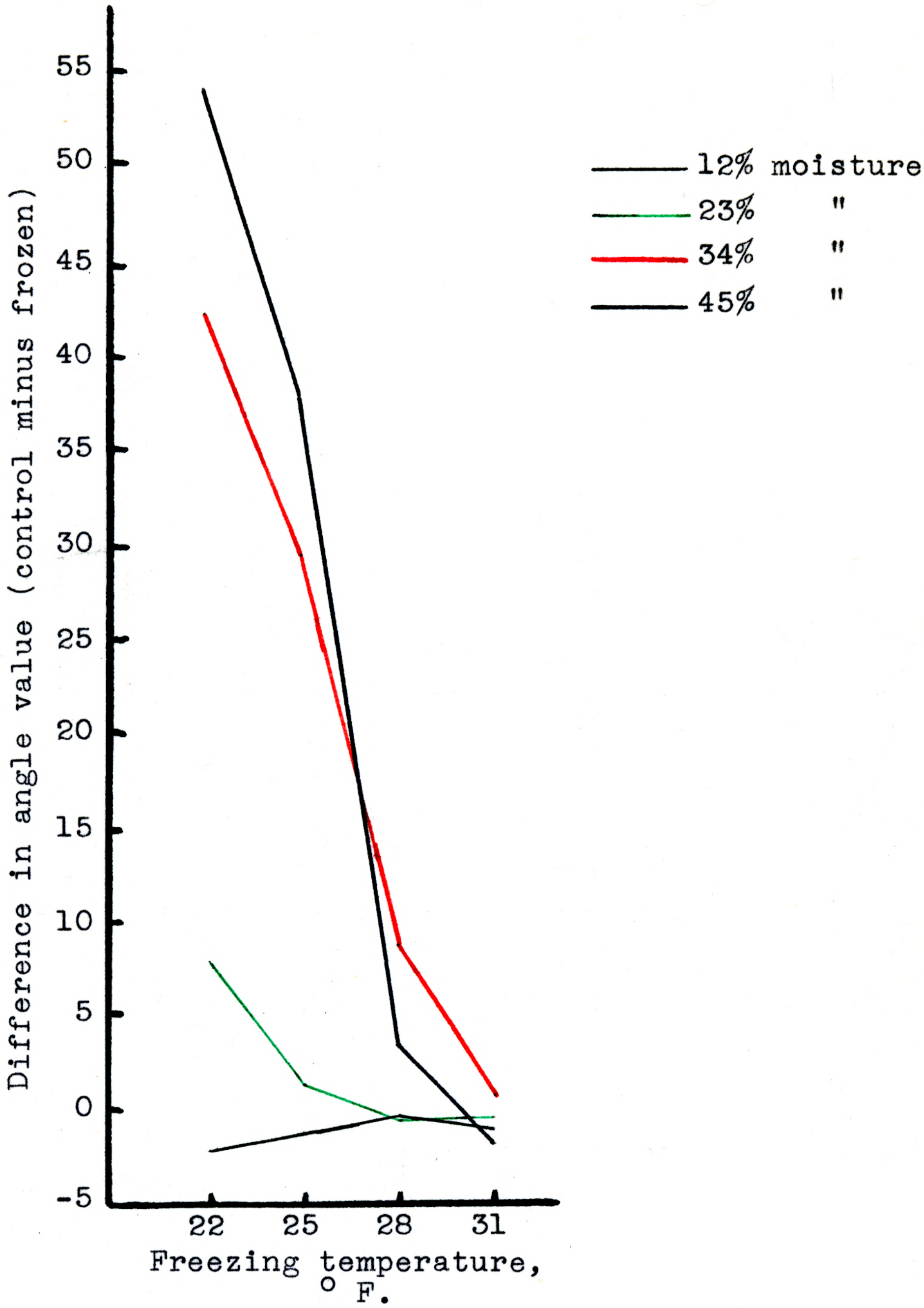

Fig. 2. The effect of different freezing temperatures on germination of sorghum seed at various moisture levels.

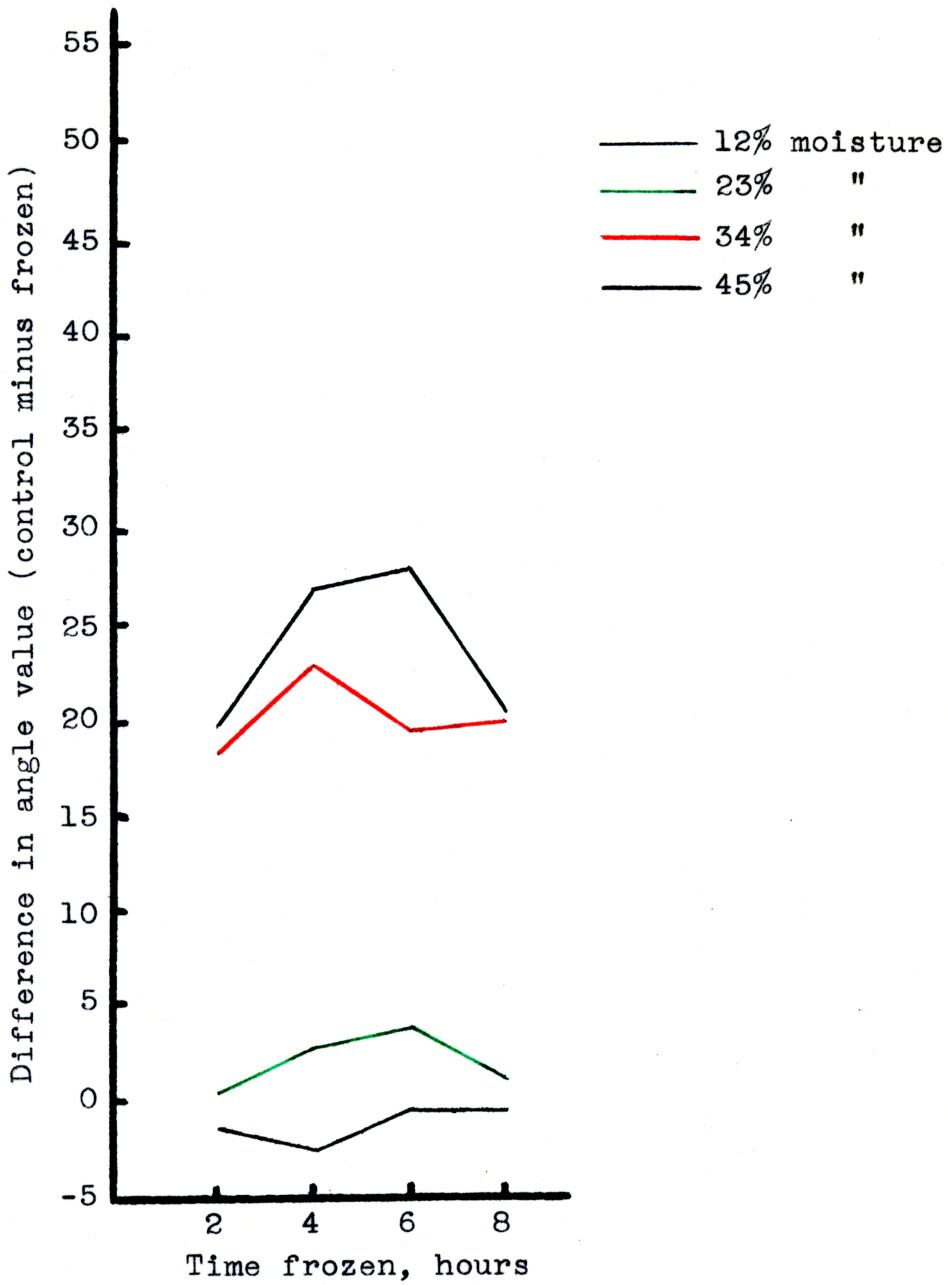

Fig. 3. The effect of length of time frozen on germination of sorghum used at various moisture levels. The differences represent an average for all freezing temperatures used: 31°, 28°, 25°, and 22° F.

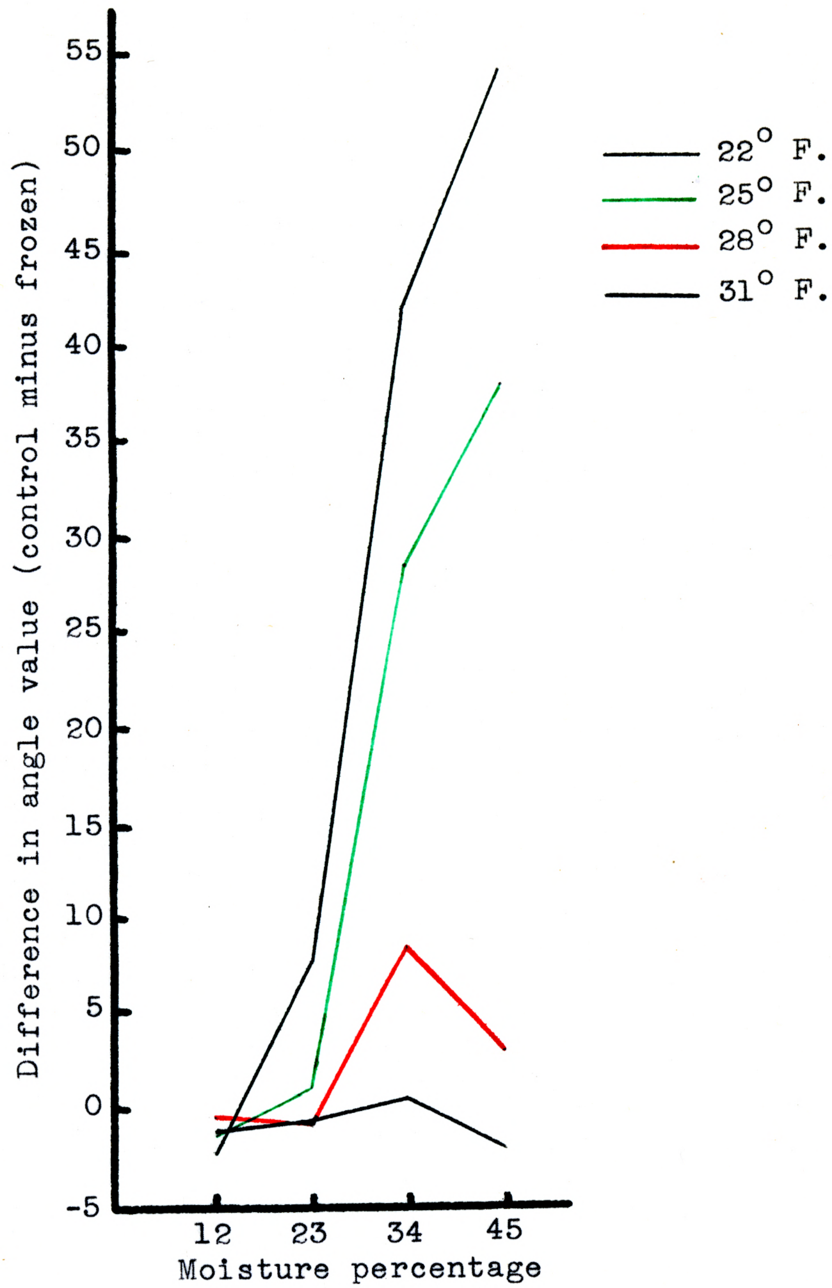

Fig. 4. The effect of different moisture percentages of the grain on the germination of sorghum seed at different freezing temperatures.

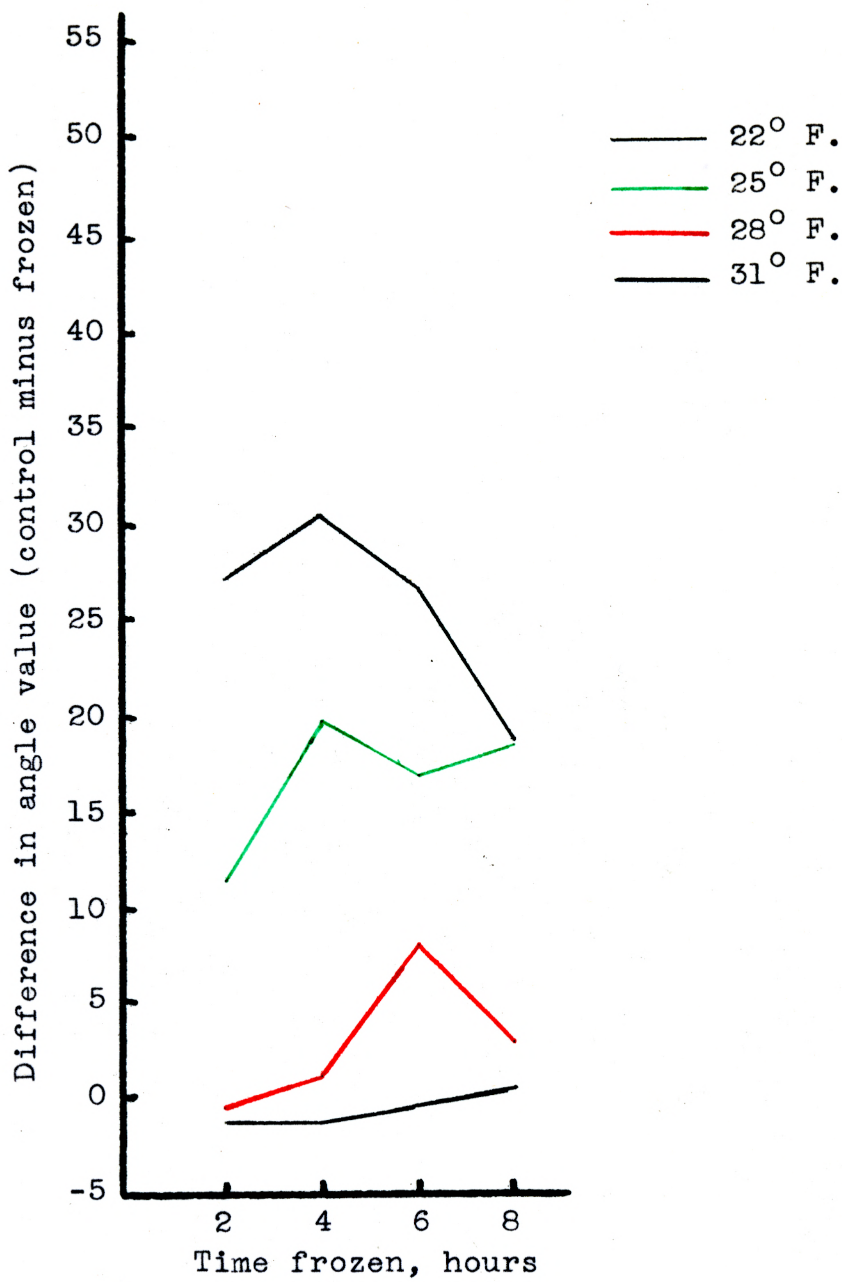

Fig. 5. The effect of length of time frozen on germination of sorghum seed at different freezing temperatures. The differences represent an average for all moisture levels used: 45, 34, 23, and 12 percent.

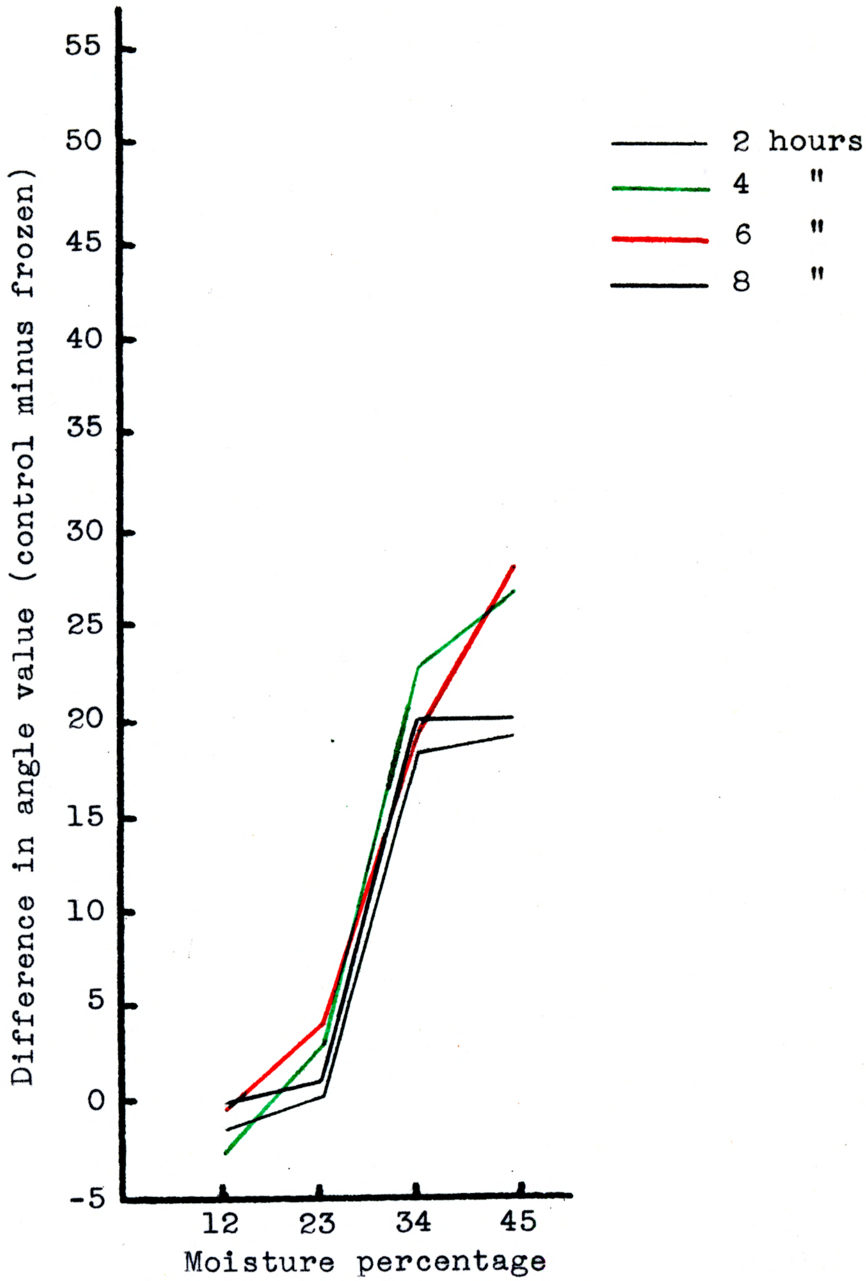

Fig. 6. The effect of different moisture levels on the germination of sorghum seed frozen for different lengths of time. The differences represent an average for all freezing temperatures used: 31°, 28°, 25°, and 22° F.

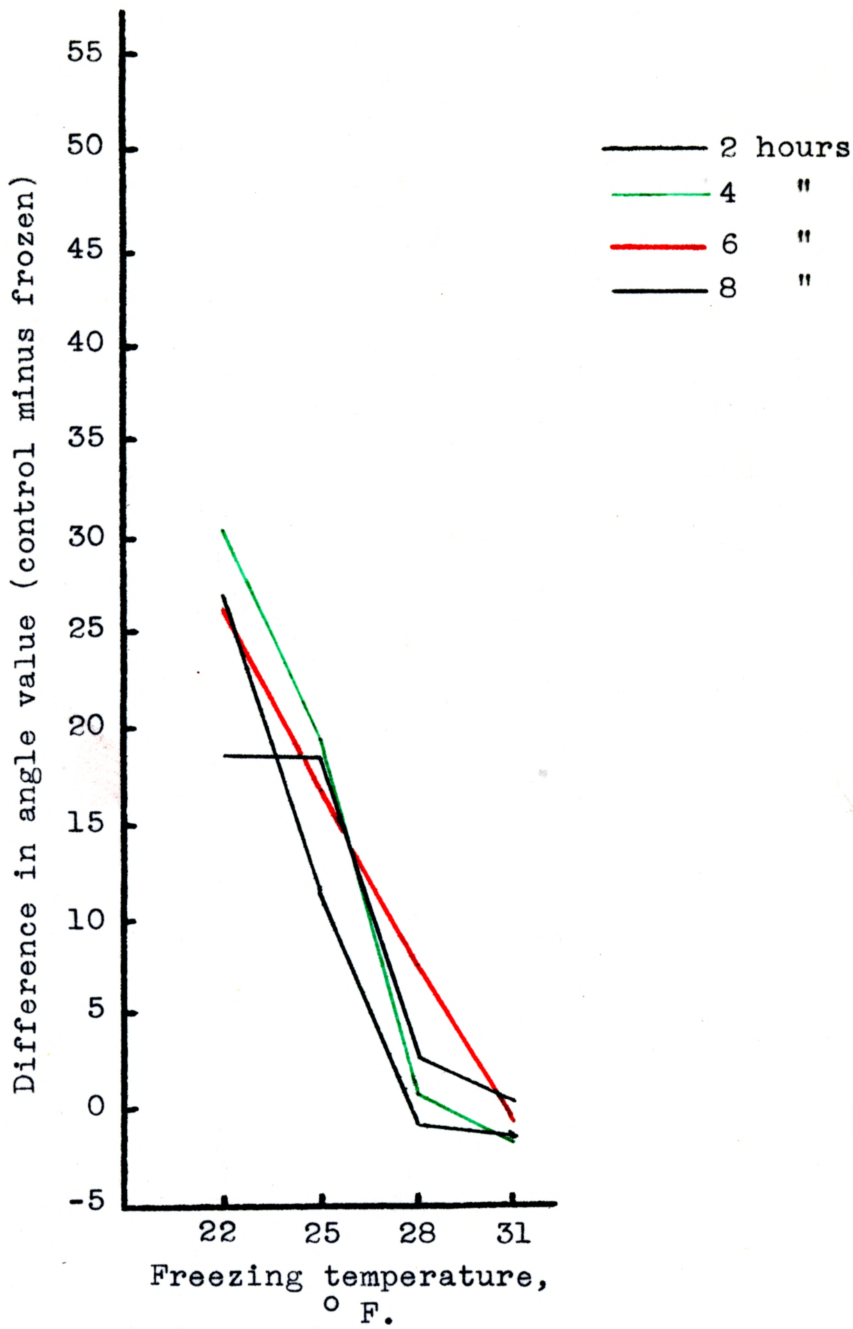

Fig. 7. The effect of different freezing temperatures on the germination of sorghum seed frozen for different lengths of time. The differences represent an average for all moisture levels used: 45, 34, 23, and 12 percent.

Table 10a. Means of differences (control minus frozen) of angle values for seed of different moisture levels, frozen for different lengths of time.

Time, hours	Moisture percentage 12	:	23	:	34	:	45
2	-1.46		+0.36	*	+18.26		+19.23
					near*		*
4	-2.61	*	+2.85	*	+22.64		+26.68
6	-0.49	*	+3.82	*	+19.32	*	+27.91
							*
8	-0.40		+1.06	*	+19.92		+20.12

LSD* = 4.53.

Note: An * between two values indicates a significant difference between those two values.

Table 10b. Identical to Table 10a, except the differences are expressed as percentages.

Time, hours	Moisture percentage 12	:	23	:	34	:	45
2	-2.1		+2.2	*	+27.9		+26.9
					near *		*
4	-4.7	*	+5.8	*	+33.8		+37.4
6	-0.7	*	+6.6	*	+29.8	*	+39.2
							*
8	-0.9		+2.9	*	+31.8		+31.3

Table 11a. Means of differences (control minus frozen) of angle values for seed with different moisture percentages that was frozen at different temperatures.

Temp.	Moisture percentage						
° F.	12		23		34		45
22	-2.22	*	+7.81	*	+42.32	*	+54.41
			*		*		*
25	-1.32		+1.28	*	+28.52	*	+37.95
					*		*
28	-0.36		-0.55	*	+ 8.66	*	+ 3.43
					*		*
31	-1.06		-0.44		+ 0.65		- 1.85

LSD* = 4.53.

Note: An * between two values indicates a significant difference between those two values.

Table 11b. Identical to Table 11a, except differences are expressed as percentages.

Temp.	Moisture percentage						
° F.	12		23		34		45
22	-4.3	*	+15.9	*	+65.2	*	+73.0
			*		*		*
25	-2.2		+ 2.3	*	+45.7	*	+59.2
					*		*
28	-0.6		- 0.4	*	+12.1	*	+ 5.4
					*		*
31	-1.3		- 0.3		+ 0.4		- 2.8

Table 12a. Means of the differences (control minus frozen) of angle values for seed frozen at different temperatures and at different lengths of time.

Temp. ^{o} F.	Time, hours: 2		4		6		8
22	+27.01		+30.47		+26.26	*	+18.64
	*		*		*		
25	+11.42		+19.59		+16.83		+18.59
	*		*		*		*
28	- 0.68		+ 0.94	*	+ 7.96	*	+ 2.96
					*		
31	- 1.34		- 1.40		- 0.48		+ 0.52

LSD* = 4.53.

Note: An * between any two values indicates a significant difference between those two values.

Table 12b. Identical to Table 12a, except the differences are expressed as percentages.

Temp. ^{o} F.	Time, hours: 2		4		6		8
22	+40.3		+42.8		+37.3	*	+29.5
	*		*		*		
25	+18.1	*	+30.8		+26.6		+29.4
	*		*		*		*
28	- 1.4		+ 0.6	*	+12.0	*	+ 5.1
31	- 2.1		- 2.0		- 1.0		+ 1.3

statistical analyses. All linear, quadratic, and cubic components in the statistical analyses were tested for significance by the error mean square in Table 3.

The effect of the different temperatures on reduction in germination at various moisture levels was largely linear, with less injury at the higher temperatures (Table 4 and Fig. 2). More injury occurred in the 34 percent than 45 percent moisture level when frozen at 28° F. Time of exposure generally was not significantly related to injury at the moisture levels used (Table 5 and Fig. 3). Eight hours of freezing produced significantly less injury than six hours at the 45 percent moisture, causing the quadratic component of 45 percent moisture to be very highly significant. The effect of moisture on injury at 22° and 25° F. was largely linear (Table 6 and Fig. 4). The effect of time of exposure on injury at different temperatures was rather erratic, but at 22° F. the trend was for the injury to be less severe at the longer exposures (Table 7 and Fig. 5). Different moistures had a significant linear effect on the reduction in germination at the different times of exposure (Table 8 and Fig. 6). The cubic component also was significant for each time of exposure which indicated a possible leveling off of the curve at high and low moistures. Temperature effects on germination reduction at the different times of exposure also were largely linear (Table 9 and Fig. 7).

A significant cubic was apparent in several cases which showed that in reaction to freezing, the sharpest change occurred when the moisture was decreased from 34 to 23 percent. The same thing was evident with the temperatures used, since the largest change occurred between the temperatures of 28° and 25° F. Table 10a showed this since, in every case, a significant difference occurred between 23 and 34 percent moisture, and in Table 12a, where a significant difference occurred in every case between 25° and 28° F. This indicated that a critical temperature occurred somewhere between 25° and 28° F. and that a critical moisture content probably occurred between 23 and 34 percent, below which little freezing injury occurred. In no cases did the 12 percent moisture group or the groups exposed to 31° F. show any significantly different response to different treatments.

The first indication of freezing injury was an increase in the percentage of abnormal seedlings (Table 13). Severe injury, however, resulted in a large increase in dead seeds. The number of dead seeds increased with increasing moisture and decreasing temperature, with 63 percent dead seeds in the 45 percent moisture seed frozen at 22° F. Many of those classed as abnormal, in the groups showing severe injury, were nearly dead, as only the primary root ever showed any sign of growth; exerting itself from one-eighth inch to just visibly breaking the seed coat before ceasing growth.

Damage to the plumule within the coleoptile was closely associated with freezing injury, as any group that showed injury to the plumules always showed a reduced germination. The plumule damage showed itself by the absence of a plumule in an otherwise

Table 13. The percentage of normal and abnormal seedlings and dead seeds at different moisture percentages, frozen at different temperatures. Similar to Table 1, except abnormal and dead are included and no distinction is made for the different lengths of time frozen.

Temp. frozen: ° F.		Moisture percentage 12			23			34			45			Average		
		Normal	Abnormal	Dead	Normal	Abnormal	Dead	Normal	Abnormal	Dead	Normal	Abnormal	Dead	Normal	Abnormal	Dead
	Control	57	25	18	61	27	12	79	15	6	77	19	4	68.5	21.5	10.0
22	Frozen	61	23	16	45	36	19	14	43	43	4	33	63	31.0	33.75	35.25
	Difference*	-4	+2	+2	+16	-9	-7	+65	-28	-37	+73	-14	-59	+37.5	-12.2	-22.25
	Control	67	22	11	62	27	11	81	17	2	82	16	2	73.0	20.5	6.5
25	Frozen	69	22	9	60	27	13	35	49	16	23	44	33	46.75	35.5	17.75
	Difference	-2	0	+2	+2	0	-2	+46	-32	-14	+59	-28	-31	+26.25	-15.0	-11.25
	Control	61	26	13	64	26	10	88	10	2	84	14	2	74.25	19.0	6.75
28	Frozen	62	23	15	64	26	10	76	21	3	79	15	6	70.25	21.25	8.5
	Difference	-1	+3	-2	0	0	0	+12	-11	-1	+5	-1	-4	+4.0	-1.25	-1.75
	Control	65	22	13	61	25	14	83	14	3	80	17	3	72.25	19.5	8.25
31	Frozen	67	21	12	61	27	12	83	14	3	83	14	3	73.5	19.0	7.5
	Difference	-2	+1	+1	0	-2	+2	0	0	0	-3	-3	0	-1.25	+0.5	+0.75
	Control	62.5	23.75	13.75	62.0	26.25	16.75	82.75	14.0	3.25	80.75	16.5	2.75			
Avg.	Frozen	64.75	22.25	13.0	57.5	29.0	13.5	52.0	31.75	16.25	47.25	26.5	26.25			
	Difference	-2.25	+1.5	+0.75	+4.5	-2.75	-1.75	+30.75	+17.75	-13.0	+33.5	-10.0	-23.0			

* Difference always means control minus frozen

normal coleoptile. In the groups showing injury from 28° F., only a few seedlings in each sample showed that condition. The absence of plumules was much more evident in the 25° F. treatment, having been very common at the high moisture levels. There was less of that condition at 22° F. at the 45 percent moisture level, as most seeds were either killed or injured so severely that the condition never became visible.

The classification of the frozen samples compared to the unfrozen, during counting, corresponded very well with the actual reduction shown by germination percentage. Zero ratings were the rule for any group with 12 percent moisture, or any group exposed to 31° F. In groups showing injury, the ratings indicated the injury, as the ratings increased with increasing injury until 2++ ratings were given to about all samples of 45 percent moisture, frozen at 22° F.

In the fall of 1959, a rerun of germination was made on 35 heads that had given inconsistent germination results in the tests the previous spring. The same number of 100-seed samples were germinated as in the spring. In general, the large variations in germination did not occur in the rerun. The average germination for all the samples showed exactly the same percentage reduction in the frozen samples; however, the average germination for both control and frozen samples was three percent lower than for those previously run. In the spring, the average germination percentage was 67.5 for control and 60.1 for frozen seed, while in the fall run, the percentages were 64.5 for control and 57.1 for the frozen samples. Germination apparently had lowered three percent in six months. The seed had been stored for four months at room temperature and two months at 39° F.

Plainsman - 1959

A few samples of Plainsman harvested in 1959 gave similar results to that harvested in 1958 (Table 14). Germination was severely reduced in the 45 percent moisture samples frozen at 22° F., but not as much injury occurred as in seed harvested in 1958. The injury at eight hours was less than at any other time of exposure, substantiating results obtained from seed harvested in 1958. The seed collected in 1959 showed much better germination than that collected in 1958.

Table 14. Comparison of Plainsman collected in 1959 with Plainsman collected in 1958, both with 45 percent moisture, and frozen at 22° F.

Variety	Time frozen, hours	Germination percentage		Percentage difference
		Control	Frozen	
Plainsman 1958	2	72.8	1.4	+71.4
	4	81.2	0.6	+80.6
	6	75.9	0.2	+75.7
	8	79.4	15.0	+64.4
	Average	77.3	4.3	+73.0
Plainsman 1959	2	95.2	31.8	+63.4
	4	92.8	24.8	+68.0
	6	87.2	17.8	+69.4
	8	97.0	60.2	+36.8
	Average	93.0	33.6	+59.4

Atlas

The Atlas harvested in 1958 was frozen at 28° F. and had an average moisture percentage of 31.1. Very little injury occurred, with an average decrease of only 2.6 percent in germination (Table 15). Atlas harvested in 1959 averaged 40.7 percent moisture and was frozen at 25° F. It showed slight injury; the frozen samples averaging 9.1 percent lower in germination (Table 16). Atlas appeared to be more tolerant to freezing than Plainsman, because at 40.6 percent moisture, it was injured much less than Plainsman with 34 percent moisture, frozen at the same temperature, which showed a 45.7 percent decrease in germination.

Table 15. Comparison of Atlas harvested in 1958 with an average of 31.1 percent moisture, with Plainsman harvested in 1958 with 34 percent moisture, both frozen at 28° F.

Variety	Time frozen, hours	Germination percentage: Control	Germination percentage: Frozen	Percentage difference
	2	88.6	89.0	- 0.4
Plainsman 1958	4	87.4	82.1	+ 5.3
	6	87.6	63.9	+23.7
	8	86.8	67.1	+19.7
	Average	87.6	75.5	+12.1
	2	75.2	74.2	+ 1.0
Atlas 1958	4	84.3	81.9	+ 2.4
	6	81.8	75.1	+ 6.7
	8	78.0	77.8	+ 0.2
	Average	79.8	77.2	+ 2.6

Table 16. Comparison of Atlas harvested in 1959 with an average of 40.7 percent moisture, with Plainsman of 45 percent moisture harvested in 1958; both frozen at 25° F.

Variety	Time frozen, hours	Germination percentage Control	Germination percentage Frozen	Percentage difference
	2	81.8	39.8	+42.0
Plainsman	4	79.8	12.9	+66.9
1958	6	86.1	17.9	+68.2
	8	80.5	21.0	+59.5
	Average	82.1	22.9	+59.2
	2	91.8	86.8	+ 5.0
Atlas	4	95.0	90.2	+ 4.8
1959	6	94.8	77.5	+17.3
	8	89.0	80.0	+ 9.0
	Average	92.7	83.6	+ 9.1

RS610

The RS610[1] reacted much the same as Plainsman to freezing, except that the injury to RS610 was less severe (Tables 17 through 21). Germination was reduced 25.7 percent when seed of 45 percent moisture was frozen at 22° F. compared to a 73 percent reduction in Plainsman. RS610 showed more tolerance to freezing than Plainsman. The germination of the control samples of RS610 was always higher than for Plainsman.

Seed grown in 1959 showed a higher germination for the control samples and less freezing injury than that grown in 1958, which indicated that possibly a weather condition in 1959 contributed to better quality seed that was more tolerant to

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[1] Refers to open-pollinated F_2 seed harvested from F_1 plants of RS610.

Table 17. Comparison of Plainsman with 45 percent moisture with RS610 of 45 percent moisture, both having the same treatment.

Temp. °F.	Time frozen hrs.	Plainsman-1958 Control	Plainsman-1958 Frozen	Plainsman-1958 Percentage difference	RS610-1959 Control	RS610-1959 Frozen	RS610-1959 Percentage difference
31	2	80.5	85.1	- 4.6	97.8	95.0	+ 2.8
	4	77.4	82.0	- 4.6	97.2	95.8	+ 1.4
28	2	85.4	86.6	- 1.2	95.8	97.0	- 1.2
	4	81.8	74.9	+ 6.9	95.8	96.8	- 1.0
25	2	81.8	39.8	+42.0	92.8	75.0	+17.8
	4	79.8	12.9	+66.9	96.0	71.2	+24.8
22	2	72.8	1.4	+71.4	97.2	48.0	+49.2
	4	81.2	0.6	+80.6	93.0	85.5	+ 7.5
	Avg.	80.1	47.9	+32.2	95.7	83.0	+12.7

Table 18. Comparison of Plainsman of 45 percent moisture with RS610 of 45 percent moisture; both frozen at 22° F.

Hybrid or variety	Time frozen, hours	Germination percentage Control	Germination percentage Frozen	Percentage difference
Plainsman 1958	2	72.8	1.4	+71.4
	4	81.2	0.6	+80.6
	6	75.9	0.2	+75.7
	8	79.4	15.0	+64.4
	Average	77.3	4.3	+73.0
RS610 1959	2	97.2	48.0	+49.2
	4	93.0	85.5	+ 7.5
	6	85.5	58.2	+27.3
	8	97.5	78.8	+18.7
	Average	93.3	67.6	+25.7

Table 19. Comparison of Plainsman at 34 percent moisture with RS610 of 38.6 percent moisture; both frozen for two hours.

Hybrid or variety	Temperature ° F.	Germination percentage Control	Frozen	Percentage difference
Plainsman 1958	31	79.1	80.2	- 1.1
	28	88.6	89.0	- 0.4
	25	83.1	40.5	+42.6
	22	80.1	9.6	+70.5
	Average	82.7	54.8	+27.9
RS610 1959	31	87.5	80.2	+ 7.3
	28	96.8	92.5	+ 4.2
	25	97.2	93.8	+ 3.4
	22	94.2	63.2	+31.0
	Average	93.9	82.4	+11.5

Table 20. Comparison of Plainsman with 34 percent moisture with RS610 with an average moisture of 39.6 percent; both frozen at 22° F.

Hybrid or variety	Time frozen, hours	Germination percentage Control	Frozen	Percentage difference
Plainsman 1958	2	80.1	9.6	+70.5
	4	81.5	6.4	+75.1
	6	75.8	16.0	+59.8
	8	78.0	22.4	+55.6
	Average	78.8	13.6	+65.2
RS610 1959	2	94.2	63.2	+31.0
	4	89.2	33.2	+56.0
	6	89.8	55.8	+34.0
	8	81.2	57.5	+23.7
	Average	88.6	52.4	+36.2

Table 21. Comparison of Plainsman with 23 percent moisture with RS610 of 29.5 percent moisture; both frozen at 28° F.

Hybrid or variety	Time frozen, hours	Germination percentage Control	Germination percentage Frozen	Percentage difference
Plainsman	2	62.0	63.4	-1.4
1958	4	61.9	63.9	-2.0
	Average	62.0	63.6	-1.6
RS610	2	95.2	94.8	+0.4
1959	4	93.5	92.5	+1.0
	Average	94.4	93.6	+0.8

freezing. The wet weather in 1958 may have been important in causing poor quality seed because of excessive weathering. However, the RS610 grown in 1959 still was injured less than Plainsman, with identical treatment, also grown in 1959, which indicated more tolerance for RS610.

DISCUSSION

Results of this study generally agree with other results reported on freezing injury to germination of sorghums and other cereals, in that seedling injury and reduced germination becomes more severe at higher moisture content of the grain and at the lower temperatures.

In this study, seed below 25 percent moisture generally was not injured, while seed with over 30 percent moisture was injured by the temperatures of 25° and 22° F. Thus, while maturing, seed became substantially more tolerant to freezing when the moisture reached 25 to 30 percent. This was slightly lower than Robbins and Porter (7), who found that sorghum seed of 30 to 35 percent moisture was not injured by freezing at 20° F.

The effect of the length of freezing was interesting, since eight hours of freezing generally resulted in less injury than freezing at six hours. Other studies, when different lengths of freezing time were used, did not show much difference, but Rossman (9), studying corn, using 2, 4, 6, 8, and 16 hours, stated that time was significant, with more injury at the longer times. Some of the results obtained fell within the limit of experimental error; however, the consistent occurrence of the results (Tables 10a and 12a), and especially since the same thing occurred in 1959, the author was led to believe that the results obtained could not be wholly attributed to experimental error. If the latter assumption was correct, that fact may possibly shed some light on the actual mechanism of freezing injury.

The seed used in the major portion of this study was not ideal. Germination of the seed was below average, especially in those samples harvested last. The average germination of all samples was slightly below 80 percent, whereas good quality seed should germinate 90 percent or better under the ideal conditions in a laboratory. The extremely wet weather during the growing season of 1958, and especially in the fall, likely contributed to the poor quality seed. Seed harvested when ripe or nearly ripe showed considerable weathering. The poor germination qualities likely had an effect upon freezing injury and probably contributed to some of the variability in germination. Seed harvested in 1959 was of better germination, and germination results tended to be more uniform. The Plainsman harvested in 1959 was injured less than that harvested in 1958, which indicated that the poor quality of seed in 1958 may have contributed to the large reductions in germination obtained.

Differences in physiological maturity may have influenced results in different years. However, since less injury occurred in seed grown in a warm, dry year, any conclusions made concerning physiological maturity would have been contrary to conclusions by Rossman (10). He stated that corn grown in hot, dry weather was less physiologically mature at a given moisture level than grain grown under favorable conditions.

The frost in the fall of 1958 apparently did not influence the study. The control samples of seed harvested after frost, germinated as well as the control samples of similar moisture percentage harvested before the frost. One sample of 23 percent moisture, and all samples with 12 percent moisture were harvested after the frost.

Individual heads with the same treatment often differed considerably in reaction to freezing. That difference likely increased the variability. Kiesselbach and Ratcliff (4) observed the same thing in corn, as a large difference often occurred in reaction to freezing of individual ears that had identical treatments.

The reason for the general occurrence of a slightly, but not significantly, higher germination in the frozen seed at the low moisture levels and the high temperatures was not known. It is possible that seed frozen at 31° F. was somehow stimulated and thus a slightly higher germination occurred. Variation in germination at the low-moisture level is attributed to experimental error.

A varietal difference apparently occurred in tolerance of immature seed to withstand freezing temperatures. This difference may occur because of differences in the general germination qualities of varieties. Atlas was more tolerant than Plainsman, and this corresponds to the generally accepted idea that Atlas has better germination properties than most grain sorghums. Also, the difference in injury to Plainsman in the two years corresponded to the differences in general germination qualities of the seed. Rossman (9) stated that tolerance of seed corn to freezing injury was significantly related to seedling vigor. Assuming that good quality seeds produce vigorous seedlings, good quality seeds should be more tolerant of freezing temperatures.

Seed of the hybrid, RS610, showed more tolerance to freezing than Plainsman. This one example does not mean that hybrid sorghums are more tolerant to freezing than standard varieties. Rossman (10) found no relation between tolerance to freezing and hybrid vigor in seed corn. The greater tolerance may have occurred mainly because of higher quality seed, or because of a genetic difference between it and Plainsman.

SUMMARY AND CONCLUSIONS

Freezing immature sorghum grain of 34 percent moisture or above at temperatures of 25° and 22° F. resulted in severe reductions in germination.

Freezing at 28° and 31° F. at moisture up to 45 percent had no important effect upon germination.

Grain with 23 percent moisture or below showed little injury at freezing temperatures as low as 22° F. for as long as eight hours. A critical moisture appeared to occur between 23 and 34 percent, probably close to 30 percent moisture.

Injury increased with lower freezing temperatures and higher moisture percentage.

Time of freezing, up to eight hours, generally had no important effect on the injury incurred.

Injury to frozen seeds was gradual; the first evidence having been an increase in abnormal seedlings, followed by seeds that were nearly dead, and finally dead seeds.

Good quality seed was injured less than poor quality seed. Thus, seed of the same variety may differ in tolerance to freezing in different years.

A varietal difference in tolerance of grain to withstand freezing temperatures was apparent. This may have been related to general germination properties of the seed of different varieties.

Atlas and grain from the hybrid, RS610, were more tolerant to freezing than Plainsman.

This study indicated that ordinary fall freezes should not materially reduce germination of grain sorghum, if temperatures don't fall below 28° F. and moisture content of the grain is no higher than 45 percent.

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FREEZING INJURY TO GERMINATION OF SORGHUM SEED WHEN FROZEN AT DIFFERENT STAGES OF MATURITY, AT DIFFERENT TEMPERATURES, AND FOR DIFFERENT LENGTHS OF TIME

by

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This study was initiated because of a need for information regarding the injury to germination that results when sorghum seed is subjected to freezing temperatures in the fall before the grain is mature. Sorghum grain was harvested at different stages of maturity, as indicated by moisture content, and subjected artificially to different freezing temperatures for different lengths of time. Moisture contents used were approximately 45, 34, 23, and 12 percent. Temperatures used were 31°, 28°, 25°, and 22° F. for two, four, six, and eight hours. The variety, Plainsman, was used for the main portion of the study, but a small amount of Atlas and the hybrid, RS610, also was used.

All heads were split; one-half was frozen, with the other half serving as the control. After freezing, heads were dried and germination tests run on the material about six months after it had been harvested. Two 100-seed samples were germinated from each half-head. Differences, control minus frozen, were calculated for each moisture, temperature, and time combination.

Seed injury became more severe as the temperature of freezing was lowered and as the moisture content of the grain became higher. Duration of freezing, ranging from two to eight hours, was not an important factor.

Seed with 34 and 45 percent moisture was severely injured by freezing temperatures of 22° and 25° F. At 25° F., germination in the 34 percent moisture group was lowered 45.7 percent while in the 45 percent group it was lowered 59.2 percent.

In seed frozen at 22° F. for 34 and 45 percent moisture groups, germination was lowered 65.2 and 73.0 percent, respectively. Seed with 23 percent moisture, frozen at 22° F., was reduced in germination by 15.9 percent. At 28° F., both 34 and 45 percent moisture groups showed slight injury; germination being reduced 12.1 percent in seed with 34 percent moisture, and 5.4 percent in seed with 45 percent moisture. No seed frozen at 31° F. was injured. Seed with 23 percent moisture showed injury only at the lowest temperature, 22° F.

Seed harvested in 1959 was more tolerant to freezing than seed harvested in 1958, which indicated that quality of seed may be an important factor in its tolerance to freezing. Seed for the main portion of the study, harvested in 1958, was of rather low quality, having lower germination than expected of good seed.

Varieties differed in their tolerance to freezing, as Atlas and the hybrid, RS610, were injured less than Plainsman.

According to the study, ordinary fall freezes should not be detrimental to seed germination, provided the temperature falls no lower than 28° F. and the moisture of the grain, when the freeze occurs, is no higher than 45 percent.